Julius-Maximilians University Würzburg
Julius von Sachs Institute
Department of Botany I – Molecular Plant Physiology and Biophysics

Nitrogen and crop productivity:

A look at mechanisms and quantitative relationships for modelling purpose

A diploma thesis
Presented by Verena Seufert

December 2009

Supervisors:

Prof. Wolfgang Cramer, Potsdam Institute for Climate Impact Research
Prof. Werner Kaiser, University of Würzburg

Abstract

The implementation of agriculture and the N cycle into global models of the terrestrial biosphere are two current important fields under construction. The integration of the combined effects of land use and human N management on the earth system into forecasting global models is of special interest, as these represent two major components of human induced global environmental change. This draws the need to first derive a sufficiently robust understanding of relevant processes. In this thesis therefore the C-N interactions in the crop plant are addressed by means of qualitative and quantitative reviews and it is examined how N constrains crop primary productivity. The crop plant is considered in an autecological context, thus emphasising on the molecular and physiological level of control. The mechanisms by which different C and N processes are regulated are identified. Special emphasis is set on the regulation of single proteins, as this allows deriving indications about primary controls imposed on plant physiology. A set of key signals involved in numerous C and N processes are distinguished, contributing to the coordinated regulation of C and N metabolisms. The linear relationship between photosynthesis and leaf N content is identified as a general and central connection between the C and the N metabolism that is however essentially empirical and most likely does not depict a primary control. The relationships dissected are then reconsidered by means of a meta-analysis. The response of important parameters describing crop physiology and growth to N limitation is analyzed. Several of the conclusions from the literature review are corroborated by the results of the meta-analysis and several open questions that emerged from the previous section can be responded. The meta-analysis however represents the first application of meta-analytical methods to the N-limitation effect and it thus includes a number of flaws that are addressed and discussed in detail, in order to allow for a future refined application of meta-analysis for the examination of the crop N response. A conceptual framework for the representation of crop C-N-interactions in a global model of the managed land surface is then proposed, based on the regulations and controls identified earlier. Several present N-inclusive models are inspected and it is evaluated if and how relevant processes and feedbacks identified in the literature review and the meta-analysis are represented. Finally the results of the thesis are placed into a broader context and issues that are in need of research are discussed.

Zusammenfassung

Die Darstellung der Landwirtschaft und des Stickstoffkreislaufs in globalen Modellen der terrestrischen Biosphäre ist ein wichtiges und sehr aktuelles Forschungsfeld. Die Integration der Folgen von Landnutzung und des durch menschliche Aktivitäten stark veränderten Stickstoffkreislaufs auf die Geosphäre in globale Modelle ist von besonderem Interesse, da diese beiden Prozesse zu den primären Treibern globaler Umweltveränderungen zählen. Für eine Integration in Modelle wird jedoch zunächst ein robustes Verständnis der primären Prozesse benötigt. In der vorliegenden Arbeit betrachte ich deshalb die Wechselwirkungen zwischen Kohlenstoff und Stickstoff in der Pflanze anhand einer klassischen, qualitativen Literaturübersicht sowie anhand einer quantitativen, statistischen Analyse experimenteller Literatur. Der Fokus liegt dabei auf landwirtschaftlichen Feldfrüchten, die in einem autökologischen Kontext untersucht werden, d.h. mit einem Schwerpunkt auf der Betrachtung der molekularen und physiologischen Ebene. Zunächst werden die Mechanismen der Regulierung von Kohlenstoff- und Stickstoffprozessen identifiziert. Dabei wird besonderer Wert gelegt auf eine Analyse der Regulierung auf Proteinebene, da dies einen Rückschluss auf die primär kontrollierenden Faktoren erlaubt. Eine Gruppe von Schlüssel-Signalen, die eine koordinierte Regulierung des Kohlenstoff- und Stickstoffmetabolismus erlauben, wird identifiziert. Die lineare Beziehung zwischen Photosynthese und dem Stickstoffgehalt des Blattes stellt eine zentrale Verbindung zwischen Kohlenstoff und Stickstoff in der Pflanze dar. Diese Beziehung ist jedoch essentiell empirisch und beschreibt keinen primären Zusammenhang. Alle Beziehungen die in der Literaturanalyse als relevant für die Zusammenarbeit von Kohlenstoff und Stickstoff im pflanzlichen Metabolismus befunden wurden, werden schließlich in einer Meta-Analyse überprüft. Dabei wird die Reaktion von Parametern die Physiologie und Wachstum von Feldfrüchten beschreiben auf Stickstofflimitierung analysiert. Zahlreiche Schlussfolgerungen aus der Literaturübersicht werden dabei bestätigt. Zudem werden auch einige offene Fragen, die sich aus der Literaturübersicht herausgebildet haben, geklärt. Die vorliegende Meta-Analyse stellt jedoch die erste Anwendung dieser Methode auf den Effekt von Stickstofflimitierung dar und sie beinhaltet daher noch einige Mängel. Diese werden ausführlich dargestellt und diskutiert, um eine zukünftige, verbesserte Anwendung meta-analytischer Methoden auf Stickstoff in Feldfrüchten zu ermöglichen. Schließlich wird ein konzeptioneller Rahmen für die Repräsentation von Stickstoff in Feldfrüchten in einem globalen Modell vorgestellt, basierend auf den Regulierungen und Kontrollen die zuvor in der Literaturübersicht und der Meta-Analyse identifiziert wurden. Die Darstellung relevanter Prozesse und Wechselwirkungen in einigen bestehenden Simulationsmodellen, die Stickstoffprozesse integrieren, wird untersucht. Zum Schluss werden die Ergebnisse der Arbeit in einen größeren Zusammenhang gestellt und Bereiche, die weitere Forschung benötigen, werden diskutiert.

Contents

List of Tables

List of Figures

Chapter 1
Introduction

Mathematical models of terrestrial ecosystems (TEMs) have now long been used to assess global carbon-climate and atmosphere-biosphere interactions. Such models are valuable tools in coping and managing human induced global environmental change, as they allow predictions about the nature of changes and about possible feedbacks, and they help to assess the contribution of different processes to the present and future alteration of terrestrial ecosystems.

The first step in the simulation of atmosphere-biosphere feedbacks was the coupling of terrestrial carbon (C) cycle models - e.g. BIOME-BGC (Running & Hunt 1993), IBIS (Foley *et al.* 1996) and LPJ (Sitch *et al.* 2003) - with global climate models (e.g. Claussen 1994; Foley *et al.* 1998; Sitch *et al.* 2005; Friedlingstein *et al.* 2006). However global environmental change does not solely consist of an increase in carbon dioxide (CO_2) concentrations and associated climate change but also in a strong alteration of the global nitrogen (N) cycle and in human land use and land cover change (Vitousek 1994; Vitousek *et al.* 1997; Foley *et al.* 2005).

Despite the great impact of human land use on earth's ecosystems and climate (Foley *et al.* 2005), global land-surface models have often not given explicit consideration to land use. Most dynamic global vegetation models (DGVMs) for example, which try to evaluate the future possible responses of ecosystem processes to climate change (e.g. net primary production, carbon storage in soil, litter and biomass, freshwater runoff), mainly describe the potential natural vegetation (Cramer *et al.* 2001). Some earth system models that do consider the role of land cover changes on global climate describe land use as deforestation, i.e. as transformation into grassland (e.g. Brovkin *et al.* 2004). But agriculture has in fact specific effects on albedo or surface roughness, and crop land differs from grassland in many aspects of the biophysical and biogeochemical cycle (e.g. Schimel 1986; Twine *et al.* 2004; Skiba *et al.* 2009). Therefore, lately crop processes have been integrated in land biosphere models (e.g. Kucharik & Brye 2003; Gervois *et al.* 2004; Bondeau *et al.* 2007; Zaehle *et al.* 2007). This not only allows considering the influence of land use on global biogeochemistry (Bondeau *et al.* 2007) but also bears the possibility to examine the impact of climate change on agricultural productivity (Rosenzweig & Parry 1994). The implementation and improvement of the representation of agriculture in global land surface models is therefore currently under intensive research.

The integration of plant-soil interactions and N processes into terrestrial global models is another current important field under construction (Ostle *et al.* 2009). The N cycle has been implemented in several terrestrial models, e.g. CN-TEM (McGuire *et al.* 1992), Hybrid (Friend *et al.* 1997), BIOME-BGC (Thornton & Rosenbloom 2005), CLM-CN (Thornton *et al.* 2007, 2009), LPJ (Xu-Ri & Prentice 2008), SDGVM (Woodward & Lomas 2004), ISAM (Jain *et al.*, in press), IGSM (Sokolov *et al.* 2008) and CN-CLASS (Wang *et al.* 2001) (see Ostle *et al.* 2009 for a recent review about the current representation of N processes in DGVMs). Comparisons of different terrestrial biosphere models reveal that the integration of N constraints and associated C-N feedbacks are responsible for large part of the variation in the prediction of gross primary productivity (GPP) between models (Cramer *et al.* 1999; Jung *et al.* 2007).

Implementation of N processes into models of the C cycle alters the sensitivity of ecosystems to temperature and precipitation and changes the magnitude of the CO_2 fertilization effect, thus leading to a decline in terrestrial C sequestration under elevated CO_2 and an increase in C sequestration under warming compared to models that do not consider C-N interactions (McGuire *et al.* 1992; Sokolov *et al.* 2008; Thornton *et al.* 2007, 2009). These results are backed up by experiments, which confirm that the CO_2-fertilization effect is limited by N availability (Reich *et al.* 2006a, b). Coupled C-N models are thus indispensable in analyzing the effect of elevated CO_2 on the biosphere, as they may lead to greatly reduced predictions of future land C sinks (Hungate *et al.* 2003).

Warming on the contrary leads to an increase in the mineralization rate and an associated increase in N availability (Melillo *et al.* 1993, 2002). The productivity of plants seems to be enhanced more strongly by this increased N than it is reduced due to increased respiration or reduced CO_2 conductance at higher temperatures (McGuire *et al.* 1992). It has been argued however that despite the higher plant biomass, ecosystem C storage is reduced at a higher N availability due to increased soil C and N losses (Mack *et al.* 2004). These contradictory considerations underline the importance of C-N cycle interactions for climate-biosphere feedbacks and the still largely missing understanding of many of those feedbacks (see Chen & Coops 2009 for a review).

Although the representation of agriculture and N processes in models of the terrestrial biosphere is improving, I am not aware of any dynamic global model that implements both crop and N processes. The combined impact of agriculture and N however is a potentially important driving variable in the earth system. The N input into the terrestrial N cycle has more than doubled through human influence and more than 85% of this anthropogenic N fixation is due to agriculture - mainly due to N-fixation by the Haber-Bosch Process for the production of industrial fertilizer and due to cultivation of leguminous crops (Vitousek *et al.* 1997). The alteration of the N cycle through food production thus represents at present one of the most important and far-reaching components of human induced environmental change (Vitousek 1994).

Human management of crop-land leads to large imbalances in the N cycle, resulting from excessive fertilization in intensive agricultural systems and simultaneous inadequate fertilization to compensate for N losses from soils through harvest in some low-input systems (Galloway *et al.* 2008; Vitousek *et al.* 2009). Excessive N leads to damages to environmental systems, e.g. (i) the acidification of soils, streams and lakes by leaching of nitrate, (ii) the euthrophication of many aquatic ecosystems, (iii) the accelerated loss of biological diversity especially in primarily N-limited ecosystems, and (iv) increased emission of the potent greenhouse gas N_2O and of the reactive trace gas NO that is involved in the formation of photosmog (Vitousek *et al.* 1997). On the other hand, inadequate N additions to fields for replenishing soil N extracted in crop harvest lead to the loss of production potential, which impedes the attainability of food security for an increasing human population (Vitousek *et al.* 2009). This emphasises the need to understand the N budgets of agricultural systems, to evaluate the impact of agriculture on global ecosystems and to optimize

agricultural N management.

N is likely to be the most limiting factor for plant growth (Agren 1985), despite the fact that N is present in large quantities in the atmosphere. However, this N source is mostly not available to plants and they instead derive their N from only a tiny fraction (0.00024%) of planetary N in the pedosphere (Miller & Cramer 2004). Most N in the atmosphere is present as N_2 but plants can only assimilate so called reactive N, i.e. oxidized and reduced inorganic and organic N forms. N constitutes the most abundant mineral element in plant tissue and it is relevant in a large part of plant processes. In particular the photosynthetic requirements for N leads to the N control on plant growth and through this to the N control of ecosystem mass and energy exchange.

To sum up, it is important to implement an explicit representation of agriculture and of the N cycle in TEMs in order to be able to assess:

I. The effect of N constraints on plant and ecosystem productivity and the resulting interaction with the effect of elevated CO_2 concentration and warming.

II. The effect of human land use on global climate-C interactions.

III. The feedbacks between climate change, N fertilization and agricultural productivity.

IV. The alteration of the N cycle through agricultural N management.

V. The climate effect of increased reactive N resulting from agriculture.

Recently the widely used Lund-Potsdam-Jena (LPJ) DGVM (Sitch *et al.* 2003) has been adapted to include a representation of the managed planetary land surface (Bondeau *et al.* 2007). The resulting LPJ managed Land (LPJmL) model allows for dynamic representation of global agriculture by using 13 different crop functional types (CFTs) in addition to the original 10 plant functional types (PFTs) of the LPJ model. Primary production of plants and crops is calculated based on the mechanistic Farquhar *et al.* (1980a) photosynthesis scheme, as generalized by Collatz *et al.* (1991, 1992) for global scale. Allocation of C to storage organs, leaves, roots, stems and reserves takes place daily for CFTs (Bondeau *et al.* 2007).

LPJmL presently does not explicitly consider the nutrient cycle. As discussed above it is however desirable to include N processes into global TEMs like LPJmL, and the implementation of N into LPJ (Xu-Ri & Prentice 2008) and LPJmL currently is under development. So far, no mechanistic simulation of N constraints on photosynthesis and plant allocation has been implemented in either of the two (Xu-Ri & Prentice 2008). The scope of the present study thus is a look at N-related crop processes with a view to a future implementation of a process-based simulation of C-N interactions in crops in the LPJmL model.

The described need for a better implementation of combined crop and N processes into global biosphere models leads to the need for understanding the basic mechanisms relating N to crop production. Although the study of plant N metabolism has a long tradition (e.g. Irving & Hankinson 1908; Clark 1936; Nightingale 1937), the quantitative understanding of crop N processes and how they are regulated is still limited (Lawlor 2002). For the implementation of crop physiology into a model however such a quantitative and mechanistic understanding is essential. Ostle *et al.* (2009) have concluded from a literature review on the representation of plant-soil interactions in global DGVMs that the supply and integration of knowledge on ecological and biological processes into global models is a major ongoing challenge in the development and validation of DGVMs.

For this reason, in chapter 2 of this study I will first review the literature with regard to the influence of N on crop physiology, and to key factors determining the C-N interactions in plants. I will - whenever possible - specifically consider regulation and coordination at the level of proteins and genes, as real and primary control can only be identified at this molecular scale. In a second

step, I will then conduct a quantitative analysis in chapter 3 – through means of a meta-analysis – of the response of physiological parameters to N limitation in crops. This second part of the study is thus intended to examine the general direction and the magnitude of responses of plants to N, without any special consideration of the mechanisms involved. The meta-analysis is also intended to lead to more synthetic conclusions about open questions emerging from the review of the literature regarding the N-limitation response of plants. However, it can just report preliminary results, as a comprehensive synthesis of literature on N-experiments would go beyond the scope of a diploma thesis. Therefore the section on meta-analysis places emphasis on the discussion of methodological aspects, in order to allow for a future refined and more complete application of meta-analysis to a synthetic review of N physiology of crops.

A full and quantitative understanding of the molecular/cellular level is a starting point and a *conditio sine qua non,* but is not sufficient to understand and to quantify the real world process. In the real world, the molecular level interferes with physiological and ecological constraints at the level of individual plants (autecology), in plant communities (synecology), and in the context of the ecosystem, that considers also the biotic and abiotic environment of the plant community (Fig. 1.1). In this thesis the focus will be on the crop plant in an autecological context, without giving further consideration to C-N interactions between the plant and its environment. Thus I will consider mainly the cellular/molecular and physiological level of N controls on crop primary production (Fig. 1.1). I will moreover focus on crops rather than natural vegetation. Many of the matters discussed however are quite general in nature and can also be said to hold true for natural vegetation.

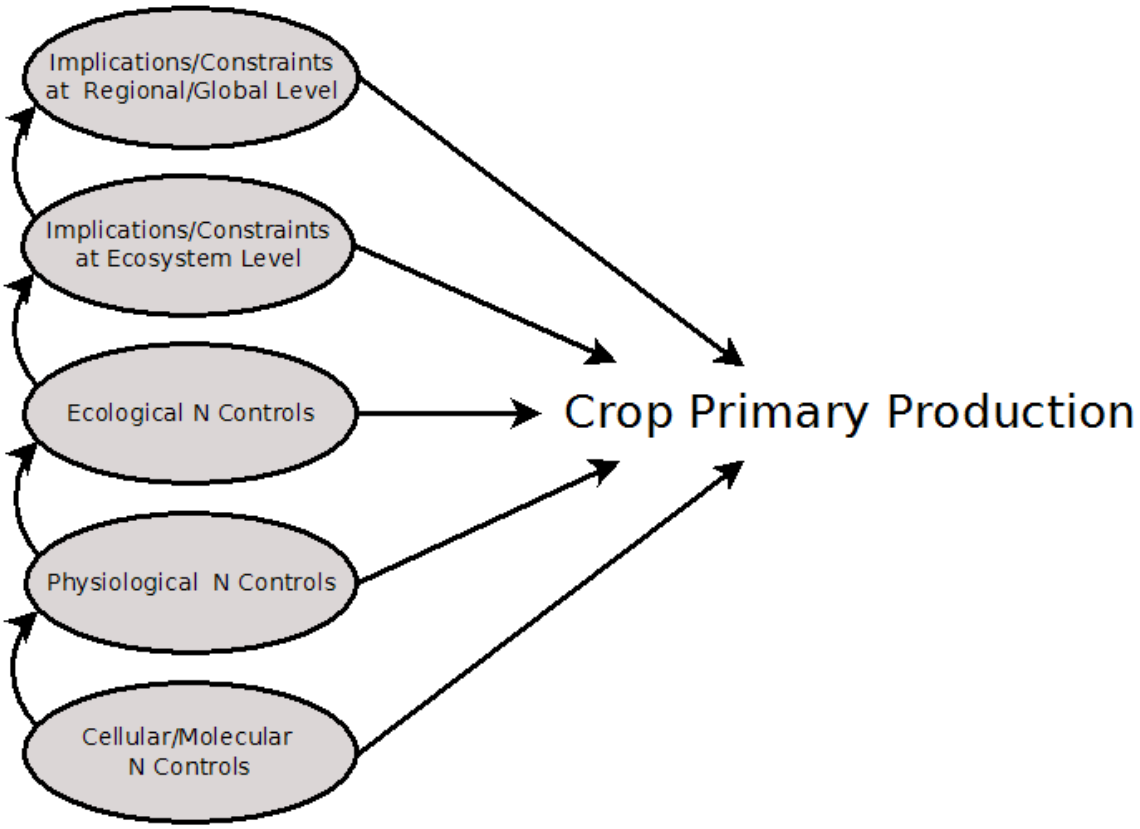


Figure 1.1: A hierarchy of explanation for N controls on crop primary production. The focal level considered in this thesis is the cellular/molecular and physiological level. Adapted from Vitousek *et al.* (2002).

Chapter 2
Coordinated regulation of C-N processes in crops: a literature review

Nitrogen constitutes the most abundant mineral element in plant tissue as it is present in organic N compounds, including amino acids (and the resulting proteins), nucleic acids, chlorophyll, phytohormones (e.g. cytokinins, auxins) and numerous secondary metabolites (e.g. alkaloids, glucosinolates). N is an extremely important nutrient for plants as it is a major component of amino acids and thus of proteins which again are the main protagonists in most plant processes. The plant therefore needs to adapt and synchronize its processes to the availability of this valuable nutrient which often is in short supply. The plant functions as a complete system that needs to balance the activities and capacities of different processes and fluxes. The mechanisms of this regulation and the restraints imposed by N on physiological processes are a key for the understanding of crop growth and production. In the following chapter I will therefore first look at the processes involved in the metabolism of N, how the plant obtains, assimilates and allocates N, how these processes are controlled and regulated to align to environmental conditions and to the plant demand. In a second part I will then look at how N controls growth, photosynthesis and other processes involved in C metabolism.

2.1 N metabolism

The focus of the description of the N metabolism will be on how different processes are regulated and controlled by plant status and environmental conditions, i.e. how the plant achieves a coordination of different activities and fluxes. The understanding of dependences and primary controls in a plants physiology are essential for the deduction of a simplified scheme of plant C-N interactions for modelling purposes. It is important to be aware of and if possible to understand the complexity of plant processes in order to be able to reduce and derive more general relations. For this reason I will also carefully look at the regulation of processes at the molecular and genetic level, as this is the level where the primary regulation of plant metabolism takes place.

In the first section I look at the uptake of N through roots (2.1.1) and shoots (2.1.2), at some general considerations on plant availability of N in soils (2.1.1.1 and 2.1.1.2), and - in more detail - at the N uptake process and at different transport systems present in plants (2.1.1.3) with finally shortly discussing the relevance of mycorrhiza for plant N acquisition (2.1.1.4). These considerations are the basis for the examination of the regulation of N uptake in section 2.1.5. Similarly in section 2.1.3 I consider some basic facts on N assimilation, which shall lay the foundations for the discussion of the regulation of N assimilation in 2.1.5. N_2 fixation is treated in section 2.1.4, with remarks on legume-rhizobia symbiosis (2.1.4.1) and on endophytic and associative N_2 fixation in Graminae (2.1.4.2). In section 2.1.5 I then discuss the regulation of the N processes described before. From the consideration of the regulation of key genes and enzymes involved in the respective processes I deduce key signals that are relevant in the control and regulation of the coherent N metabolism. Lastly I will look at some more general aspects of N allocation (2.1.6). N allocation is treated differently from other N processes as it is much more diffuse as it involves several different sub-processes and it cannot be reduced to a number of distinct enzymes.

2.1.1 N root uptake

Plants take up nutrients from the soil mainly through their rooting system. Only a small proportion of the nutrients in the soil is available to plants. The larger part (98%) is bound in minerals, compounds of low solubility, humus and other organic material and cannot be taken up by the roots (Larcher 1976). The remaining nutrients are either bound as cations or anions by adsorption to the surface of colloid soil particles (mainly clay minerals and humus, 2%) or dissolved in the watery soil solution (< 0.2%) (Larcher 1976). Only the latter are easily available to plant roots; the former can just be made available through exchange adsorption against ions released by the plant.

Nitrogen – as the most important plant nutrient - is available in the soil to plants mainly in the form of nitrate (NO_3^-), ammonium (NH_4^+) and amino acids. The N status of the soil from plant view is mainly dependent on the balance between mineralisation (i.e. the conversion of organic molecules into ammonium by bacteria and fungi), nitrification (i.e. the oxidation of ammonium into nitrate by nitrifying bacteria) and denitrification (i.e. the conversion of nitrate into N gases under anaerobic conditions by bacteria). All of these processes are dependent on environmental factors influencing microbial activity, like soil temperature, soil moisture, acidity and aeration (Miller & Cramer 2004). As these environmental factors vary spatially between sites and temporally within sites, the distribution of different N forms in the soil is very heterogeneous and quantities vary across several orders of magnitude.

The rate of N uptake by the root depends both on the supply of N to the root surface, on the active uptake rate of the root cells and on the volume of soil exploited by roots and rooting density

(Lawlor 2002). The N supply to the root surface again depends on soil characteristics like (i) the N concentrations in the soil solution, (ii) the buffering power of the soil (i.e. capacity of soil pools to replenish the N compounds taken up from the soil solution), and (iii) the N transportation rate to the root surface by diffusion or by mass flow of soil water (Chapin 1980).

2.1.1.1 Inorganic N supply

Concentrations in the soil solution

As the dissolved inorganic N (DIN) is the most important N supply for plants, plant available N mainly derives from the decomposition of organic matter. Microbial mineralization of organic N thus is considered as the bottleneck in the flux of N in terrestrial ecosystems (Chapin *et al.* 2002). DIN occurs in soils mainly in the form of nitrate and ammonium. In well-aerated agricultural soils nitrate is the most abundant form of available N, occurring in concentrations ranging approx. between 0.5 and 10 mM (Marschner 1995). This high availability of nitrate is due to an increase in ammonification and subsequent nitrification in cultivated soils (Radin & Elmore 1980). Ammonium concentrations in agricultural soils are 10 to 1000 times lower than nitrate concentrations and range from 20 to 200 µM (Owen & Jones 2001). But even under comparatively controlled, manipulated agricultural conditions, plant roots experience an enormous heterogeneity of N concentrations in the soil solution. In 77 well-aerated agricultural soils in New Zealand, Australia and the USA the concentrations of nitrate and ammonium in the soil solution was 4.5 ± 9.8 and 0.78 ± 1.5 mM respectively (mean ± standard deviation), ranging across three to four orders of magnitude (Wolt 1994).

Ammonium can dominate the plant available N supply in soils if nitrification is inhibited by low temperatures, waterlogging, high acidity or the presence of allelopathic chemicals (Britto & Kronzucker 2002). Still the concentrations of the different N forms in a soil do not necessarily determine which N form is predominantly taken up by plants inhabiting the soil. Even if nitrate dominates as N supply form, the uptake of ammonium can still greatly exceed that of nitrate. In a study with tomatoes (*Lycopersicon esculentum*) for example, plants covered 50% of their N demand through uptake of NH_4^+ although ammonium represented only 10% of available N (the remaining available N being nitrate) (Glass *et al.* 2002).

Many plants preferentially take up ammonium when both DIN forms are available. The advantage of ammonium is that its oxidation state allows the plant cell to avoid the energy-requiring reduction during the N assimilation process (see 2.1.3). Instead ammonium can be immediately used in the synthesis of amino acids. But when ammonium occurs in very high concentrations or as the only available N form, NH_4^+ can have toxic effects on many plant species (Britto & Kronzucker 2002). Nitrate on the contrary is not toxic to plants even at high concentrations. Possible processes which could be responsible for the toxic effect of ammonium include (i) a resulting charge imbalance in the plant, as plants take up much more positively charged ammonium ions than anions, (ii) an acidification of the rizosphere, resulting from the uptake of NH_4^+ and the excretion of protons to compensate for the charge imbalance in the plant tissue, (iii) the decline in essential cations like K^+, Ca^{2+} and Mg^{2+} in the plant tissue because of an excess concentration of the cation NH^+, (iv) lack of downregulation of NH_4^+ uptake systems even at a high N status of the plant and the resulting energy-demanding active efflux of cytosolic NH_4^+ (Britto & Kronzucker 2002). Although tomato plants for example prefer ammonium over nitrate under a non-optimal N supply (see above), optimal growth of tomato occurs in soils with a ratio of nitrate to ammonium of 3:1 and is inhibited if the concentration of ammonium is too high (Bloom *et al.* 1993). The toxic effect of ammonium may be relevant for all plant species at high ammonium concentrations, but the threshold at which toxicity begins varies widely among species (Britto & Kronzucker 2002).

The example of ammonium toxicity demonstrates how difficult it can be to draw any general conclusions about the response of plants to external conditions, e.g. to make predictions about how a certain N supply affects plant growth. For a certain species a high ammonium concentration might stimulate growth, as this leads to a competitive advantage of that species. But even in a monoculture one crop species might benefit from the addition of ammonium fertilizer while another crop species might reduce growth if supplied with ammonium fertilizer due to a low threshold for ammonium toxicity. It is not possible to describe a general preference of crops for nitrate or ammonium. No particular N form is more readily usable than another. Most plant species grow optimally when supplied with both N forms simultaneously and in most soils both N forms are produced and by far most species are able to use both N forms (Runge 1983).

Transport in the soil

Uptake of N occurs, compared to other mineral nutrients, relatively fast. This leads to an impoverishment of N in the medium directly surrounding the root surface. Therefore the transportation of N to the root surface often is the limiting variable for N uptake. Still this does not necessarily hold true for highly fertile or fertilized soils, in which N concentrations are high enough to enable an adequate nutrient transport to the root surface (Marschner 1995). Under non-limiting N supply the transport rate of N to the root is much faster than maximum rates of N uptake by the plant. Under such conditions N uptake is limited by the N-demand and by the uptake mechanisms of the plant and is independent of soil N concentrations (Seligman *et al.* 1975).

Nutrients can reach the root surface through the processes of mass flow, diffusion and root interception, i.e. growth of the root to the nutrient (Marschner 1995). Root interception is the dominant process in the uptake of sparingly soluble nutrients like phosphorus, while N is mainly delivered to the root surface through mass flow and diffusion (Wiren *et al.* 1997).

The rate of mass flow of water and dissolved nutrient is dependent on the transpirational water stream to the root surface. Diffusion on the other hand is dependent on the concentration gradient and the diffusion coefficient of the particular form of N (Miller & Cramer 2004). The contribution of the different transport forms to total supply is not only dependent on the form of mineral nutrient and soil characteristics but also on the plant species. While the N demand of a maize crop for example is met to approximately 80% by mass flow (diffusion contributing the remaining 20%), the relative contribution of mass flow to the N supply of onions might be even higher, as onion roots have a higher water uptake per unit length (Marschner 1995).

Because of the dependence of the plant available N supply on mass flow and on the accessibility of N in the soil solution, N resources are incompletely exploited during periods of severe water shortage. Still this restricted N availability is not the cause of limited growth during periods of water shortage, as growth is restricted by the water shortage directly (Runge 1983).

As most soil particles have a negative charge, the negatively charged nitrate has a much greater mobility in soils than the positively charged ammonium – which is to a great extent absorbed by the soil particles (Runge 1983). The diffusion coefficient of nitrate in soils is therefore ca. 10-fold to 100-fold greater than that of ammonium (Owen & Jones 2001). One the one hand nitrate can thus be leached more easily and lost from the soil than ammonium, but on the other hand, because of its higher diffusion coefficient, nitrate is more available to plant roots through diffusion. Because of the high mobility of nitrate in the soil, nitrate uptake is to a certain degree independent of root density. Still the competitive ability of plants for the utilization of soil nitrate is strongly conditioned by the root system (Runge 1983). Ammonium has a lower mobility and the ammonium resources near the roots are thus more rapidly exploited. Utilization of ammonium is therefore more strongly dependent on root growth and density than nitrate uptake (Runge 1983).

2.1.1.2 Amino acid supply

The uptake of amino acids occurs in many plant species over a wide range of ecosystems and several plant species have even been shown to take up amino acids preferentially over DIN sources (Lipson & Näsholm 2001). The concentration of free amino acids in the soil solutions of a series of different ecosystems in Southern Ireland ranged from 0.1 to 50 µM and typically constituted 10-40% of total soluble N (Jones *et al.* 2002). Amino acids mainly enter the soil solution through the activity of decomposers which secrete extracellular enzymes (proteases) and thus break down organic molecules into their amino acid fractions (Lipson & Näsholm 2001). When mineralization and nitrification rates are low because of soil acidity, low temperatures or anaerobic conditions (e.g. in alpine, arctic or boreal regions), amino acids can even become the dominant N supply form (Atkin 1996). In a study of arctic soils, water-extractable free amino acid concentrations ranged from 11 to 26.5 µM, while the ammonium concentrations were slightly lower and ranged between 8 and 22 µM (Kielland 1994, as cited by Atkin 1996). Kielland (1994) calculated that the uptake of amino acids may account for 10 to 82% of the N demand of several arctic species.

In agricultural soils concentrations of amino acids range approximately between 20 and 100 µM (Monreal & McGill 1985). Yet Owen and Jones (2001) conclude that despite the partially high concentrations, organic N is of only limited consequence for plants in agricultural systems with high N inputs as most of the amino acids in the soil are taken up by microorganisms. The diffusion coefficients of amino acids in soils are low and lie in the range of those of ammonium (Owen & Jones 2001). This low mobility of amino acids limits the rate of amino acid supply to the root surface through diffusion, making it more likely that the amino acids are consumed by microbes than taken up by plant roots (Miller & Cramer 2004). Plants seem to be able to compete for the N in amino acids above all in environments where microbial activity and decomposition is limited by physical or biological factors (Neff *et al.* 2003).

2.1.1.3 Uptake process

As discussed above, plant roots are exposed to a huge spatial and temporal heterogeneity of N concentrations. They must therefore be able to respond to changes and to optimize their N uptake according to the form and concentration of the available N supply in the soil solution. N concentrations in the soil are several orders of magnitude smaller than the concentrations in plant tissue (Finck 2007). These differences in concentration of N from the soil to the plant as well as the necessity for the plant to regulate N uptake according to N demand and N availability imply an active uptake mechanism for N into the plant tissue.

In fact, after entering the apoplasm of the root rhizodermal and cortical cells N is transported into the symplasm through several transporters located in the plasma membrane. In order to be able to control the influx of N into the stele, from where it is transported to other parts of the plant, the endodermis forms an effective barrier against passive inflow, so that here at the latest, N has to cross the transport proteins situated in the plasma membrane. Higher plants exhibit several putative high- and low-affinity transport systems for the different N forms available to the plants in the soil.

Nitrate transport systems

Net nitrate uptake is the balance between nitrate influx and nitrate efflux. While nitrate efflux is a passive process possibly mediated by nitrate inducible anion channels (Miller & Cramer 2004), thermodynamic calculations as well as empirical observations suggest that nitrate influx requires energy, even under the highest nitrate concentrations experienced in the soil (Crawford & Glass

1998). The energy required for nitrate uptake derives from a proton gradient across the plasma membrane created by the H^+-ATPase[1] (Miller & Smith 1996). The transport systems that catalyze nitrate influx are a combination of high- and low-affinity transport systems (HATS and LATS respectively), being active at low and high substrate concentrations respectively (Glass *et al.* 2002). The HATS takes up the majority of nitrate; in rape (*Brassica napus*) HATS contributed with 89% to total N uptake, while LATS was only important in the early development stage and immediately after N fertilization (Malagoli *et al.* 2008). Three kinetically distinct nitrate transporters have been characterized: two HATS operating at low external nitrate concentrations with low transport capacity and one LATS operating at high external nitrate concentrations with high transport capacity (Forde 2000; Malagoli *et al.* 2008). The inducible HATS (iHATS) is strongly induced in the presence of external nitrate while the constitutive HATS (cHATS) is constitutively expressed (i.e. expressed in the absence of nitrate), but also upregulated by exposure to nitrate (Glass *et al.* 2001). The induction times and capacities of the different transport systems vary both within and between species (Crawford & Glass 1998; Glass *et al.* 2002). The cHATS has a higher affinity to nitrate – with K_m[2] values ranging from 6 to 20 μM (Forde 2000), compared to 13-85 μM in the iHATS (Forde 2000; Malagoli *et al.* 2008). But the iHATS has a much greater capacity for nitrate uptake: in rape the V_{max}[3] of iHATS was over 5fold higher than that of cHATS (Malagoli *et al.* 2008), while in barley (*Hordeum vulgare*) iHATS increased to 30 times the cHATS activity after provision of nitrate (Siddiqi *et al.* 1990). The constitutively expressed LATS is most important at high external nitrate concentrations (generally > 1 mM). In spite of the linear (i.e. non-saturable) response of LATS to concentration, thermodynamic evaluations demonstrate that nitrate uptake through LATS is also active (Forde 2000).

Physiological studies suggest that each of the observed kinetically distinct transport systems result from a single species of transporter. Yet molecular studies indicate that both HATS and LATS are encoded by multiple gene family members (Glass *et al.* 2001). To date two families of nitrate transporter genes have been identified: the NRT1 – which is part of the PTR family of peptide transporters - and NRT2 families (Forde 2000). It seems that the NRT2 family encodes nitrate-inducible, high-affinity nitrate transporters, while the NRT1 family may be involved in low-affinity nitrate transport (Crawford & Glass 1998; Forde 2000,). Still many questions regarding the characterization of genes involved in nitrate transport, making up the iHATS, cHATS and LATS, about how they function and how they are regulated remain to be answered (Forde 2000).

Ammonium transport systems

The uptake of ammonium shows – as that of nitrate – a concentration-dependent multiphasic pattern and requires energy (Runge 1983). Again both types of transport systems – HATS and LATS – contribute to ammonium influx (Glass *et al.* 2001). Kinetic analysis of high affinity ammonium influx shows Michaelis Menten curves and suggests that a single transport protein predominates (Glass *et al*. 2001). The structure of the high-affinity ammonium transporters still is relatively unclear. Electrophysiological studies suggest that ammonium influx may be mediated by cotransport with protons but it could also occur through a channel, driven by the negative membrane potential of the plant cell (Miller & Cramer 2004). Ammonium uptake at high external concentrations via LATS instead shows – like the nitrate LATS - a linear concentration response, and – unlike the nitrate LATS – it seems to be driven by a passive process (Glass et al. 2002).

[1] An enzyme located in the plasma membrane that catalyzes the transport of H^+ against a concentration gradient through hydrolysis of ATP into ADP and inorganic phosphate.
[2] K_m is the Michaelis-Menten constant, denoting the substrate ion concentration giving half the maximal enzyme velocity (i.e. in the case of uptake the half-maximum transport rate).
[3] V_{max} is the maximum enzyme velocity (i.e. here the maximal transport rate).

Physiological studies demonstrate that LATS is expressed constitutively but its genetic component still has not been identified (Howitt & Udvardi 2000).

Two distinct groups of genes of the AMT family, AMT1 and AMT2, have been identified as being involved in root ammonium uptake but their specific role has not yet clearly been established (Miller & Cramer 2004). Several features of the AMT1 family indicate that it is involved in the HATS ammonium influx (Rawat *et al.* 1999; Howitt & Udvardi 2000). Studies with AMT1 mutants suggest that the transporters encoded by this gene family are redundant (Glass et al. 2001). Still it seems that different family members of the AMT1 multigene family fulfil slightly different physiological roles: while the *Arabidopsis thaliana* gene AtAMT1.1 may play a major role in ammonium uptake at low N availability, AtAMT1.3 may coordinate ammonium uptake with C metabolism and may be responsible for the diurnal cycle of ammonium uptake (Howitt & Udvardi 2000). Some AMT genes are constitutively expressed, but most are only induced in the presence of ammonium (Miller & Cramer 2004).

In addition to ammonium uptake through transporters also pH-dependent NH_3 diffusion across the plasma membrane occurs. Yet this N uptake plays no significant role and is – compared to NH_4^+ uptake through transporters - the by far less efficient path (Wiren *et al.* 1997).

Amino acid transport systems

Amino acid uptake shows multiphasic kinetics, including high-affinity uptake in the range of amino acid concentrations found in the soil (Lipson & Näsholm 2001). Amino acid transport occurrs through active proton symport (Lipson & Näsholm 2001). There have been several amino acid transporters identified in plants, encoded by multiple gene families (Ortiz-Lopez *et al.* 2000). Still the concrete function and role of most of these transporter proteins remains largely unknown. The *Arabidopsis thaliana* transporter encoded by the gene AAP3 - an amino acid permease belonging to the amino acid transporter family (ATF) - is expressed in roots and could be a candidate for a role in amino acid uptake (Ortiz-Lopez *et al.* 2000). Many of the identified amino acid transporters presumably possess the capacity to transport acidic, neutral, and basic amino acids (Lipson & Näsholm 2001).

2.1.1.4 Mycorrhizal N acquisition

While the main nutritional benefit plants derive from mycorrhizal association is enhanced P acquisition, ectomycorrhizas and ericoid mycorrhizas also contribute to plant N nutrition (Hodge *et al.* 2000a; Miller & Cramer 2004). The presence of ectomycorrhizal fungi on the root surface improves amino acid acquisition of plant roots (Miller & Cramer 2004). This phenomenon seems to be mainly due to the increased absorptive area and proteolytic activity through the mycorrhizal infection and not as much due to an increase in K_m values – as the affinity for amino acid uptake of infected roots is similar to that of uninfected roots (Lipson & Näsholm 2001). Ectomycorrhizas are also capable of increasing the uptake of ammonium through extensive growth of soil mycelia from ammonium depleted zones into ammonium rich zones (Miller & Cramer 2004). Still the role of arbuscular mycorrhiza – which is the most common type of mycorrhizal association – in accessing soil N resources is controversial (Hodge *et al.* 2000a). While some field- and laboratory studies show direct uptake of serine and glycine by arbuscular mycorrhiza (Cliquet *et al.* 1997; Näsholm *et al.* 1998), studies with a more complex substrate do not show any direct uptake of intact organic N and total N acquisition in these studies does not increase by inoculation with an arbuscular mycorrhiza (Hodge *et al.* 2000b). Hodge *et al.* (2000a) therefore conclude that as – contrary to ectomycorrhizal and ericoid associations – the arbuscular mycorrhizal association did not evolve in

N-limited environments but in P-limited soils, it is unlikely that fungal N capture by arbuscular mycorrhiza contributes substantially to plant N acquisition.

2.1.1.5 Conclusion

Nitrate is the most abundant N form in agricultural soils. Yet ammonium and amino acids do also occur in considerable concentrations. The rapid microbial turnover of organic N in agricultural soils and the low diffusion coefficients of amino acids suggest that plants cannot compete with microbes for organic N in agricultural soils. Ammonium – which can have toxic effects on plant growth at very high concentrations - may contribute substantially to the N uptake of crops, as many plant species preferentially take up ammonium over nitrate. Yet ammonium occurs in smaller concentrations than nitrate and is less easily available to plant roots because of its smaller mobility in the soil. Nitrate therefore is the most abundant N form taken up by crops.

The transportation of N to the root surface typically is the limiting step for N uptake. Diffusion and mass flow are the dominant processes in the transportation of N to the root, with their relative contribution varying between sites and species. Still highly fertile agricultural soils may have high enough N concentrations to provide an adequate nutrient transport to the root surface, turning the N uptake capacity of the root membranes into the limiting step for crop N uptake.

Uptake of N shows multiphasic kinetics and is mediated via several energy requiring transport systems situated in the plasma membrane of root rhizodermal and cortical cells. Different transport systems operate at different external concentrations and show different uptake capacities. Each physiologically defined nitrate and ammonium transporter is encoded by multiple members of the corresponding gene family. The exact structure and function of most of the putative N transport proteins identified to date remains largely unknown.

Ectomycorrhiza – which occurs mainly in woody species - and ericoid mycorrhiza – which occurs in Ericacea – may contribute to plant N uptake, while arbuscular mycorrhiza – which is the most common mycorrhizal association occurring also in agroecosystems – seems not to affect plant N acquisition.

2.1.2 N shoot uptake

N can also be taken up from the atmosphere and under some conditions this uptake can contribute substantially to N nutrition (Raven 1988). In the atmosphere N is provided mainly in the form of molecular N_2 (78.09% of air volume) but plants cannot directly access this N form. Some plant families and species can make use of molecular N through a symbiosis with N fixing microorganisms who are capable of reducing the chemically inert triple bond of N_2 (see 2.1.4). Other more labile N forms occur in the atmosphere in much lower and more variable concentrations but are available to all plants and can be taken up through the shoots. As plant shoots not only gain but also loose N, thus both acting as N sinks and N sources, net N accumulation through the shoot is the result of release and sorption (Raven 1988).

Gaseous ammonia (NH_3) occurs in the atmosphere in concentrations of approx. 1-10 µg m^{-3} gas (Yin *et al.* 1996), corresponding to partial pressures of about 8 µPa and 0.15-1.5 mPa respectively. Ammonia can be absorbed through the cuticle of plants but it mainly enters the plant via the stomata (Marschner 1995). The „ammonia compensation point“, i.e. the minimum partial pressure of ammonia in the air from which net uptake of ammonia into the leaves takes place, of several

species lies near the low partial pressure of ammonia found in unpolluted air (e.g. 0.25 mPa in *Phaesolus vulgaris*, Farquhar *et al.* 1980b). Thus aerial shoots probably act as net sink for NH_3 under the normal range of atmospheric NH_3 concentrations. However, the net uptake rate of gaseous ammonia under natural conditions is low (lying in the nmol m^{-2} s^{-1} range, Raven 1988) because of the small concentration difference between the atmosphere and the intercellular space driving NH_3 diffusion into the stomata. The contribution of NH_3 to the N requirements of plants under unpolluted conditions is estimated to be less than 0.1% (Raven 1988). Under experimental conditions (plant growth chambers with high NH_3 concentrations of 520 µg m^{-3}) instead N derived from ammonia uptake can provide up to 50% of total plant N at low soil N availability and 35% of total plant N at a higher soil N status (Lockyer & Whitehead 1986). As ammonia concentrations in the air above grassland fertilized with cattle and pig slurry can periodically reach 70-4750 µg m^{-3} due to losses through ammonia volatilisation (Pain *et al.* 1989), ammonia uptake of plants in agricultural land fertilized with animal waste could contribute much more substantially to the N requirements of plants than under natural conditions. Still it is unlikely that the proportion of N derived from shoot acquisition of ammonia in field crops will reach values similar to those obtained in growth chamber experiments, as high concentrations of gaseous ammonia in the field always goes along with a high soil N status (Raven 1988).

While plants are unable to use N_2O, NO_x can provide a N source for plants (Raven 1988). Under natural conditions NO_2 occurs in the atmosphere in concentrations of up to 6 µg m^{-3}, or 0.32 mPa (Raven 1988). The NO_2 compensation point of spruce trees (*Picea abies*) was determined to be 3,5 µg m^{-3} and thus lower than most ambient NO_2 concentrations (Geßler *et al.* 2002). Like with ammonia, under experimental conditions with severe N limitation in the soil and elevated atmospheric NO_2 concentrations, the uptake of gaseous NO_2 can provide an important contribution to the N nutrition of plants (Rowland *et al.* 1987: Weber & Rennenberg 1996). While species vary greatly in their capacity to assimilate NO_2 from the air, some species (e.g. *Eucalyptus viminalis, Nicotiana tabacum*) may derive more than 10% of their N from NO_2 (Lambers *et al.* 2008). Short term (< 24 h) and very low level exposures to NO_x generally lead to beneficial effects on plant growth while long term exposure (> 48 h) usually leads to reductions in growth (Wellburn 1990). This adverse effect of N oxides is due to resulting changes in the cellular pH and in the general N metabolism of plants (Wellburn 1990). Both the uptake of NH_3, NO_2 and NO change the acid-base balance of the whole plant (Raven 1988). NO_2 and NO presumably dissolve into HNO_2 and HNO_3 in the aqueous phase of the apoplast and lead to additional acidity in the plant tissue (Wellburn 1990). Adsorption of NH_3 alkanises leaf cells, as ammonia is dissolved and protonated in aqueous leaf compartments (Yin *et al.* 1996). Accumulation of NH_x in the leaves can also be toxic by acting as decoupler of the electron transport and by leading to membrane disfunctions (Fangmeier *et al.* 1994).

Anthropogenic ammonia and NO_x emmissions have increased the deposition of N on natural vegetation drastically. Anthropogenic ammonia emissions – which by far exceed natural emissions in developed regions like Europe - mainly result from livestock management and to a minor part from fertilizer application (Fangmeier *et al.* 1994). The main source of NO_x instead is fossil fuel combustion (Wellburn 1990). Additionally to the physiological effects of N deposition resulting from enhanced N uptake through leaves described above, deposition of dry and wet NH_x and NO_x also has major ecological effects, especially on nutrient poor habitats (Fenn *et al.* 2003).

2.1.2.1 Conclusion

Although shoots mostly act as sinks of NH_3 and NO_x compounds, uptake of N through the shoot plays a minor role in plant N nutrition under natural conditions. Under severe N deposition and/or N

fertilization, atmospheric NH_3 and NO_x concentrations can reach levels at which uptake of gaseous N through the leaves could be more substantial. Yet N uptake through the shoot contributes significantly to plant N nutrition only when air N supply is high and simultaneously soil N supply is low, e.g. in N-limited forests exposed to high N deposition through anthropogenic pollution.

The effect of the uptake of gaseous N on plant physiology varies and is dependent on the N supply from the soil and the amount of NH_3 and NO_x taken up. N uptake through leaves can have a fertilizing effect on plant growth but it can also be toxic and reduce growth.

A substantial shoot N uptake in crops seems to occur only under certain specific conditions, e.g. high application of animal manure or foliar application of N fertilizer. Yet under such conditions usually soil N supply is also high and thus the contribution of aerial N to the N balance of crops may be restricted. On a global scale the process of N uptake through the shoot does not seem to be of any considerable importance for the N budget of crops. And as the focus of this thesis is the crop in an autecological context, shoot N uptake will not be considered further here. If however one wanted to simulate the effects of N deposition on natural vegetation or to model bi-directional N fluxes between the biosphere and atmosphere, plant shoot uptake should be considered.

2.1.3 N assimilation

Inorganic N in the form of nitrate or ammonium has to be incorporated into organic compounds in order to be further used in the plant N metabolism. Nitrate must first be reduced to ammonium, which then has to be attached to a C skeleton to be further used in biosynthesis.

2.1.3.1 Nitrate reduction and assimilation

As nitrate enters the rhizodermal and cortical symplasm it can (i) be mobilized into the xylem for long-distance transport to the shoot, (ii) be transported into the vacuole for storage, (iii) flow back across the plasma membrane to the apoplasm, or (iv) be reduced to nitrite (Crawford & Glass 1998). According to Marschner (1995), the reduction and assimilation of nitrate – as the most important plant N source - is of similar importance for plant metabolism as the reduction and assimilation of CO_2 in photosynthesis. Nitrate first has to be reduced to ammonium, before it can be incorporated into organic compounds. This reductive step is catalysed by the enzymes nitrate reductase (NR) and nitrite reductase (NiR) (Fig. 2.1). NR is a complex enzyme localised in the cytoplasm of higher plants, made up of two identical subunits, catalysing the transfer of two electrons from NAD(P)H to a nitrate ion via several redox centres composed of three prostethic groups (Marschner 1995; Miller & Cramer 2004). NiR instead is a monomeric polypetide containing two redox centres and localized in the chloroplats in leaves and in the proplastids in roots and other nongreen tissue (Marschner 1995). The electron-donor for the six-electron reduction of nitrite to ammonia catalyzed by NiR is either reduced ferredoxin (generated in green leaves in the light by photosystem I) or - in the dark and in non-green tissue - a ferredoxin-like electron carrier, with the energy for its production provided by glycolysis (Marschner 1995; Miller & Cramer 2004).

Thus the principal reaction for nitrate reduction to nitrite catalyzed by NR is:

$$NO_3^- + 2e^- + 2H^+ \rightarrow NO_2^- + H_2O \quad (2.1)$$

And the reaction of nitrite reduction to ammonia catalyzed by NiR is:

$$NO_2^- + 6e^- + 6H^+ \rightarrow NH_3 + H_2O + OH^- \quad (2.2)$$

In most plants nitrate reduction occurrs in both root and shoots, with roots contributing between 5 and 95% of total nitrate reduction (Marschner 1995). The variation in this distribution of nitrate reduction between roots and shoots depends on factors like the level of nitrate supply, the plant species and the plant development stage, and it has important consequences for the N and C economy of plants (Marschner 1995). In most agricultural plants nitrate is transported to the leaves for assimilation (Radin & Elmor 1980).

In the roots the energy for nitrate and ammonium assimilation comes from respiration (see 2.2.2). In leaves instead the reducing equivalents for nitrate assimilation can be provided by photosynthesis and nitrate reduction can be adapted to photosynthetic activity (see also 2.1.5.2.1). In fact a close correlation exists between light intensity and nitrate reduction in leaves – with nitrate reduction showing a strong diurnal pattern in leaves but not in roots (Marschner 1995).

2.1.3.2 Ammonium assimilation

While nitrate is readily mobile in the xylem and can also be stored in the vacuoles of the different plant organs, most of the ammonium has to be assimilated to amino acids directly in the root, as ammonium – and its equilibrium partner ammonia – is toxic at quite low concentrations (Marschner 1995). Ammonium in plants derives not only from ammonium uptake and nitrate reduction but also from photorespiration. Ammonium release from photorespiration even exceeds that from primary nitrate reduction (Somerville & Ogren 1980).

Ammonium is combined with a C-skeleton to form an amino acid by the enzyme glutamine synthetase (GS). GS occurs in two isoforms, the plastidic GS2 and the cytosolic GS1. GS2 is the major isoform in leaves, but is also present in plastids in roots. GS1 is the predominant GS isoenzyme in the root cortex, but is again also present in leaves – here being located in vascular tissue and in mesophyll cells - as well as in root-nodules of legumes (Miflin & Habash 2002).

GS aminates (i.e. adds an $-NH_2$ group) the amino acid glutamate under consumption of ATP to produce glutamine (Fig. 2.1). As GS has a very high affinity for ammonium (low K_m value) it is capable of incorporating ammonium even if present at very low concentrations (Marschner 1995; Cren & Hirel 1999; Lancien *et al.* 2000; Miller & Cramer 2004,). GS2 is encoded by a single gene, while GS1 belongs to a small multigene family (Lancien *et al.* 2000; Sugiyama & Sakakibara 2002). It is suggested that GS1 and GS2 differ in their physiological functions, with GS1 being involved mainly in the primary assimilation of external ammonium ions and in the re-assimilation of ammonium released during N remobilization and GS2 being responsible for the assimilation of ammonium derived from nitrate reduction as well as with the assimilation of photorespiratory ammonium in leaves (Sugiyama & Sakakibara 2002).

In a next step the enzyme glutamine:oxoglutarate aminotransferase (or glutamate synthase, GOGAT) catalyses the transfer of an amide group ($-NH_2$) from glutamine to 2-oxoglutarate to produce two molecules of glutamate. 2-Oxoglutarate – which brings in the C skeletons for the assimilation of ammonium – is provided by the tricarboxylic acid cycle (TCA) (Marschner 1995; see also 2.2.3.2). The results of the reaction are two molecules of glutamate; one has to be reutilized in the ammonium assimilation cycle, the other can be transported to other parts of the plant and utilized for example for the biosynthesis of proteins (Fig. 2.1) (Marschner 1995).

There are two types of GOGAT which can use either NAD(P)H (from respiration) or reduced ferredoxin (from photosystem I) as the electron donor for the amination of 2-oxoglutarate (Marschner 1995; Temple et al. 1998; Miller & Cramer 2004). The activity of NADH-GOGAT is usually 2- to 52-fold lower than that of Fd-GOGAT (Lancien *et al.* 2000; Miller & Cramer 2004).

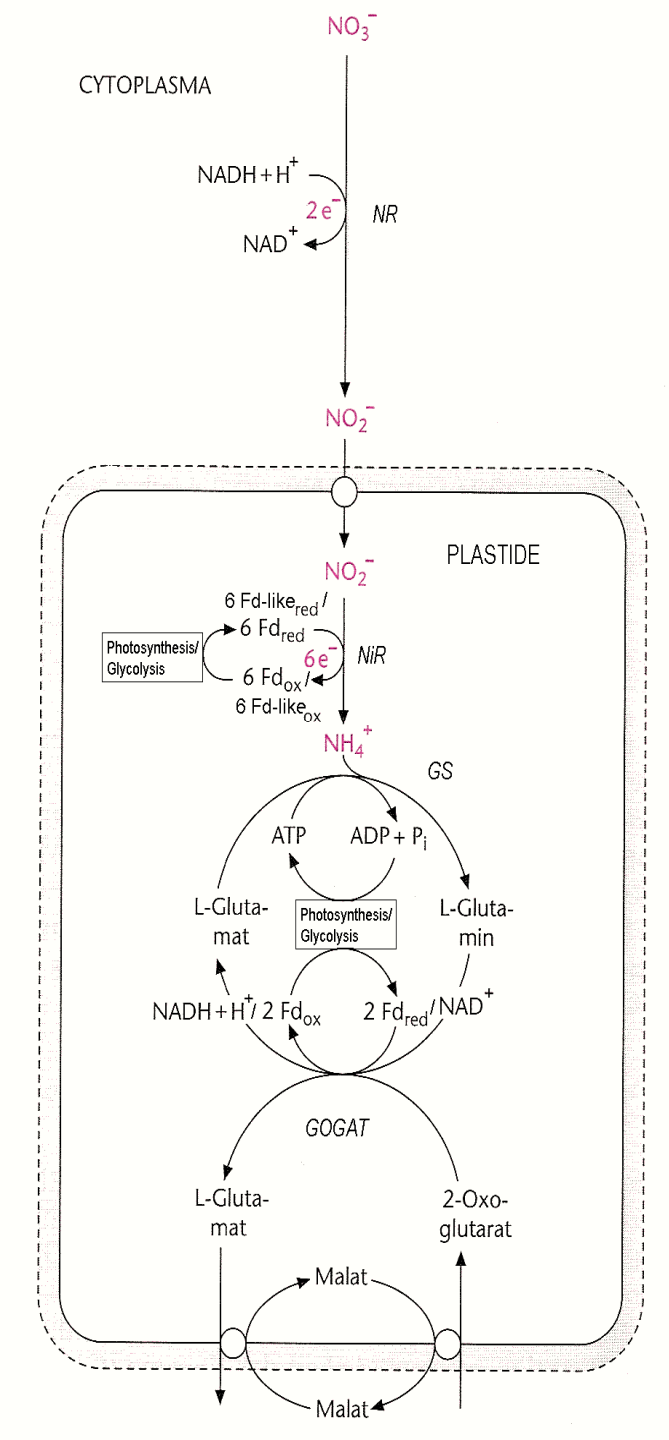


Figure 2.1: Processes involved in N assimilation. Adapted from Strasburger *et al.* (2002). In leaves the reducing equivalents derive from photosynthesis, in root and other non-green tissue instead from glycolysis (see text). The enzyme GS occurs both in the cytoplasma and in plastides. Thus the amination of glutamat can also occur in the cytoplasma.

Both GOGAT enzymes are usually located in plastides but they differ in their structure and function. Fd-GOGAT catalyzes the assimilation of ammonium derived from both the light-dependent reduction of nitrate and the ammonium generated during photorespiration, as well as – in a distinct Fd-GOGAT isoform – the assimilation of ammonium derived from soil nitrate. NADH-GOGAT instead is involved in ammonium assimilation in N-fixing legume nodules and in non-legumes it functions in primary assimilation of ammonium derived from nitrate uptake, in the reassimilation of ammonium released during amino acid catabolism, in the synthesis of glutamate from the glutamine released from senescing tissues, and/or in the reassimilation of ammonium released during seed germination (Temple *et al.* 1998).

Fd-GOGAT occurs in two isoforms, encoded by two distinct genes: the GLU1 gene which is expressed predominantly in leaves and GLU2 which is more abundant in roots (Temple *et al.* 1998; Miller & Cramer 2004).

2.1.3.3 Conclusion

Nitrate reductase (NR) and nitrite reductase (NiR) catalyze the two-step reduction of nitrate to ammonium. Ammonium is then converted to the amino acid glutamate in the GS-GOGAT-cycle by the enzymes glutamine synthetase (GS) and glutamate synthase (GOGAT). The different steps of N assimilation are located in different plant compartments, with NR occurring only in the cytoplasm, NiR and GOGAT being located in plastids and GS occurring in two distinct isoforms in both cytoplasm and plastids. All steps of N assimilation can be carried out in both leaves and roots, but only the starting substance nitrate, the intermediate glutamine and the end product glutamate can be transported in the stele and thus circulate in the plant, while the intermediate products nitrite and ammonium have to be processed immediately as they are toxic to the plant cell even at low concentrations. Nitrate in the plant derives from direct uptake or from storage and translocation, while ammonium derives from direct uptake, nitrate reduction, photorespiration, N remobilization or in legume nodules also from N_2 fixation. N assimilation requires energy in the form of ATP and electron donors, derived from photosynthesis in green leaves and from respiration in non-green tissues.

2.1.4 N_2 fixation

Biological fixation of atmospheric N_2 (biological N fixation, BNF) contributes substantially to terrestrial input of N. Overall estimates suggest that worldwide natural BNF in terrestrial ecosystems accounts for 90-140 Tg N per year (Vitousek *et al.* 1997), with BNF in agricultural systems contributing 50-70 Tg N per year (Herridge *et al.* 2008), compared to an N input through industrial fertilizers of estimated 101 Tg N per year (FAOSTAT 2007). While in the industrialised countries, under intensive agricultural production, the agricultural dependence on BNF has declined due to increased rates of N fertilization and N deposition from the atmosphere, BNF is still the dominant input into agricultural systems in developing countries (Graham & Vance 2000).

Procaryotes – namely bacteria and blue-green algae (Cyanobacteria) - are the only organisms capable of N_2 fixation. The most important BNF system in terrestrial ecosystems yet are not free-living procaryotes but symbioses between vascular plants and bacteria – namely between legumes and rhizobia and between a number of plants and the actinomycete *Frankia* (Marschner 1995; Vitousek *et al.* 2002). This holds also true when looking just at agricultural systems, where the symbiotic associations between legumes and rhizobia are the most important N_2 fixing agents (Herridge *et al.* 2008, see below).

The reduction of N_2 to NH_3 is a highly endergonic process requiring much energy. The only enzyme capable of catalyzing this reaction is the enzyme complex nitrogenase which is unique to N-fixing procaryotes (Marschner 1995). It consists of two nonheme iron proteins – with the smaller Fe^4 protein consisting of two subunits and the larger $MoFe^5$ protein consisting of four subunits. The reduction of N_2 through the nitrogenase complex requires energy in the form of ATP (from

[4] iron
[5] molybdenum-iron

respiration) and reducing equivalents (i.e. electron donors) usually in the form of ferredoxin (from photosynthesis).

The principal reaction of the N_2 reduction to ammonia catalyzed by nitrogenase is:

$$N_2 + 8H^+ + 8e^- + 16Mg \cdot ATP \rightarrow 2NH_3 + H_2 + 16Mg \cdot ADP + 16P_i \quad (2.3)$$

In addition to the reduction of N_2, nitrogenase also catalyzes the reduction of several other substrates, including H^+ and acetylene (C_2H_2). As the nitrogenase enzyme is extremely sensitive to oxygen and is irreversibly inactivated by O_2, but at the same time needs ATP from aerobic respiration, N_2 fixing systems require a delicate regulation of O_2 fluxes (Marschner 1995).

There are several N_2 fixing agents of varying importance in agricultural systems (see Herridge *et al.* 2008 for a review) but as the focus of this study is the plant system, only those agents involving a close interaction with crop plants including direct transfer of fixed N_2 products to the plant host (i.e. only symbiosis) are considered here. The role and function of free-living diazotrophic microorganisms in agricultural systems and of N_2 fixing symbioses not involving the crop plant directly (e.g. the symbiosis between the cyanobacteria *Anabaena azollae* and the water fern *Azolla*, which is important in paddy rice cultures) have to be discussed in the context of rhizosphere N processes elsewhere. The N_2 fixing symbioses between nodulated nonleguminous species (e.g. *Alnus, Myrica, Rosaceae*) and the actinomycete *Frankia* is left out as it is restricted to woody, perennial species occurring mainly in natural systems. The symbiosis between cycads and the cyanobacteria *Nostoc* is also left out as it is restricted to natural systems (Vessey *et al.* 2004; Herridge *et al.* 2008).

2.1.4.1 N_2 fixation in legume-rhizobia symbiosis

Plants of the family Fabaceae form symbioses with N_2-fixing microorganisms of the genera *Rhizobium* and *Bradyrhizobium*. It is estimated that 58-66% of global N_2 fixation in agricultural systems is carried out by legume-rhizobia symbioses (Herridge *et al.* 2008). Many leguminous crops, like soybean and groundnut, derive in experiments up to 95% of their N from fixation of atmospheric N_2, while under real field conditions on average 58% of the N incorporated in soybean and groundnut derives from N_2 fixation (Herridge *et al.* 2008).

Legume-rhizobia symbioses are not only the most important but also the most effective N_2 fixing agents. Crop legumes in agricultural systems fix estimated 115 kg N ha^{-1} $year^{-1}$, compared to a rate of maximal 25 kg N ha^{-1} $year^{-1}$ in endophytic, associative and free-living bacteria (Herridge *et al.* 2008). The effectiveness of rhizobia-legume symbioses in N_2 fixation is mainly based on three factors: (i) direct supply of photosynthates to the N_2-fixing bacteroids in the nodules, (ii) effective maintenance of very low O_2 concentrations in the interior of the nodule for protection of the nitrogenase, and (iii) rapid export of the fixed N (Marschner 1995).

The microsymbionts are located in specialized root organs, the root nodules. These are formed by the plant from unique zones of cell division in the root cortex (Vessey *et al.* 2004). The recognition of the host, the attachment of the rhizobe to the root hair, the growth, division and differentiation of root cells and the development of the root nodule and finally the invasion of the nodule by the rhizobia are mediated by an exhange of signals between the eukaryotic host and the prokaryotic symbiont (see Long 1996 for a detailed description of the processes involved; Fig. 2.2). The plant roots exude flavonoids which activate the expression of nodulation genes (so called *nod* genes) in the rhizobia, resulting in the production of the rhizobial lipochitooligosaccharide (Nod factors). This signal exchange shows a high degree of host specifity – with each leguminous plant species producing only a limited number of different flavonoids, the *nod* genes of each rhizobia being

induced only by specific flavonoids and producing a specific Nod factor that is again recognized only by a particular legume (Long 1996; Schultze & Kondorosi 1998).

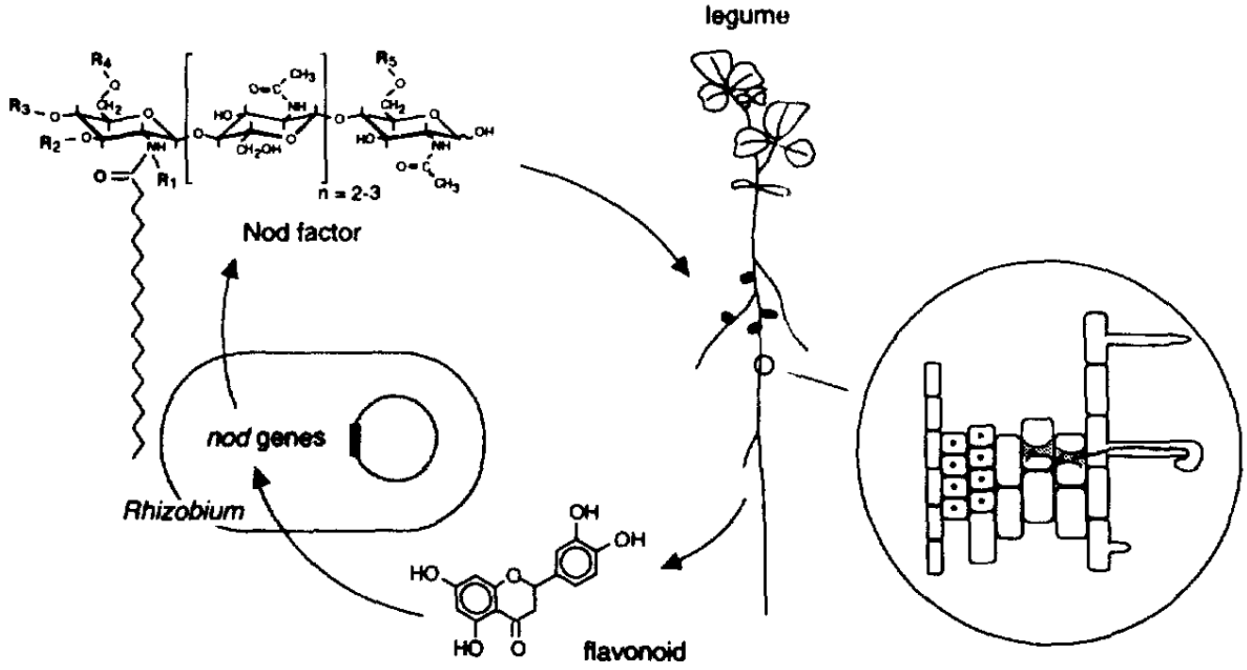


Figure 2.2: Signal exchange in the rhizobium-legume symbiosis. The legume roots exudate flavonoids that induce the rhizobial *nod* genes. This leads to the production of the nodule-inducing Nod factors. The insert shows an infection thread passing the root cortex toward a cluster of dividing cells that will become a nodule primordium. From Schultze & Kondorosi (1998).

Inside the nodule cells the rhizobia are packaged inside a plant-derived peribacteroid membrane and there they form bacteroids, which are several times larger than the original bacteria and devoid of a cell wall (Marschner 1995). During nodule development several adaptations to prevent aerobic damage to the oxygen-sensitive nitrogenase enzyme are implemented: (i) elaboration by the plant of an O_2 diffusion barrier in the nodule cortex which limits influx of O_2 to infected cells; (ii) synthesis of the O_2-binding protein leghemoglobin within nodules, which facilitates O_2 diffusion to bacteroids and perhaps plant mitochondria within the infected zone; (iii) plant redirection of glycolysis to malate with subsequent reductive formation of succinate under microaerobic conditions; (iv) bacteroid utilization of C4-dicarboxylic acids rather than mono- and disaccharides to fuel nitrogenase; and (v) bacteroid ATP formation coupled to a high-O_2-affinity terminal oxidase (Vance & Heichel 1991).

The bacteroids in the nodules start at the earliest between 10 and 21 days after infection with the N_2 fixation. During this phase the host plant has to deliver mineral nutrients, photosynthates and amino acids required for the growth of the rhizobia and the root nodules, without any direct benefit to the host. Only after this lag-phase the rhizobia begin to fix atmospheric N_2 and supply their host with reduced N in the form of ammonia (Marschner 1995). In the cytosol of the nodule cells the ammonia derived from the N_2-fixing bacteroids is then rapidly assimilated to amino acids. The enzymes responsible for this assimilation are the isoenzymes GS1 and NADH-GOGAT (Temple *et al.* 1998; Lima *et al.* 2006b; see 2.1.3).

Symbiotic N fixation genes can in the broadest sense be divided into *nod* genes – involved in

nodule development (see above) -, *nif* genes and *fix* genes (Fischer 1994). *Nif* genes mainly encode components of the nitrogenase complex, with the exception of *nifA* which encodes an important positive regulator of *nif*, *fix* and other genes. The products of *fix* genes are essential for N fixation but have varying functions, including genes involved in development and metabolism of bacteroids (Fischer 1994).

2.1.4.2 Endophytic and associative N_2 fixation in Gramineae

There is now increasing evidence that indicates that non-legumes can fix agriculturally important amounts of N_2. Yet this evidence originates mostly from pot experiments or carefully controlled conditions and is difficult to extrapolate to real conditions (Peoples & Craswell 1992). Several gramineous species, including sugarcane (*Saccharum officinarum*) and rice (*Oryza sativa*), derive considerable amounts of N from atmospheric N_2 fixation. In wetland rice N balance experiments suggest that crops may be able to obtain 30 to 60 kg N ha^{-1} $crop^{-1}$ from BNF (Boddey *et al.* 1995). Still the contribution of plant-associated BNF was estimated to be as low as 5 to 8 kg N ha^{-1} (Boddey *et al.* 1995).This shows that in many instances the actual microorganisms responsible for the N_2 fixation have not been isolated or identified. The source of this N may be free-living diazotrophs, associative N_2-fixing bacteria, living on the root surface and feeding from root exudates of the plant, and/or endophytic diazotrophs, living in the interior of the plants (Boddey *et al.* 1995).

While it is undisputed that large and diverse populations of heterotrophic diazotrophs are located in the rhizospheres, on the root surfaces and in intercellular spaces, vascular tissue, aerenchyma, and dead cells of sugarcane and rice (James 2000), the question, how the plant benefits from these associations, remains open. If the nature of the interaction between these tropical grasses and the diazotrophs is an association, then the plant benefit is mainly indirect, as approximately 90% of the fixed N becomes only available to the plant after the death of the bacteria (Marschner 1995). If instead it is a symbiotic relationship, then direct transfer of fixed N to the host plant should occurr. Yet so far no evidence of such a transfer has been found (James 2000). Evidence that raises doubts about a hypothetic symbiotic BNF in grasses includes the fact that so far endophytic diazotrophs have not been observed in living cells of the gramineous species. In addition most of the N derived from N_2 fixation remained in the root and was not translocated to the shoot, which suggests that much of the supposed plant-associated N_2 fixation is probably due to uptake of N from mineralized free-living diazotrophs (James 2000). Still, the possibility of a symbiotic interaction between Gramineae and N_2 fixing microorganisms cannot yet be totally ruled out.

Several Brazilian varieties of sugarcane, that have been bred for high yields with low fertilizer N inputs, have been shown to be capable of obtaining between 40 and 60% of their N (i.e. > 150 kg N ha^{-1} $year^{-1}$) from BNF (Boddey *et al.* 1995). But – as in rice – it is not yet established which microorganisms and what type of relationship, is responsible for the observed BNF. Several species of endophytic diazotrophs have been discovered in sugarcane, but still it is not known which of these contribute to the supply of N derived from BNF in the plant and in what site within the plant the N_2 fixation mainly occurs (Boddey *et al.* 2003). Based on strongly varying figures obtained in field studies with sugar cane in different world regions – with the figures for Brazilian sugar cane stated above marking the upper limit of these values -, Herridge *et al.* (2008) estimated that BNF in sugarcane – be it derived from endophytic, associative or free-living bacteria - globally may fix 25 kg N ha^{-1} $year^{-1}$.

2.1.4.3 Conclusion

N_2 fixation represents an important N input into agricultural systems, especially in extensive agriculture. The enzyme nitrogenase is the only enzyme capable of breaking the triple bond of N_2 and of reducing atmospheric N_2 to ammonia and it is only located in diazotrophic procaryotes. The symbiosis between legumes and rhizobia is the most important N_2 fixing system. It is based on a tightly synchronized signal exchange between the two symbionts resulting in the development of bacteroids located in root nodules of the plant host. The protection of the oxygen-sensitive nitrogenase enzyme of the bacteroid from aerobic damage is an important part of the symbiosis. There are also several other biological N_2-fixing agents, but only the N_2 fixation associated with several gramineous species, including sugarcane and rice, is of agricultural importance and involves crops directly. The exact role in agriculture as well as the nature of this N_2 fixing system still remains a controversial issue. It is not yet established what type of relationship the grasses and cereals form with the diazotrophs and how much N they really derive from atmospheric N_2 fixation. Because of the missing knowledge about the processes involved and about the contribution of associative and/or endophytic N_2 fixation to the N balance of these species under field conditions, it would not be possible to integrate this N_2 fixing system into a concept for a global model. Therefore the N_2 fixation in gramineous species will not be considered further in the context of this thesis.

2.1.5 Regulation of N metabolism

As N is one of the most important nutrients in plant metabolism, the regulation of processes like N uptake, N assimilation and N allocation is of outmost importance for plant growth and development. Through internal regulation the plant is able to synchronize different mutually dependent processes in different parts of the plant. At the same time the plant has to react and adapt to external, environmental factors and regulate its physiological processes accordingly.

2.1.5.1 N uptake

The whole pathway of N assimilation is highly regulated, but the influx of N seems to be the single most important regulatory step (Forde 2002). Although the N uptake rate is mainly governed by external N supply (Lambers *et al.* 2008) it must also be regulated through internal signals in order to integrate the N uptake with the demand for N imposed by shoot growth (Crawford & Glass 1998). Split-root experiments have shown that this regulation involves both local and long-range signalling pathways: when one half of a split-root system is deprived of N (-N) while the other half remains well-supplied with N (+N), then the N uptake capacity of the +N half is upregulated, although there are no changes in the external N supply to these roots (e.g. Öhlen & Larsson 1992; Laine *et al.* 1995; Gansel *et al.* 2001). Thus N uptake responds to external as well as to internal N concentration and to growth requirements. The regulation of amino acid uptake will not be further discussed as the transport proteins involved in it have not yet been identified (see 2.1.1.3) and as amino acids are of little importance for crops on agricultural soils (see 2.1.1.2).

Local signals

Local signals that influence N uptake directly include N concentrations in the medium surrounding the root, inducing the N uptake systems, and N concentrations in the root tissue itself.

External ammonium concentrations influence nitrate uptake, but not the other way round (Runge 1983). External ammonium has a long-term as well as in some higher plants a short-term inhibitory effect on nitrate uptake (Crawford & Glass 1998). Nitrate uptake is also inhibited by internal ammonium concentrations; the expression of NRT2 genes encoding high-affinity nitrate transporters is strongly downregulated by the presence of high levels of ammonium (Glass *et al.* 2001).

Some ammonium transporters are constitutively expressed, but for most the expression depends on induction through external ammonium (Miller & Cramer 2004). Still this induction does not result directly from the external ammonium supply but from a derepression of the uptake system by internal ammonium deficiency (Forde 2000). Ammonium influx through the HATS is directly regulated by ammonium tissue concentrations: it is downregulated at high cytoplasmatic ammonium concentrations and upregulated at low internal ammonium concentrations (Rawat *et al*. 1999; Glass *et al*. 2001). This regulation does not work at the level of AMT1 gene expression – transcript levels of the AtAMT1 gene, encoding a putative high-affinity ammonium transporter in *Arabidopsis thaliana,* are not affected by ammonium itself – but may work through direct (e.g. allosteric effects) or via post-translational events (Rawat *et al.* 1999). The ammonium influx through LATS instead is not downregulated by accumulated ammonium. It even seems that ammonium LATS influx is altogether insensible to N regulation (Howitt & Udvardi 2000). This missing downregulation and the resulting excessive ammonium accumulation or the energetic requirements for pumping ammonium out of the cells may contribute to the toxic effects of elevated external ammonium concentrations (Glass *et al.* 2001; Miller & Cramer 2004).

External nitrate induces nitrate influx through HATS rapidly (see 2.1.1.3). Both the expression of NRT2 - presumably encoding the nitrate high-affinity transporter - and NRT1 genes - presumably encoding the nitrate low-affinity transporter - are strongly induced by external nitrate (Forde 2000). Internal root nitrate concentration instead is assumed to decrease net nitrate uptake by downregulating nitrate HATS influx directly through mechanisms such as allosteric effects on transporters or protein phosphorylation and also by increasing nitrate efflux (Glass *et al.* 2001). But internal nitrate concentrations do not seem to act on the transcriptional level on nitrate transporter genes (Gansel *et al.* 2001). While in ammonium influx internal nitrate seems to act itself as a signal downregulating the expression of AtAMT1.1 (Gansel *et al.* 2001).

Another difference between nitrate and ammonium transporter genes is that gene expression of AtNRT2.1 is strongly upregulated by moderate N limitation while AtAMT1.1 expression is upregulated only under severe N deficiency. This suggests that the ammonium uptake system is much less efficient than the nitrate uptake system to compensate for restricted N availability (Gansel *et al*. 2001).

Long-range signals

The internal N status of the plant also regulates nitrate uptake in order to coordinate nitrate uptake with the N demand of the plant. This feedback is regulated by long-range signals from the shoot, so that a particular root will increase its uptake rate when the whole plant N status is sub-optimal even if itself has an optimal N status (Forde 2002).

Ammonium influx through HATS is downregulated when whole plant N status is high and upregulated when plants are deprived of sufficient N (Glass et al. 2001). This implies a regulation of the ammonium transporters in the root by long-range signals from the shoot. Yet a study on *Arabidopsis thaliana* using short-term split-root experiments demonstrated that the expression of the AtAMT1.2 gene responded to the local concentration of N in the root but not to long-range signals from the shoot (Gansel *et al.* 2001). While an earlier study revealed that transcript levels of

AtAMT1 were repressed by glutamine (Rawat *et al.* 1999). Regarding these results it is hypothesized that AtAMT1 is under dual regulation by both local and global signals of N status – but with the former being more effective, at least under short-term conditions (Gansel *et al.* 2001; Miller & Cramer 2004).

The nitrate transporter gene AtNRT2.1 – a putative high-affinity transporter gene in *Arabidopsis thaliana* - instead seems to be mainly regulated by long-distance signals from the shoot and local N concentration signals seem to be of minor importance for the regulation of AtNRT2.1 expression (Gansel *et al.* 2001). While the AtNRT1.1 gene – presumably involved in low-affinity nitrate uptake in *Arabidopsis thaliana* - does not appear to be influenced by any kind of feedback regulation by downstream N metabolites (Forde 2000; Forde 2002).

Phloem-translocated amino acids have often been proposed to play a key role in the control of N uptake at the level of the whole plant and to constitute long-range signals that communicate shoot N demand to the root (Cooper & Clarkson 1989; Muller & Touraine 1992). Amino acids are rapidly cycled between shoot and root and thus can communicate changes in the N status of the shoot promptly to the root (Forde 2002). Glutamine has been identified as the root to shoot signal of N status regulating nitrate IHATS through downregulation of NRT2 gene expression (Glass *et al.* 2001). Yet the role of other amino acids, like aspartate, glutamate and asparigine, which might also participate in long-range signaling of plant N status, remains to be unveiled (Glass *et al.* 2001; Forde 2002). Also the existence of long-distance signals other than amino acids, like plant hormones, peptides or RNA molecules, is possible (Forde 2002).

Figures 2.3 and 2.4 summarize the observations about the regulation of different nitrate and ammonium transport systems through both local and long-range N signals.

Carbohydrates

The uptake rate both of nitrate and ammonium shows a diurnal cycle and a dependency on light intensity (Forde 2002; Malagoli *et al.* 2008). This indicates that the uptake rate is also dependent on the C status of the plant and on photosynthetic activity. This relationship seems obvious as N assimilation is dependent on the supply of carbohydrates. Soluble carbohydrates are required as C-skeletons for the synthesis of amino acids and as respiratory substrates needed for the generation of energy that is required for the uptake, reduction and assimilation of inorganic N in the root (Runge 1983). Sucrose has been identified as a signal involved in this regulation, influencing the expression of NRT1 and NRT2 genes and thus helping to coordinate the processes of root nitrate uptake and leaf photosynthesis (Forde 2000; Forde 2002). The fact that AtNRT2.1 and AtNrt1.1 are both positively regulated by sucrose supply but respond differently to feedback regulation through amino acids (see above), suggests that the effect of sucrose is not mediated through its influence on the overall N/C balance (Forde 2002).

As with any process that is dependent on respiratory energy, low temperatures directly reduce N uptake (Macduff *et al.* 1987). Nitrate uptake seems to be more sensitive to temperature than ammonium uptake (Macduff *et al.* 1987), so that at low temperature the contribution of ammonium to total N uptake increases (Wiren *et al.* 1997). Yet this temperature control on N uptake seems not to work directly, but seems to be mediated indirectly through the N demand of the shoot, which markedly decreases at low temperatures due to a general decrease in growth (Wiren *et al.* 1997). Similarly, decreased N uptake under water limitation is mediated through decreased growth and the resulting decreased N demand of the shoot and not through a direct effect of water on N uptake (Lambers *et al.* 2008). And the N demand of the shoot is, as outlined above, signalled to the root by concentrations of sugars and amino acids in the phloem.

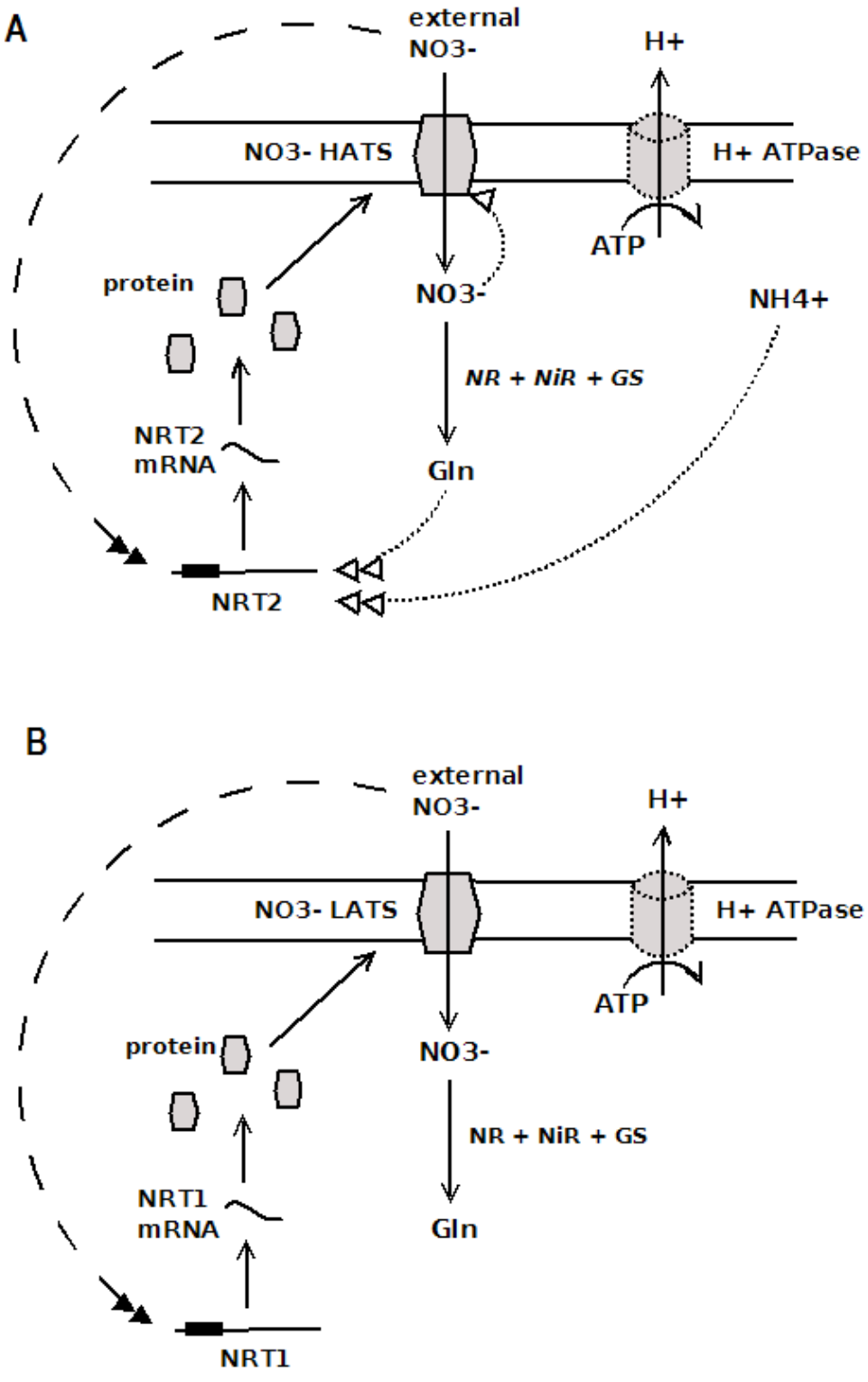


Figure 2.3: A model describing the N regulation of nitrate transporters, at the level of gene transcription (two arrows, ►►) or at the level of the protein (single arrow, ►). Dotted lines (····) with white arrow indicate a negative feedback (i.e. downregulation), broken lines (- - -) with black arrows indicate a positive feedback (i.e. upregulation). Fig. 1a shows the regulation of the high-affinity nitrate uptake system, Fig. 1b that of the low-affinity nitrate uptake system. The nitrate LATS seems to be active but not regulated by N metabolites (see text). NR: nitrate reductase, NiR: nitrite reductase, GS: glutamine synthetase, Gln: glutamine.

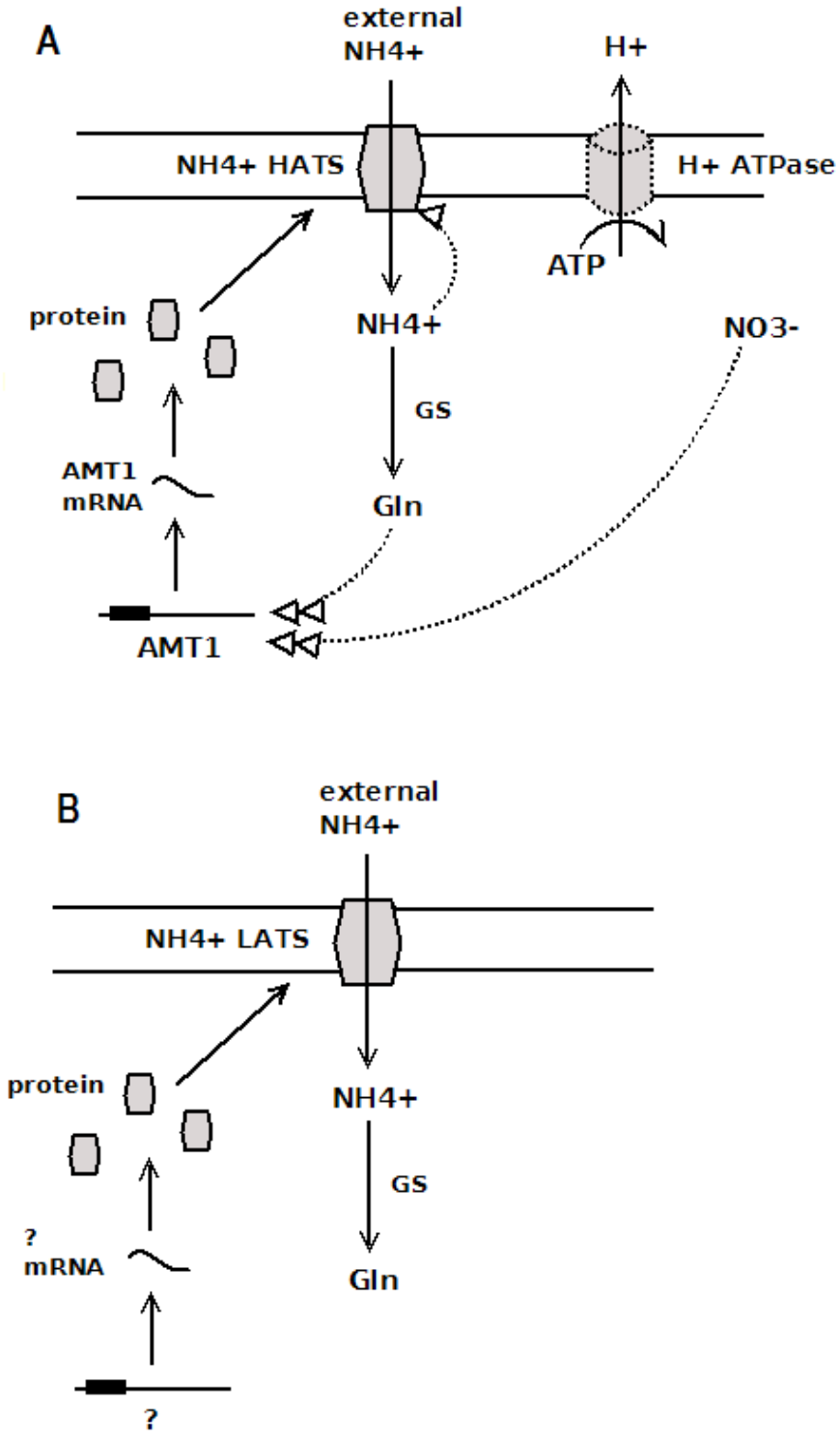


Figure 2.4: A model describing the N regulation of ammonium transporters, at the level of gene transcription (two arrows, ►►) or at the level of the protein (single arrow, ►). Dotted lines (····) with white arrow indicate a negative feedback (i.e. downregulation). Fig. 2a shows the regulation of the high-affinity ammonium uptake system, Fig. 2b that of the low-affinity ammonium uptake system. The ammonium LATS seems to be a passive system, with no N regulation (see text). GS: glutamine synthetase, Gln: glutamine.

Integrative model of the regulation of N uptake

It can be concluded that there do exist substantial differences in the regulation of different transport systems. While the nitrate HATS for example is downregulated by internal amino acid concentrations, the nitrate LATS mainly responds to external nitrate concentrations and is not controlled by plant N status (see Fig. 2.3). However there can also be observed some common patterns and several signals are relevant for several different N transport systems (e.g. amino acids for both nitrate- and ammonium-HATS). For a truly mechanistic model of N uptake based on the kinetics of different transport systems one should thus consider the differences in the regulation of different transport systems. For a coarser process-based model that considers N uptake driven by external N availability and plant status it is however possible to integrate different transport systems and to reduce a more general picture of the regulation of N uptake. Figure 2.5 shows a simplified model summarizing the regulation of N uptake by environmental conditions and the plant C and N status.

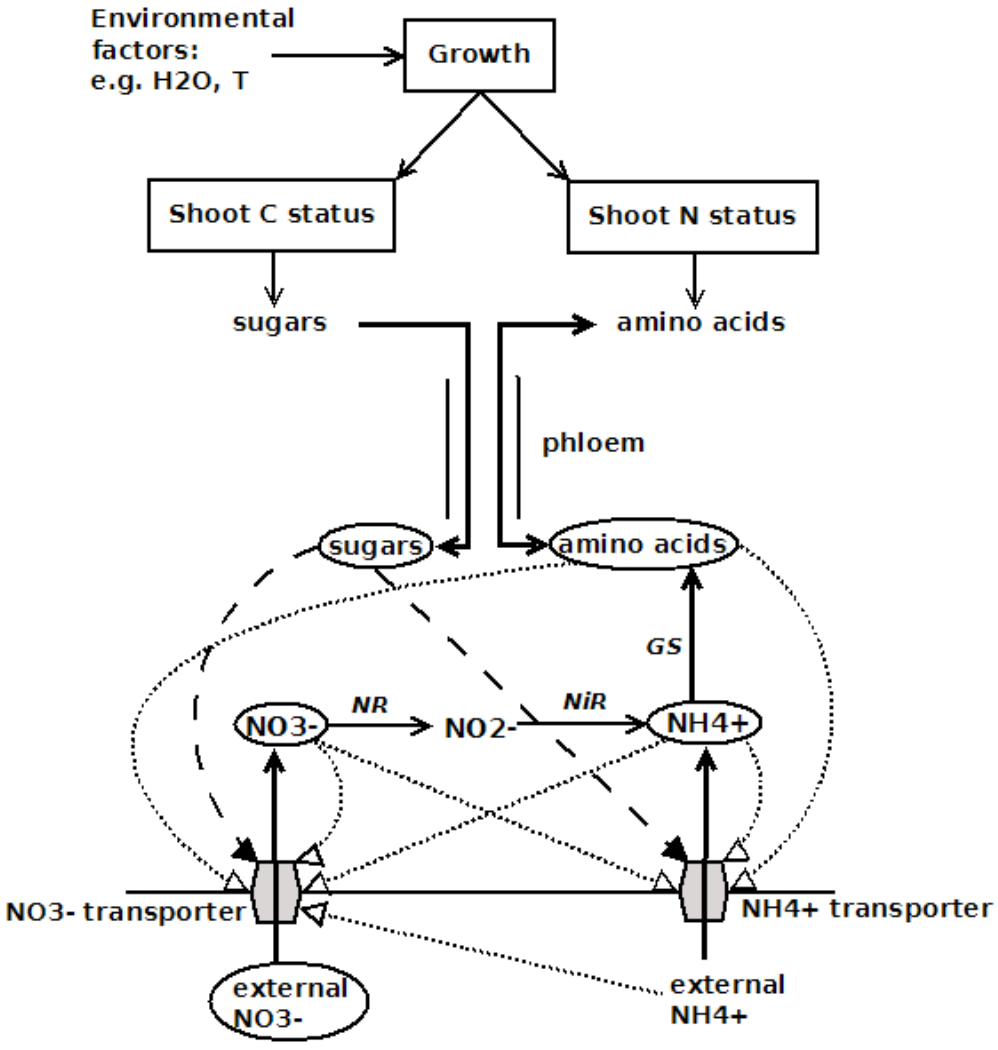


Figure 2.5: A model summarizing the local and long-range regulation of nitrate and ammonium uptake by plant status. Dotted lines (····) with white arrow indicate a negative feedback (i.e. downregulation), broken lines (- - -) with black arrows indicate a positive feedback (i.e. upregulation). Signals involved in this regulation are encircled.

2.1.5.2 N assimilation

The assimilation of N requires C skeletons and energy. Nitrate reduction and assimilation is – especially when carried out in the roots – a costly process for plants. When expressed in ATP equivalents the reduction of nitrate requires 15 mol ATP and the assimilation of ammonia an additional 5 mol ATP (Marschner 1995). In addition the assimilation of ammonia has a high demand for C skeletons and it seems that there is a competition between sucrose synthesis and amino acid synthesis (Marschner 1995). There is therefore a close connection between N assimilation and C metabolism (Runge 1983). This connection is not only mediated indirectly by the quantity of the supply of C skeletons and energy from photosynthesis and respiration, but also directly through a tight regulation of N assimilation. The several steps in N assimilation catalyzed by enzymes (see 2.1.4) are prominent targets for this regulation (Fig. 2.6).

Nitrate reductase

The NR is a key enzyme in N metabolism. It catalyzes the rate-limiting step in the process of nitrate assimilation, which in turn often limits plant growth and productivity (Kaiser *et al.* 1999; Tischner 2000). As NR determines the rate of nitrate assimilation and as different plant species show considerable differences regarding the maximum possible activity level of NR, the occurrence and activity of NR are of ecological importance (Runge 1983).

The enzyme has a half-life of only a few hours; it is induced and degraded quickly, thus has a high turnover rate (Marschner 1995; Campbell 1999). This is a prerequisite for an efficient control of the NR protein (Kaiser *et al.* 1999). The total nitrate-reducing capacity of a plant system depends on: (i) availability of the substrates in the cytoplasm (steady-state concentrations of NAD(P)H and nitrate), (ii) the level of functional NR (amount of NR polypeptide and availability of cofactors and metal ions), and (iii) the activity level of the functional NR (Campbell 1999). Thus NR is mainly regulated at two levels: a more long-term regulation of NR at the level of gene expression - determining the level of functional NR - and a rapid regulation through reversible protein phosphorylation - determining the activity level of the functional NR (Kaiser *et al.* 1999).

Under sub-optimal photosynthetic conditions and especially in the dark, in leaves NADH rather than the cytosolic nitrate concentration appears to be the principal factor limiting nitrate reduction, while under high illumination, the cytosolic nitrate concentration may drop below the level saturating for NR (Kaiser *et al.* 2002).

The expression of the NR gene is induced by nitrate. This induction is very fast and requires only low concentrations of nitrate (Miller & Cramer 2004). Yet the amount of NR and of nitrate in leaves vary independently on one another. This indicates that the synthesis of NR in leaves is primarily controlled by the flux of nitrate from the roots and not by the nitrate content (Runge 1983). Still the presence of nitrate seems not to be an absolute prerequisite for the expression of the NR gene since this possibly is also induced by other N sources (Tischner 2000). The NR gene is up-regulated by a variety of signals, including light, plant hormones such as cytokinin and C metabolites. The N metabolite glutamine instead acts as an inhibitor of NR gene expression (Campbell 1999; Tischner 2000; Sugiyama & Sakakibara 2002). Ammonium also affects the level of NR expression but this seems not due to a direct effect but may be based on the reduced availability of nitrate due to the downregulation of nitrate uptake (Tischner 2000). NR regulation at the translational level is probable but not yet well studied (Campbell 1999).

The post-translational modulation of the NR protein occurs through a reversible phosphorylation. The free and active NR enzyme is phosphorylated by a Ca^{2+} dependent protein kinase, with ATP acting as the donor of the phosphate group. The phosphorylated site of the NR enzyme is then

recognized by a 14-3-3 protein which – in the presence of a divalent cation - binds and thereby leads the NR into an inactive state. The inactive and phosphorylated NR can be activated again through dephosphorylation by a protein phosphatase (Kaiser & Huber 2001). The activation state of NR shows a diurnal cycle and this is most probably mediated by photosynthates (possibly sugar phosphates) – this regulation acting quickly in leaves, in order to match nitrate reduction with photosynthesis, and slowly in roots through translocation of C assimilates from the shoot (Kaiser *et al.* 1999). NR is also activated by several other signals which influence either the NR protein kinase, the 14-3-3 protein or the NR protein phosphatase, including tissue acidification – deriving from inhibition of respiration – and cytosolic Ca^{2+} concentrations (Kaiser *et al.* 1999). While nitrate concentrations itself seems not to change the NR activity state (Kaiser & Huber 2001). It seems that the NR activity at substrate saturation is in excess of the actual nitrate reduction rate (Kaiser *et al.* 2002) and that the level of NR is above that required for actual N assimilation (Foyer *et al.* 1994a), enabling the plant to respond immediately to increased reductant availability.

NR is especially sensitive to temperature – with increasing temperatures decreasing NR activity – but this correlation has to be interpreted with caution, as NR activity varies corresponding to the true causes of growth reduction without being itself one of these causes (Runge 1983). The activity of NR is also reduced with decreasing water status of the plant. Yet this is not due to a direct effect of water on NR activity, but due to the diminished nitrate influx under water stress and the resulting restricted synthesis of NR (Runge 1983).

Nitrite reductase

The NiR gene is also controlled at the transcriptional and post-transcriptional level. The expression of the NiR is induced by nitrate and light and repressed by glutamine and asparagine (Sugiyama & Sakakibara 2002; Miller & Cramer 2004). The degree of dependence of NiR induction on nitrate and light varies considerably between species (Miller & Cramer 2004). The response of NiR expression to carbohydrates varies between species; while in maize (*Zea mays*) sucrose enhances induction, in tobacco (*Nicotiana tabacum*) it is unresponsive to glucose (Miller & Cramer 2004). The mechanisms operating at the post-transcriptional level have not yet been identified (Miller & Cramer 2004). The activity of NiR is always higher than the activity of NR in order to prevent the accumulation of nitrite (Hoff *et al.* 1994).

Glutamine synthethase

GS – as a multi-gene product (see 2.1.3) – is under complex transcriptional and post-transcriptional control with considerable differences between species (Cren & Hirel 1999; Miller & Cramer 2004). Ammonium for example enhances GS2 gene expression in rice (*Oryza sativa*) and tobacco but not in several other plant species (Cren & Hirel 1999).

Light upregulation of GS2 expression occurs both directly - mediated via phytochrome - and indirectly through sugar concentrations (Oliveira *et al.* 2001). The expression level of GS1 instead is not significantly affected by light directly but is upregulated by the C metabolites sucrose and 2-oxoglutarate (Oliveira *et al.* 2001; Miflin & Habash 2002).

In leaves and roots of rape a post-translational regulation of GS1, involving the phosphorylation of GS1 and interaction with a 14-3-3 protein, has been discovered. The model for this regulation proposes that in the dark the ATP/AMP ratio is high and GS1 is phosphorylated and binds a 14-3-3 protein, which serves as a protection against degradation. This suggests that in the dark GS1 has its highest activity, while in the light – in an un-phosphorylated state – it is degraded more easily (Finnemann & Schjoerring 2000). It is hypothesized that this regulation plays a role during

senescence and N remobilization (Finnemann & Schjoerring 2000). In *Medicago truncatula* on the contrary, GS1 was phosphorylated but did not interact with a 14-3-3 protein. The affinity of GS1 to its substrate glutamate was increased by the phosphorylation, but V_{max} of the enzyme was decreased. Phosphorylation of GS1 in *M. truncatula* – unlike in rape - was increased by light and by active N fixation in root nodules (Lima *et al.* 2006b).

GS2 is also a phospho-protein interacting with 14-3-3 proteins but its phosphorylation seems not to be regulated by light (Finnemann & Schjoerring 2000; Lima *et al.* 2006b). In contrast to GS1, GS2 is inactivated by the interaction with the 14-3-3 protein, as this leads to selective proteolysis of GS2 (Lima et al. 2006a, Man & Kaiser 2001). It seems that although there is general agreement that GS enzymes are phosphorylated and that they do interact with 14-3-3 proteins, the exact regulation and effects of this interaction in different plant species are not yet understood (Huber *et al.* 2002).

Amino acids – especially glutamine and asparagine – inhibit GS gene expression (Cren & Hirel 1999; Oliveira *et al.* 2001; Miflin & Habash 2002,), while nitrate enhances transcription of GS1 and GS2 genes through direct signalling by nitrate itself (Krapp *et al.* 2002).

Glutamate synthase

The transcription of the Fd-GOGAT isoform GLU1 is induced directly by light, mediated via phytochrome, in the presence of nitrate or ammonium, while GLU2 expression also shows a light dependence, probably mediated by concentrations of C metabolites (Temple *et al.* 1998). Nitrate has been shown to enhance transcripts encoding Fd-GOGAT directly (Temple *et al.* 1998; Krapp *et al.* 2002).

NADH-GOGAT on the contrary shows no light dependence (Suzuki *et al.* 2001) but its transcription is induced by ammonium supply, with the signal triggering this response possibly being ammonium itself or a downstream product of its metabolism like glutamine (Temple *et al.* 1998; Miller & Cramer 2004).

Integrative model of the regulation of N assimilation

Similar to the N transport proteins also N assimilatory enzymes are thus regulated by a combination of N and C signals. The picture that emerges about the regulation of NR, NiR, GS and GOGAT is however much more complex. While all N assimilatory enzymes are regulated by nitrate and those in leaves are all regulated by light, sugars do only control GS and NR and root-GOGAT, while glutamine upregulates NR, NiR and GS, but downregulates GOGAT. Figure 2.6 depicts the combined regulation of N assimilatory enzymes in plant leaves and roots by different signals.

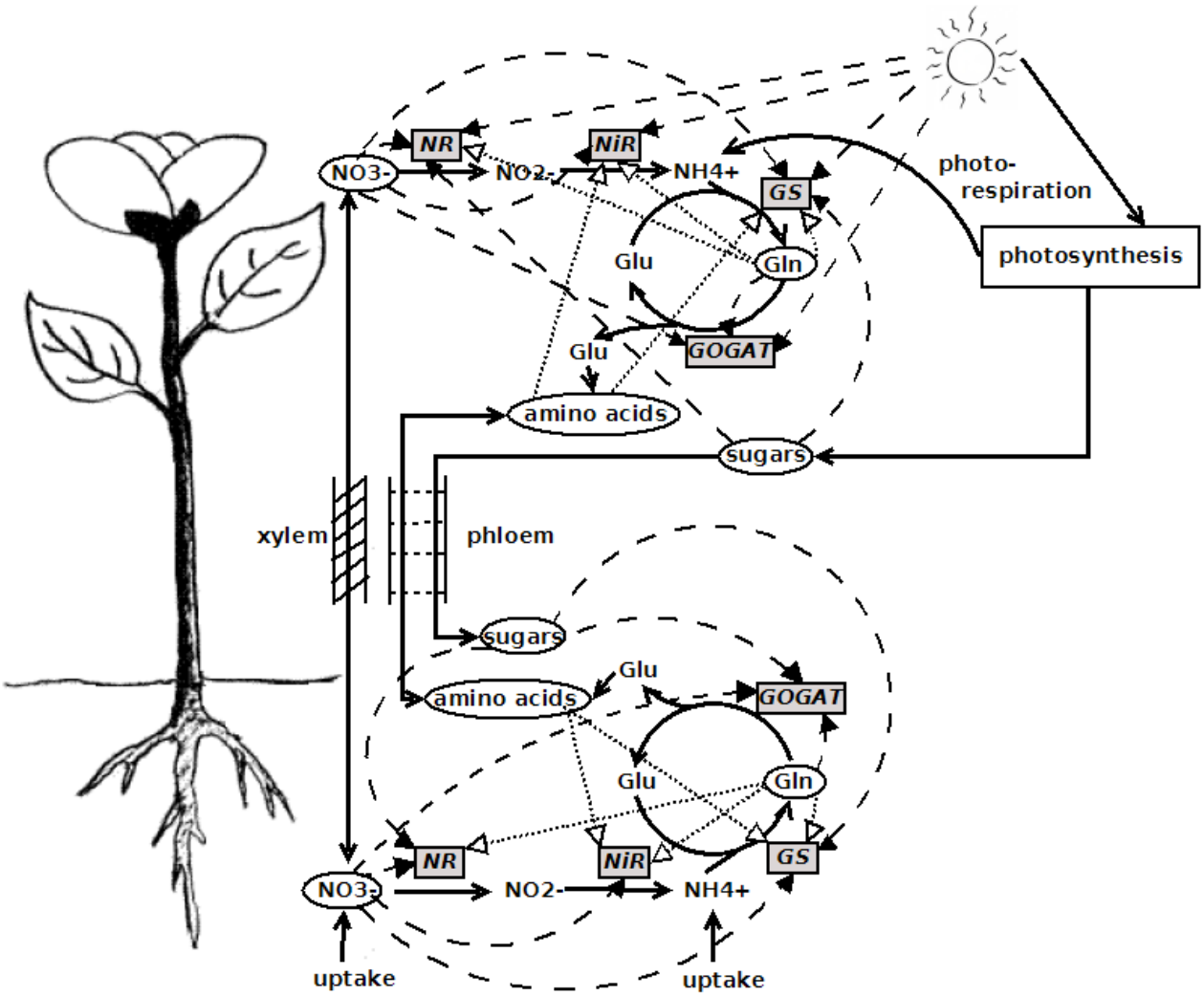


Figure 2.6: A model summarizing the regulation of enzymes involved in N assimilation by plant status. Solid lines denote the movement of molecules or electrons. Dotted lines (····) with white arrow indicate a negative feedback (i.e. downregulation), broken lines (- - -) with black arrows indicate a positive feedback (i.e. upregulation). Signals involved in this regulation are encircled. Some enzymes (e.g. GS and GOGAT) are present in the plant in different isoforms encoded by different genes, these responding to different signals. In the model such differences are not depicted, as generally the direction and nature of the regulation of the isoforms is consistent. The decrease in GS1 activity through light is left aside in the model, as this mechanism is probably restricted to senescing leaves (see text).

2.1.5.3 N_2 fixation

Regulation of nodule development

The release of flavonoids to attract rhizobes to the root hairs is the first step in the interaction between legumes and rhizobia and it is also the first step where regulation and control of this interaction can occur. The release of flavonoids is strongly dependent on calcium concentrations and inhibited by low pH (Marschner 1995).

Nod gene expression in rhizobia, hence Nod signal abundance and quality, is tightly regulated both by positive regulation, being induced e.g. by the legume flavonoids, and by negative control (Schultze & Kondorosi 1998). Downregulation of nod genes is important possibly because excess amounts of Nod factors elicit plant defence reactions. The signal that actually triggers this downregulation has not yet been determined but a possible role of dicarboxylic acids is suggested (Schultze & Kondorosi 1998).

Nod factors act as external growth factors triggering an endogenous nodulation program in the host plant (see 2.1.3.1). However they are not sufficient to allow the formation of nodules. Plants are able to control nodule induction, and to prevent nodule development even when Nod factors from rhizobia are present. This ability to control nodulation is important in order to adapt nodule formation to physiological conditions of the plant.

Nodule formation is suppressed by high concentrations of reduced N – with nitrate acting as a signal directly – while adequate concentrations of photosynthates are a requirement for efficient nodulation (Schultze & Kondorosi 1998). Phytohormones – probably opposing gradients of auxins and cytokinins – are involved in the determination of the location of nodule development in the rooting system. While cytokinin enhances genes involved in nodule formation – probably being part of or influencing the same signal transduction as the bacterial Nod factors -, auxin acts as an inhibitor. It is suggested that the cell divisions induced by Nod factors are mediated by a perturbation of the auxin flow (Schultze & Kondorosi 1998; Stougaard 2000). The plant hormone ethylene suppresses nodule formation, probably playing a role in the local autoregulatory control by stopping rhizobial infections. Only those cells preconditioned by the gradients of the different endogenous factors discussed above will divide in response to Nod factors (Schultze & Kondorosi 1998).

Regulation of N_2 fixation

In the functional root nodule N_2 fixation through the bacteroid is also susceptible to regulation. As the enzyme nitrogenase has a relatively slow turnover time and therefore is required to be synthesizes in large quantities, the transcription of nitrogenase genes is an important regulatory step in N_2 fixation (Dixon & Kahn 2004).

The main factors controlling the expression of *nif* genes in diazotrophs are environmental N and oxygen concentrations. Under aerobic conditions the synthesis of the oxygen-sensitive nitrogenase complex is inhibited in all diazotrophs (Fischer 1994). This regulation is mediated by the regulatory *nifA* gene, which controls expression of other *nif* and *fix* genes and which is only expressed in a low-O_2 environment (Fischer 1994).

The N regulation of nitrogenase expression shows differences between different N_2 fixing agents: In contrast to free-living diazotrophs, rhizobial nitrogenase genes seem not to be under significant control by N status, as the bacteroides are committed to provide fixed N for the benefit of the plant (Fischer 1994; Dixon & Kahn 2004).

The N_2 fixation rate is closely correlated with the supply of newly fixed carbohydrates to the root nodules. It shows diurnal fluctuations and a dependence on light availability (Marschner 1995). Experiments have further shown that CO_2 fertilization of legumes increase not only nodule mass and N accumulation but also specific nitrogenase activity (Vance & Heichel 1991). These observations raise the possibility that photosynthesis may limit N_2 fixation by (i) supply and/or availability of C substrates and reductants for the nitrogenase enzyme, and/or (ii) quantity of nitrogenase in the nodules (Vance & Heichel 1991).

Yet legume nodules seldom have excess nitrogenase capacity that could be activated by an increased photosynthetic capacity of the plant. In addition short-term CO_2 fertilization rarely enhances nitrogenase specific activity. And all legume species store starch in their actively N_2-fixing root nodules, which is generally interpreted as excess carbohydrate. These and other observations lead Vance & Heichel (1991) to the conclusion that photosynthetic production is sufficient to provide energy and reductants for nitrogenase and sufficient for the synthesis of nitrogenase. The increased nodule mass and N_2 fixation rate under long-term CO_2 fertilization instead are attributed to the long term coordinated increase in the mass of all plant organs under enhanced supply of photosynthetic products (Vance & Heichel 1991). Rather it is suggested that N_2 fixation in nodules is limited primarily by utilization of C within the nodule, which is again limited by O_2 availability. As oxygen is damaging for nitrogenase, the O_2 concentration in infected nodule cells has to be held at a level that supports a sub-optimal metabolic rate in the nodule (Vance & Heichel 1991; Hunt & Layzell 1993). The primary limitation causing the observed decrease in N_2 fixation rate through reduced photosynthate transport to the nodules is not due to a limited photosynthate supply for N_2 fixation but due to a decreased nodule permeability for O_2, causing a decrease in O_2 concentrations in the nodule cells, and a corresponding decrease in nodule respiration and nitrogenase activity (Hunt & Layzell 1993). The diurnal fluctuations in N_2 fixation rate again may also be primarily due to O_2 limitation as they may reflect fluctuations in soil temperature and corresponding differences in O_2 diffusion rates into the nodules (Marschner 1995).

Similar mechanisms are thought to mediate drought induced inhibition of nitrogenase activity. As with reduced nitrogenase activity following low carbohydrate supply, also the drought-reduced nitrogenase activity could be recovered by elevating O_2 partial pressure. A hypothesis for the mechanism of O_2 limitation of N_2 fixation during drought is that concentrations of leghemoglobin, which decline in water-stressed nodules, restrict facilitated diffusion of O_2 to the bacteroids (Hunt & Layzell 1993; Fig. 2.7). Restricted photosynthate supply to the nodules resulting from a decline in photosynthetic rate instead is not considered as the primary cause of nitrogenase inhibition under drought conditions (Hunt & Layzell 1993).

Nitrate has an inhibitory effect on nitrogenase activity but without affecting the expression of nitrogenase genes in bacteroides (see above). The exact mechanism underlying the nitrate inhibition of nitrogenase activity remains obscure but as with photosynthates, the effect seems to be due to resulting O_2 limitation and not due to a direct effect of nitrate (Fig. 2.7). It is hypothesized that nitrate may cause an increase in the diffusion barrier resistance for O_2 by acting as an osmotically active ion, or by causing an osmotic adjustment of the diffusion barrier through diversion of carbohydrates in the phloem sap from the nodule to the root tissue and resulting changes in nodule sucrose concentration. The resulting decreased nodule permeability then leads to a decrease in O_2 concentrations in the nodule cells, which again limits respiration and nitrogenase activity (Hunt & Layzell 1993).

Feedback regulation of N_2 fixation rate through recently fixed N may be another important regulation mechanism (Marschner 1995). Parsons *et al.* (1993) propose a model in which concentrations of reduced N compounds in the phloem sap are sensed by the nodules and growth and activity of the nodules adjusted accordingly. They suggest that the mature, lower leaves have

little requirements for additional nutrient and thus do not longer utilize N delivered in the xylem and so export it in the phloem. As almost all import into nodules originates from phloem sap transported from these lower leaves, under adequate N supply, nodules may perceive high concentrations of N compounds in the phloem. A key N compound in the phloem – probably an amino acid - could then regulate nodule growth and nitrogenase activity through a mechanism that affects the diffusion of O_2 into the nodules (see above, Parsons *et al.* 1993).

Integrative model of the regulation of N_2 fixation in nodules

The O_2 concentration in nodules is the key factor in the regulation of N_2 fixation. Several mechanisms of this regulation are still poorly understood, but the drivers of the regulation are largely identified. Figure 2.7 shows a model of the regulation of N_2 fixation and nitrogenase activity through the influence of different signals on O_2 diffusion into the nodules.

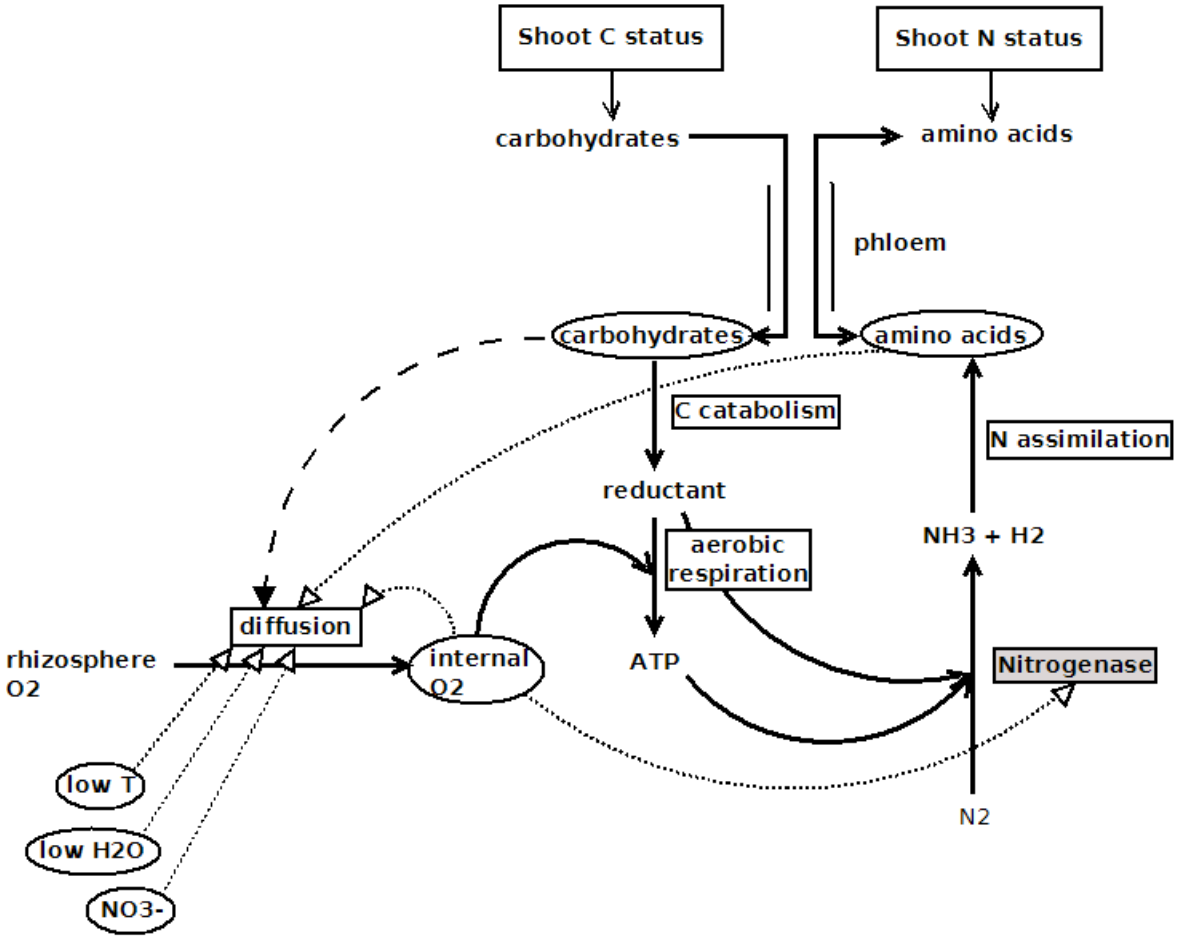


Figure 2.7: A model summarizing the regulation of N_2 fixation in legume-rhizobia symbioses. Solid lines denote the movement of molecules or electrons. Dotted lines (····) with white arrow indicate a negative feedback (i.e. downregulation), broken lines (- - -) with black arrows indicate a positive feedback (i.e. upregulation). Signals involved in this regulation are encircled.

2.1.5.4 Conclusion

N uptake

The uptake process is affected both by environmental conditions – influencing the N supply in the soil available to the plant - as by the physiological status of the plant. The transcription of genes encoding ammonium and nitrate transporters is downregulated strongly by amino acids, with these acting as a long-range signal mediating the whole-plant N status to the root. At the same time transporter activity is also regulated directly by nitrate and ammonium concentrations in the root. In addition the transporters show a diurnal regulation, probably mediated by carbohydrate availability. The effect of several environmental factors, like temperature and water status, on N uptake does not work directly, but is mediated by the resulting variations in growth and concomitant changes in shoot C and N status.

N assimilation

The enzymes of the N assimilatory pathway are upregulated by photosynthates (in leaves also by light directly), in order to match N assimilation to the C metabolism, as well as by the substrate of N assimilation nitrate (and in the case of GOGAT also by its substrate glutamine). Downregulation instead occurs by feedback regulation through the products of N assimilation - namely amino acids – with glutamine being the most important feedback signal.

N_2 fixation

Nodule development depends on a close signal exchange between rhizobes and legumes. In addition it is controlled by the physiological status of the plant. While nitrate inhibits nodule development, photosynthates are required for the process. Only those cells determined by a gradient of different opposing phytohormones and specific physiological conditions (low N, high C), have the competence for cell division and can develop nodules.

Nitrogenase activity is both oxygen sensitive and oxygen demanding. This ridge walk between the damaging effects of O_2 on nitrogenase and the O_2 requirements of aerobic respiration to provide energy for N_2 fixation is the major starting point for regulation of N_2 fixation in root nodules. Low soil temperatures, drought, low photosynthate, and high amino acid transport to the nodules as well as high nitrate concentrations are all proposed to inhibit the O_2 diffusion into the nodule cells through varying mechanisms, thereby decreasing the activity of the nitrogenase enzyme.

Integrated view of the regulation of N metabolism

Summing up, it is possible to reduce from the review of the regulation of different N processes a relatively simple picture of the regulation of the N metabolism by the C and N status of the plant (Fig. 2.8). The products of N assimilation have a negative feedback on N uptake, N_2 fixation and N assimilation itself. Similarly the products of N uptake (internal ammonium and nitrate) inhibit further N uptake. N_2 fixation in addition is inhibited by the alternative N supply nitrate. The substrates of N uptake (external ammonium and nitrate) and of N assimilation (internal nitrate) instead induce these processes. The coordination of the N processes with the C metabolism of the plant occurs through regulation by the products of C assimilation. Photosynthetic activity stimulates N uptake, N assimilation and N_2 fixation through concentrations of photosynthates in the phloem. Environmental factors like temperature and water status control N uptake and N assimilation through their effects on growth, while N_2 fixation is affected more directly.

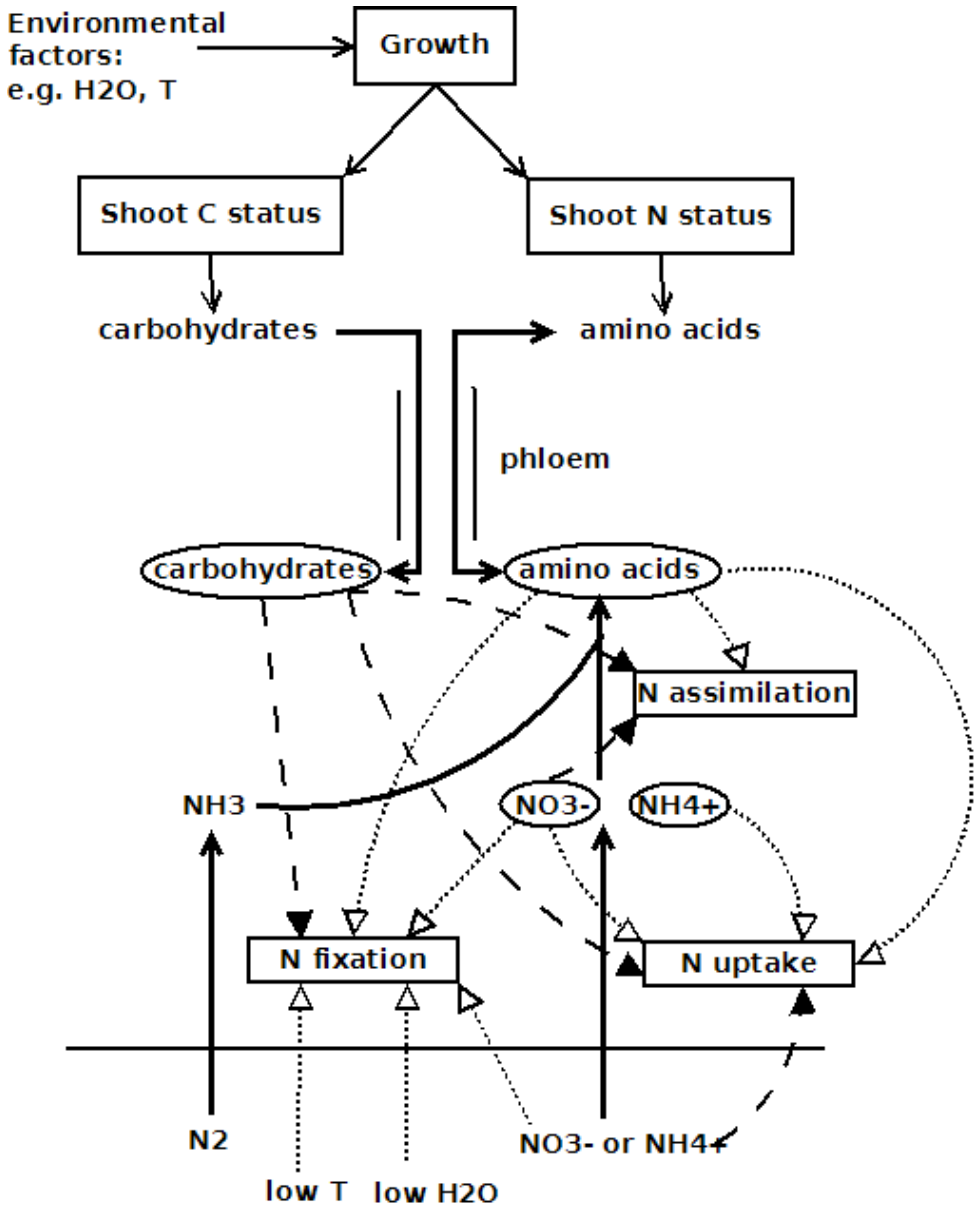


Figure 2.8: A model summarizing the integrated regulation of the N metabolism. Dotted lines (····) with white arrow indicate a negative feedback (i.e. downregulation), broken lines (- - -) with black arrows indicate a positive feedback (i.e. upregulation). N assimilation in the shoot is not depicted for simplification.

2.1.6 N allocation

Unlike the processes discussed so far, N allocation is not a process that can be defined clearly and it cannot be constrained to a couple of proteins encoded by a couple of genes. N allocation is not an independent process but the sum of many different anabolic and catabolic processes involving nitrogenous compounds. N allocation is not static but involves the continuous turnover of N in the plant from one structure and function to another, with a mobile phase in between in which N can be translocated to different parts of the plant.

N is present in the plant in organic and inorganic compounds. The only inorganic compound that is present in considerable concentrations is nitrate, as nitrate is – in contrast to ammonium, ammonia or nitrite – not toxic at high concentrations. Organic N compounds in plants include proteins and amino acids, nucleic acids, chlorophyll, phytohormones (e.g. cytokinin, auxin) and many secondary metabolites, especially alkaloids (e.g. nicotine). The variety of compounds containing N indicates that N is used and needed in numerous processes. N compounds take on numerous roles in the plant metabolism, including not only nutritional but also osmotic, signalling and storage functions.

N allocation is coordinated with C allocation, as growth and biomass increase depend on N-rich compounds. Thus the general patterns of allocation of N to different plant organs will be addressed later in the context of C allocation (see 2.2.3), while here the focus will be on those instances where N allocation is uncoupled from C allocation. The allocation of N to different photosynthetic proteins will be discussed in the context of N controls on photosynthesis (see 2.2.1).

The partial asymmetry between C and N allocation is demonstrated by the large variations in the N content of tissues, i.e. the N/C ratio. Under uniform growth conditions this differs among (i) species (up to 200%), (ii) genotypes of a species (up to 50%), (iii) tissues of a single plant (up to 300%), and (iv) development stage of a given tissue (up to 50%) (Bloom *et al.* 1985). At this point the differences between species will not be considered further; instead I will look at the differences in N content between tissues, the changes in N allocation during development and at the storage of N compounds.

2.1.6.1 N content of plant tissues

Depending on the plant species, development stage and organ, the N content required for optimal growth varies between 2 and 5% of the plant dry weight (Marschner 1995). N contents of different plant tissues can range from 0.03 to 7.0% of dry weight (Mattson 1980). Although the N concentration in plant tissues of a given species does thus vary with time (see 2.1.6.2) and also with environmental conditions (e.g. with N supply, see part meta-analysis), this variation always remains within a relatively narrow range.

The N content of plant organs is strongly dependent on the amount of metabolic tissue, as enzymes, especially those involved in photosynthesis, constitute a large proportion of total N in plants (see 2.2.1). Highest concentrations (3-7%) of N are thus found in young, actively growing tissues, which require high levels of N to support rapid protein synthesis, or in storage tissues (e.g. seeds). Lowest concentrations (0.5-1.5%) instead are found in senescing tissues, where new protein synthesis is minimal, large parts of the proteins are hydrolysed and N is translocated to other parts of the plant (Mattson 1980). From these considerations follows that plant organs with high turnover rates and thus rapid growth and decay cycles (i.e. flowers, fruits, seeds, leaves, fine roots and cambial tissue) have higher N concentrations than more stable and quiescent tissues (e.g. stems, roots) (Mattson 1980).

N content thus varies with the metabolic activity of tissues. This is also evidenced in the

partitioning of N within a canopy. A large body of data indicates that the N distribution between leaves of a canopy is not uniform, in dense stands more strongly so than in open stands (Werger & Hirose 1991; Grindlay 1997). Individual leaves in a canopy experience different light environments due to shading by upper leaves. It has been shown in numerous experiments that the N content of leaves declines, in parallel to the light intensity, with increasing depth in the canopy (e.g. Hirose & Werger 1987a; Lemaire *et al.* 1991). In birch seedlings (*Betula verrucosa*) for example N was preferentially distributed to the uppermost, youngest leaves at the cost of the N content in the lower, older leaves, with 63-86% of total leaf N being located in the 7 youngest (from 13-16) leaves (Ingestad 1979). Even monocotyledous stands – which normally do not have several layers of vertical leaves but erect leaves - have been observed to distribute their N according to light availability, so that N contents varied from bottom to the top within a single leaf (Pons *et al.* 1993). Leaves in a canopy also differ in age, with the youngest leaves situated at the top of the canopy. And the N content of leaves declines with increasing age (Field & Mooney 1983).

From this experimental evidence combined with the observations suggesting a strong dependence of photosynthesis on leaf N content (see 2.2.1) and from other theoretical considerations (e.g. Field 1983; Anten *et al.* 1995) the N optimization theory was established. This theory states that the N content of leaves should be adjusted according to the light intensity experienced during growth, in order to make full use of intercepted radiation (Hirose & Werger 1987b). If leaf N content is smaller than this optimal value, then the incident light cannot be used completely. If instead the leaf N content is higher than this optimal value, the costs of the excess N (in terms of maintenance respiration, N assimilatory costs and herbivory risk) are high, without bringing any benefit for photosynthesis and thus for growth (Hirose & Werger 1987b). The leaf N content should thus not only be optimized during growth and development of leaves, but also after leaf maturity. As lower leaves in a canopy become increasingly shaded during canopy growth, their N should be translocated to the leaves higher up in the canopy (Werger & Hirose 1991). The optimization theory has been successfully utilized to describe N distribution patterns observed in real canopies and many plants appear to distribute leaf N close to the calculated theoretical optimum distribution (e.g. Hirose & Werger 1987b; Schieving *et al.* 1992). It thus appears that plants allocate their N in order to optimize total whole canopy photosynthesis. Yet the optimization theory encounters several difficulties (discussed in Reynolds & Chen 1996), including the lacking insight into the mechanisms involved in the proposed translocation of N within the canopy, as well as time constraints: it is difficult to imagine how plants should foresee environmental patterns in order to adapt their N allocation, as the time scale of adjustment of leaf N content is apparently days to weeks (Reich *et al.* 1991). Thus other theories have been established, proposing possible mechanisms by which the near-optimal N distribution in canopies could be maintained (e.g. Chen *et al.* 1993).

Although the different concepts generally predict the N distribution within the canopy well, they do not account for the physiological mechanisms behind. Lambers *et al.* (2008) therefore propose a model for the physiological regulation by which the N gradient in the canopy could be achieved (Fig. 2.9): Leaves higher up in the canopy have higher transpiration rates than the shaded leaves lower down in the canopy. This probably is due to three factors: (i) stomata respond to the level of irradiance, (ii) higher in the canopy there is a greater water vapor difference between the leaf and the air, and (iii) the temperature of the top leaves is higher which increases the partial pressure of water vapor inside the leaf (Lambers *et al.* 2008). This higher transpiration rate causes a greater influx of solutes via the xylem, including amino acids and root-produced phytohormones like cytokinins. In the top leaves the greater inflow of cytokinins enhances the incorporation of N into the photosynthetic apparatus (see 2.2.1) as well as the leaf expansion rate (see 2.2.3.2). In the absence of a large inflow of cytokinins because of a reduced transpiration rate instead, much of the N compounds imported via the xylem are exported again via the phloem.

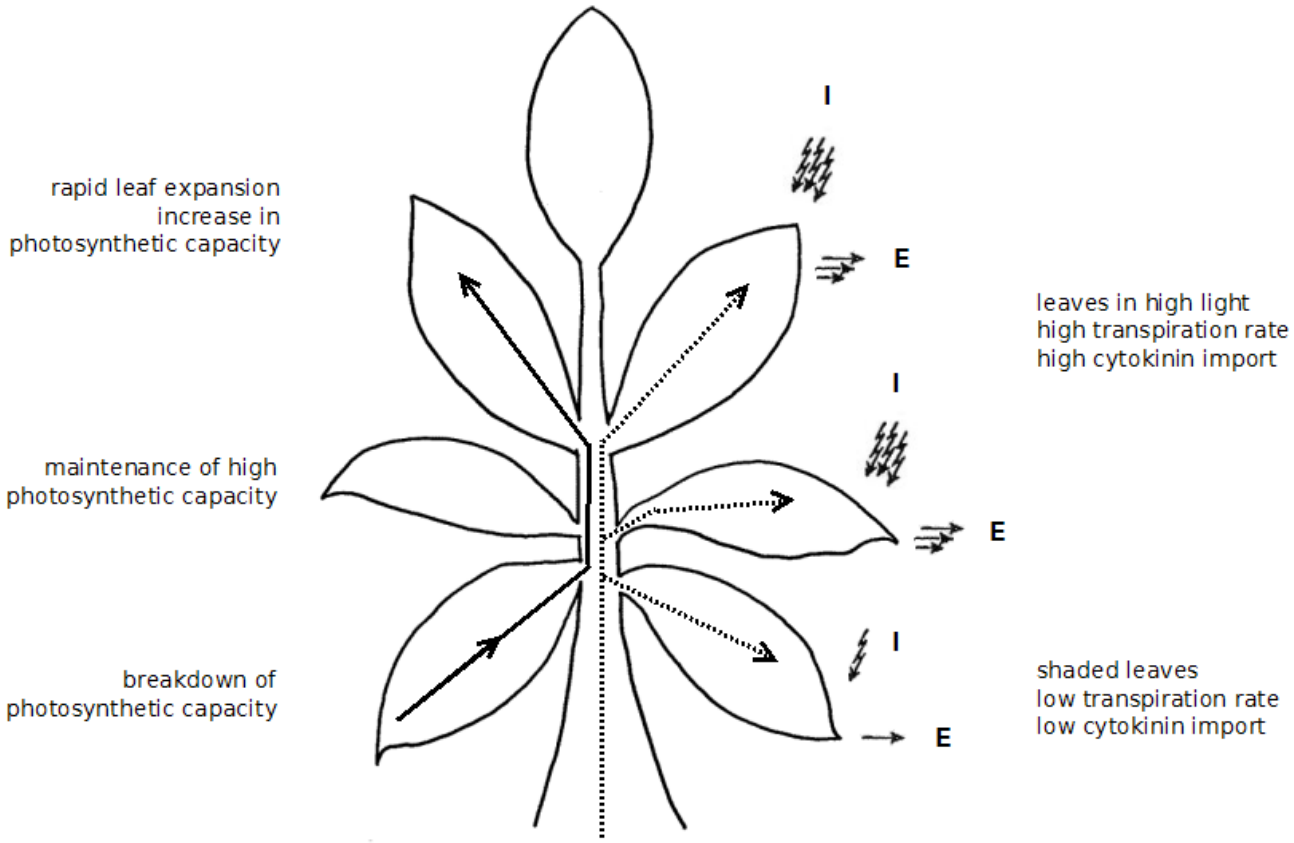


Figure 2.9: A hypothetical model to account for the differential allocation of N to leaves exposed to high or low levels of irradiance within a canopy. The broken line (- - -) represents transpirational water stream with cytokinins, the solid line represents net transport of amino acids. I stands for irradiance, E stands for transpiration. For explanations see text. Redrawn from Lambers *et al.* (2008).

This model thus suggests that the variation in the allocation of N to leaves at different depths in the canopy is due to variations in the import of cytokinins and resulting variations in photosynthetic capacity. High import of cytokinins in leaves high up in the canopy due to a higher transpiration rate leads to a stimulation of photosynthetic genes (see 2.2.3) and thus to an enhanced incorporation of amino acids into photosynthetic proteins.

2.1.6.2 N allocation during development

The time course of N allocation to the different plant parts shows a pattern with two distinct phases (see Fig. 2.10). The partitioning pattern for wheat (*Triticum aestivum*) shown in Figure 2.10 is a fairly typical one for annual crops. Till the onset of grain production the biomass as well as the total N in leaves, stem and roots increases in a similar manner, indicating a fairly constant N content. After heading yet there is a slight negative growth of stem and leaves, due to mobilization and translocation of carbohydrates to the grain, and a significant decrease in their N content. This suggests that remobilization of C from vegetative tissue to support reproductive growth is considerably smaller than remobilization of N (see also 2.1.6.3).

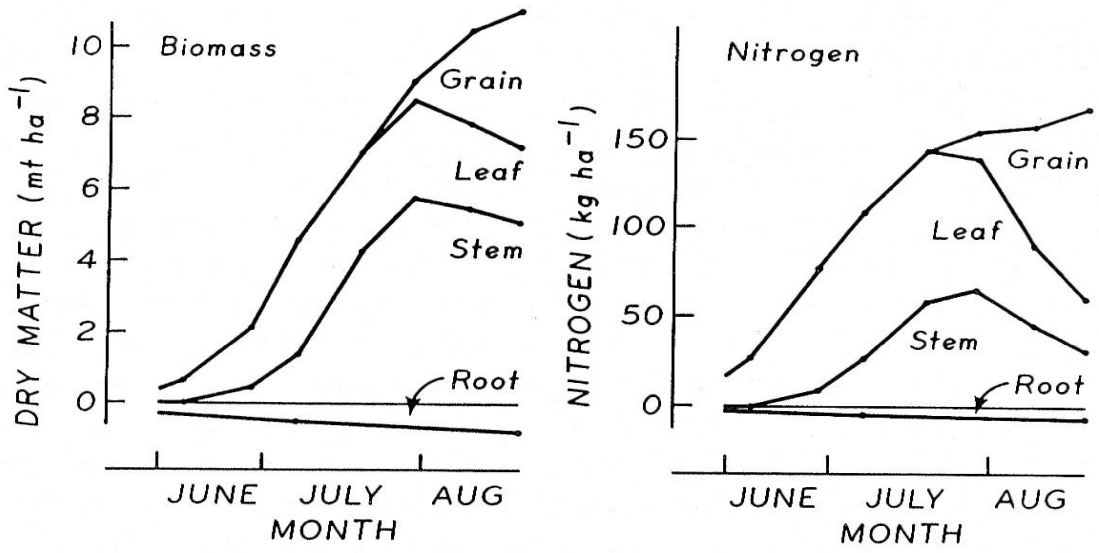


Figure 2.10: Time course of dry matter (left) and N (right) accumulation by wheat and their distribution among various plant parts. The observations are means for five cultivars grown in a replicated trial under dryland conditions under moderate fertility in Montana, USA. From McNeal *et al.* 1966.

Thus the common pattern observed for the partitioning of dry matter in annual crops, also appears to apply to the allocation of N during development: In the juvenile stage, growth is dominated by allocation to leaf, stems and root tissue. In the reproductive stage instead photosynthates and N compounds flow primarily to reproductive organs (grains, beans etc.) or to vegetative storage organs (potato, sugar beet). While C compounds allocated to reproductive and vegetative storage organs derive mainly from new C assimilation – although sometimes even negative growth of stem and leaves occurs (as in Fig. 2.10), indicating remobilization of carbohydrates –, the N compounds allocated to these tissues, derive mainly from remobilization.

In fact the remobilization of N from vegetative parts to reproductive growth is quite dramatic: N concentration in leaves and stems reached a peak shortly before grain production (2% of weight) and then declined to 0.8% by the final harvest. At this point the total plant N concentration was 1.6%, but 62% of this N was sequestered in the grains (McNeal *et al.* 1966). In a study of N allocation during development in rape (*Brassica napus*), leaves and taproots were permanent sources of N, with the N from these sources being allocated mainly to the stem and later to flowers (Malagoli *et al.* 2008). The status of the stem changed during floral transition from sink to source, while the flowers remained sinks till the end of the flowering period, from which point on they became N sources. During the pod filling stage all vegetative tissue behaved as source of N for pod-filling, with N coming mainly from green leaves (36%), followed closely by stem (34%), inflorescences (22%) and the taproot (8%). The pod derived 73% of its final N content from endogenous N remobilization, with the remaining 27% deriving from N uptake (Malagoli *et al.* 2008).

The time course of biomass and N accumulation depicted in Fig. 2.10 further shows that N accumulation does not keep pace with biomass accumulation. This holds true even under an optimal N supply (Seligman *et al.* 1975; Greenwood *et al.* 1990). While in young plants the increase in N has a more or less linear relation with biomass increase, whole plant N concentration relative to plant dry mass decreases as it matures. This decline of the N content with time can also be depicted as a decline of N content with increasing biomass, often considered as a “dilution” phenomenon of plant N by C assimilates (e.g. Justes *et al.* 1994). This “dilution” of N is due to two

processes:

- The proportion of N-rich metabolic (e.g. young leaves) to N-poor structural tissues (e.g. stem) decreases with plant age (Seligman *et al.* 1975; Caloin & Yu 1984; Greenwood *et al.* 1990; Lemaire & Gastal 1997).
- With increasing leaf area and canopy size, N is recycled and translocated from shaded, lower parts to the sun-lit upper parts of the canopy (see above), thus providing N for new growth (Lemaire & Gastal 1997).

Thus the N level in young leaves stays fairly constant even as the whole plant ages, while the N concentrations in the stem decreases to a much larger degree (Gastal & Lemaire 2002). The form of the decline in N concentration with increasing age and increasing biomass is similar between different crop types and also between C3 and C4 plants (Greenwood *et al.* 1990).

2.1.6.3 N storage

Storage of N enables the plant to uncouple N uptake from N utilization. Chapin *et al.* (1990) define storage as resources that build up in the plant and can be mobilized in the future to support biosynthesis. They distinguish between three general categories of storage:

I. Accumulation is the increase in compounds that do not directly promote growth. Accumulation occurs when resource acquisition exceeds demands for growth and maintenance.
II. Reserve formation involves the metabolically regulated synthesis of storage compounds that might otherwise directly promote growth. Reserve formation may compete for resources with growth and defence.
III. Recycling is the reutilization of compounds whose immediate physiological function contributes to growth or defence, but which can subsequently be broken down to support growth.

Accumulation occurs when the supply of a compound exceeds the capacity of the plant to utilize it in growth as another factor is limiting. Thus accumulation is relevant when the capacity for N uptake and for photosynthesis differ, as supplies of C and N change asynchronously, e.g. under rainy weather, when N supply might increase due to an improved soil water status, while the C supply decreases due to reduced light availability. Accumulation, also termed “interim deposition”, accounts for much of the short-term fluctuations in the chemical composition of plants, yet it is less important over time scales of weeks to years. Over these longer time scales, capacities for photosynthesis and N uptake adjust to plant demand, thus minimizing a large long-term imbalance between C and N stores (Lambers *et al.* 2008).

Stored reserves, which are formed directly from newly acquired C and N, often in competition to growth, make a plant less dependent on current photosynthesis and N uptake and provide resources at times were either of those are limited, e.g. in early spring in cold climates. The term “luxury consumption” sometimes used for storage of nitrogenous compounds is misleading, as N-deficient plants also store some N, which they later use to support reproductive growth (Lambers *et al.* 2008). Chicory (*Cichorium intybus*) for example builds up stores of N reserves in the tuberized roots, even when plants are grown on a limiting N supply (Améziane *et al.* 1997). These reserves are essential for biennial plants like chicory that do not flower in the first year of their life cycle, as the stored reserves are used for regrowth in the second year.

Recycling of compounds following leaf senescence is an important source of N – as it allows the

reutilization of about half of the N originally contained in the leaf – while it is a relatively unimportant source for C (Lambers *et al.* 2008). In burdock (*Arctium tomentosum*) a single molecule of reduced N can be reutilized up to six times in a single growing season (Heilmeier *et al.* 1986). Recycling can take place within a canopy from old leaves to young leaves, or from shaded parts to more sun-lit leaves, as well during different development stages, from leaves to flowers and finally to the seeds (see 2.1.5.2) (Chapin *et al.* 1990).

This concept of storage describes functional categories and not specific forms of storage. The specific forms of N storage in plants are: (i) nitrate, when plants are supplied with high levels of nitrate from the soil, (ii) amino acids, amides (asparagine and glutamine), or proteins (e.g. Rubisco) at moderate or low N availability (Lambers *et al.* 2008).

Nitrate – as most storage compounds – is divided in the plant between a metabolic and a storage pool (Millard 1988). As nitrate is taken up by the root and transported in the xylem, many tissues which have low transpiration rates contain no significant quantities of nitrate (Millard 1988). Nitrate content of plant tissues varies enormously (Stitt & Krapp 1999), yet cytosolic concentrations of nitrate are relatively constant and probably are regulated within relatively narrow limits (Miller & Smith 1996; van der Leij *et al.* 1998). Most of the nitrate is in fact located in vacuoles (Miller & Smith 1996). If thus most of the stored nitrate is localized in the vacuole, the accessibility of this store depends on the rate at which nitrate can be transported across the tonoplast (van der Leij *et al.* 1998). The remobilization of nitrate stores seems to be regulated by down-stream signals generated during nitrate assimilation (Stitt & Krapp 1999). How changes in the external N supply are sensed and translated into increased vacuolar nitrate accumulation and which transport proteins are involved in the nitrate transport across the tonoplast, remains to be elucidated (Miller & Smith 1996).

Vacuolar nitrate stores could both be due to accumulation, thus constituting a short-term storage of excess N, as due to reserve formation, thus being created in competition to growth requirements and constituting a more long-term storage for future use in plant growth. Yet laboratory studies have revealed that vacuolar nitrate stores can buffer the cytoplasm against short-term N shortages and that they are depleted within a few days (e.g. van der Leij *et al.* 1998). Under field conditions they might be even more limited and deplete even faster than under laboratory conditions. As the osmotic and charge-balancing function of nitrate in vacuoles must be replaced by alternative compounds when the vacuolar nitrate stores are used for nutritional purposes, plants typically respond to N shortages long before the vacuolar stores are exhausted (Glass *et al.* 2002). In addition nitrate concentrations in leaves often show a diurnal pattern, being accumulated at night, when the supply of reductants from photosynthesis is limited, and depleted during the day, when photosynthesis is at work (Chapin *et al.* 1990). Thus vacuolar nitrate seems to constitute mainly an "interim deposition" and not a long-term N reserve.

Proteins and amino acids instead can take over roles in all of the different components of storage; they are accumulated under excess N supply, they are stored as reserves in competition to a use for growth and are used in the recycling of N compounds (Chapin *et al.* 1990).

Amino acids and amides can be stored in high concentrations in vegetative tissue. Amide accumulation is common when plants experience an excess N supply due to a constraint upon their growth, e.g. by deficiencies of other mineral nutrients like sulphur or copper (Millard 1988). Amino acids and amides accumulate for example under salinity stress, resulting from new synthesis of amino acids that cannot be further used in growth due to a growth reduction caused by the salinity stress as well as resulting from protein degradation (Gilbert *et al.* 1998). Root-shoot cycling of amino acids in the phloem is an important, dynamic and readily-accessible N store (Millard 1988). The main transport amino acids are asparagine and arginine, which of all protein amino acids have the highest N/C ratio and are thus the most economic in their use of C (Miflin & Lea 1977; Radin &

Elmor 1980). Several free amino acids, e.g. arginine, seem also to be stored as overwintering compounds (Miflin & Lea 1977).

Proteins can accumulate in considerable quantities under luxury N supply. In leaves nearly the entire organic N accumulating under excess N is stored as soluble proteins (Millard 1988). There are storage proteins, which are essentially synthesized for the purpose of being stored and that lack any other metabolic or structural role (e.g. patatin and sporamin in potato tubers, Staswick 1994), as well as proteins that normally take over different roles in the plants physiology but that can be accumulated for storage purposes.

Storage proteins have to be protected against premature degradation. This is achieved by sequestering the storage proteins into specialized vacuoles called "protein bodies" that are separated from the metabolically active cytoplasm (Müntz 1994; Staswick 1994). The reactivation of these protein reserves thus occurs through a controlled release of the storage proteins from the protein bodies. While the storage of the protein reserves is regulated through the tissue-specific and developmentally-regulated gene expression (Müntz 1994).

In many growth conditions part of the investment in Rubisco, which is the most abundant protein in leaves (see 2.2.1), may be viewed as N store (Millard 1988; Stitt & Schulze 1994). Under high N supply Rubisco protein was accumulated without a concomitant increase in the rate of C assimilation in wheat leaves (Lawlor *et al.* 1987b). This lead the authors to suggest that under high N some 50% of the Rubisco protein could be inactivated, or that only half of the catalytic sites were functional, indicating accumulation of the protein for storage purposes (Lawlor *et al.* 1987b). Although C4 plants contain substantially less Rubisco than C3 plants (see 2.2.1), they also appear to have the capacity to accumulate N in Rubisco, but to a lesser extent than C3 plants (Millard 1988).

Organic N can be stored in substantial quantities for reproductive growth. Much of this N comes from recycling of N from soluble proteins from vegetative tissue, which is then translocated for use in reproductive organs (Millard 1988). Rubisco for example is an important source for recycling of N and can play a significant role in the N-filling of reproductive root organs: during tuber growth of potatoes (*Solanum tuberosum*), 11-15% of the N content of the tubers appeared to derive from N mobilized from Rubisco in leaves (Millard & Catt 1988). It is possible that chlorophyll is – similarly to Rubisco – also mobilized rapidly from senescing leaves and its N recycled for use in other plant parts (Millard 1988).

Although the energy cost of storing N as protein is much higher than for nitrate, there are several advantages of storing N in an organic form: Firstly proteins affect osmolarity to a much smaller extent than nitrate (Staswick 1994). Polymerizing organic compounds to form high-molecular weight compounds for storage purposes is an important adaptation to avoid osmotic problems associated with the accumulation of low-molecular weight compounds (Müntz 1994). In addition, by storing N in a catalytically active enzyme, the plant would be able to rapidly access the functional enzyme under N limitation and thus for example maximize the potential for C assimilation under N shortage (Millard 1988). And lastly: N mobilization occurs predominantly from older, senescing leaves, which are often shaded. Yet for the release of nitrate from storage pools light is needed, and the reduction and assimilation of nitrate to organic N is also regulated by light and by the availability of energy and reducing equivalents (see 2.1.5.2). Thus mobilization of N from older, shaded leaves would be constrained if N was stored as nitrate (Millard 1988).

Figure 2.11 shows the different forms of storage in plants with the associated N compounds that make out these storage pools (adapted from Chapin *et al.* 1990).

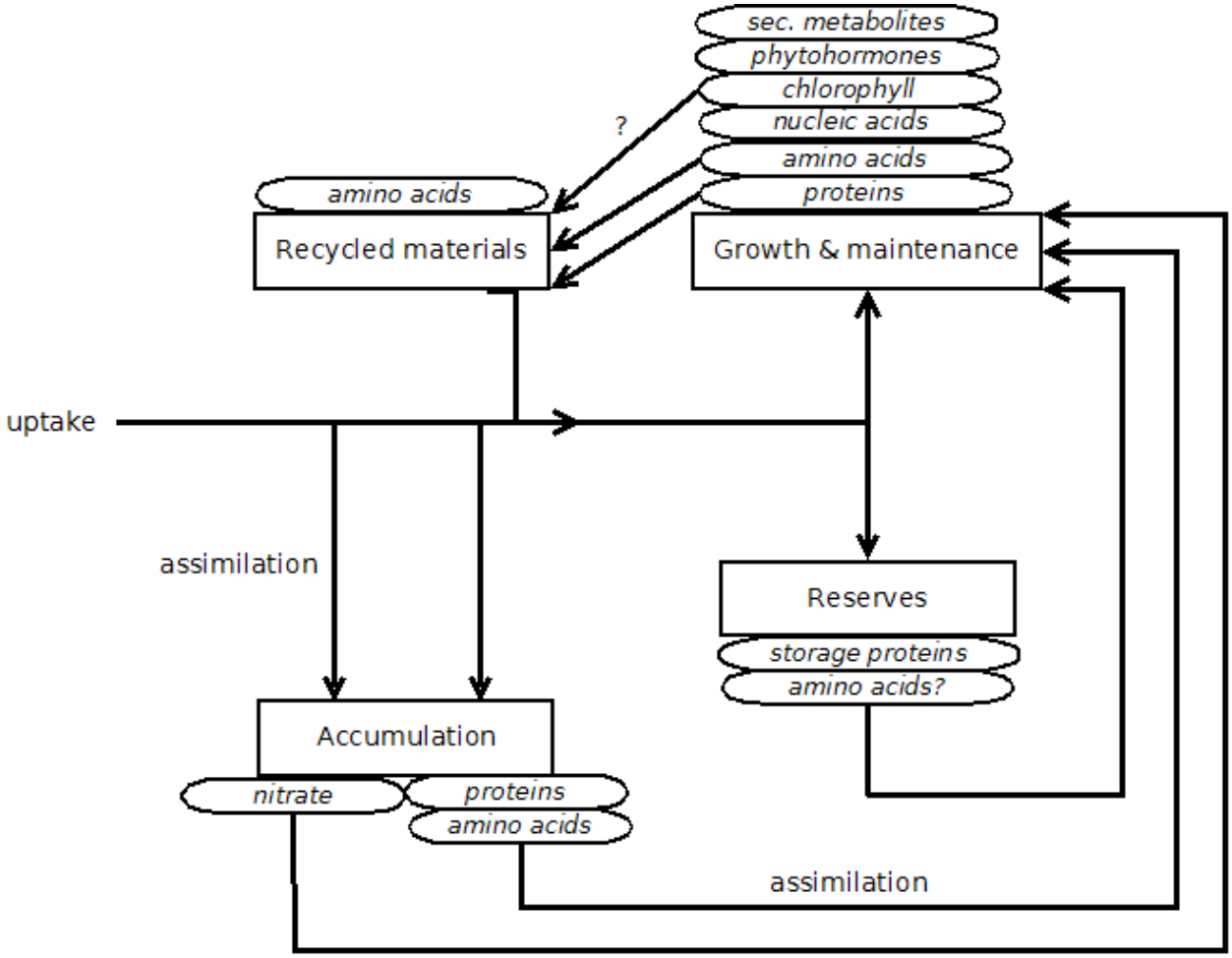


Figure 2.11: A model of pools (boxes) and fluxes associated with storage. The bubbles show the N compounds associated with different pools. See text for further explanations. Adapted from Chapin *et al.* (1990).

2.1.6.4 Conclusion

N content of plant tissues

The N content of plant tissues varies with their metabolic activity, with metabolically active tissues containing considerably more N due to high concentrations of N-rich proteins involved in metabolism.

Despite some uncertainties about the mechanisms involved and the true driving forces, it is undisputed that plants allocate N within the canopy in a non-uniformous pattern, with old, shaded plants at the bottom of the canopy containing considerably less N than young, sun-lit plants at the top of the canopy. This distribution seems to have causal connection with the light environment and the age of the leaves. The optimization theory tries to explain this N distribution pattern and states that plants should allocate N within the canopy in such a way as to maximize photosynthetic C gain and minimize costs of excess N content. It is proposed that the observed allocation of N within the canopy could be driven by differences in the transpiration rates of leaves and associated differences

in import and concentrations of cytokinins.

N allocation during development

N allocation during development shows two distinct phases: During the vegetative juvenile phase N is allocated to leaf, stem and root tissue, in relative concordance with the allocation of biomass. In the reproductive stage instead N flows primarily to reproductive organs and a large proportion of this N comes from N taken up earlier in the season and remobilized from vegetative plant organs.

With increasing age and increasing biomass the whole plant N content declines, as the proportion of N-poor structural tissue to N-rich metabolic tissue increases and as N for new growth is mobilized and reutilized from old tissue e.g. from senescencing leaves.

N storage

Storage can be divided into three functional categories: (i) accumulation of N under excess N supply, when N acquisition exceeds the ability of the plant to use N for growth, (ii) formation of N reserves - in competition to utilization of this N in current growth - for later use in reproductive growth or for outlasting resource-poor periods, e.g. winter in cold climates, and (iii) recycling of previously acquisitioned N to support new current growth.

Nitrate seems to be the major N storage compound that is accumulated under excess N supply. Yet due to several disadvantages of nitrate as N store it does not play a significant role in more long-term reserve formation or in recycling and remobilization of N. Many structural and functional proteins represent a N store that can be recycled when necessitated. Organic N in the form of soluble proteins seems to be the major N storage form that is recycled and translocated to those plant organs requiring N for new growth, e.g. in the remobilization of N from vegetative to reproductive tissue. Organic N is also accumulated under excess N supply, in the leaves mainly in the form of Rubisco. Long term N reserves, that are built up for special purposes in competition to growth, also seem to be mainly in the form of proteins, often in distinct storage proteins that do not have any metabolic function. Soluble amino acids are accumulated in the plant under excess N, when growth is limited by other factors, and in the phloem they represent a mobile, readily accessible N store.

2.2 N controls on C metabolism

While N acquisition is dependent on C metabolism for the disposal of energy and C skeletons (see 2.1), C acquisition is in return dependent on N metabolism for the provision of proteins and other N compounds. The comparison of the relative cost of C and N acquisition, in terms of C and N compounds, confirms this strong mutual dependence:

IV. The N cost of C acquisition is higher than the N cost of N acquisition.

V. The C cost of N acquisition is higher than the C cost of C acquisition.

The first assertion is based on the observations that N concentrations are greater in leaves than in roots and that C acquisition is directly proportional to leaf N content (see 2.2.1), whereas N influx is inversely related to N root concentrations (see 2.1.5.1). The second assertion is base on the observation that the rate of maintenance respiration is much higher in roots than in shoots and that the energy required for assimilation per unit nitrate is at least twice as great as that required per unit CO_2 (Bloom *et al*. 1985). The integrated process of growth of a plant is also dependent on the simultaneous provision of sucrose and amino acids.

From these considerations, i.e. the C requirements of N assimilation, the N requirements of C assimilation and the N and C requirements of growth, results a picture of a very close interaction between C and N metabolism. This mutual dependence requires a concerted repertoire of signals that allows the balanced regulation of processes of C and N metabolism. Nitrate is such an integrated signal, acting not only as a resource but also directly or indirectly as a signal modulating gene expression, metabolism and development (Hoff *et al*. 1994; Crawford 1995; Stitt 1999; Forde 2002; Krapp *et al*. 2002; for a discussion of molecular mechanisms of N signal perception see Krapp *et al*. 2002). Over 1000 different nitrate-induced genes have been found in *Arabidopsis thaliana*, including genes involved in the uptake and assimilation of N (see 2.1) as well as metabolic genes involved in the oxidative pentose phosphate pathway, reduction of ferredoxin, organic acid and starch metabolism, glycolysis and sulphur metabolism and several regulatory genes (mainly transcription factors and protein kinases) (Wang *et al*. 2003). Similarly many amino acids, especially glutamine, are thought of acting as signal molecules for the control of several metabolic processes (see also 2.1.5) (Krapp *et al*. 2002).

Here both the effect of N as a nutrient and as a plant signal on several processes of C metabolism will be discussed. I will look at how N supply poses a constraint on C metabolism and plant growth and how C processes like photosynthesis and respiration are regulated by N signals and the N status of the plant. First photosynthesis will be discussed (2.2.1) by looking at the photosynthesis-N relationship (2.2.1.1 and 2.2.1.2) and at the underlying reason for this relation, i.e. the importance of N in the photosynthetic apparatus (2.2.1.3). The role of N in respiration is considered briefly (2.2.2), while the regulation of different C processes by N signals is discussed in more depth (2.2.3). Finally the dependence of biomass allocation to different organs on N supply and N signals is examined in section 2.2.4.

2.2.1 Photosynthesis

2.2.1.1 Photosynthesis-N relationship

Photosynthesis is strongly dependent on N, as N is an important component of the photosynthetic apparatus (see 2.2.1.3). The importance of N for photosynthesis is evidenced by the strong correlation between leaf photosynthesis and leaf N content (Field & Mooney 1986; Evans 1989). The light-saturated rate of photosynthesis expressed on a leaf area basis (A_{max}), which is frequently used to characterize leaf photosynthesis, increases linearly with increasing leaf N content (N_L), regardless of whether the variation in leaf N is caused by differences in soil N availability, growth irradiance or leaf age (e.g. Reich *et al.* 1991; Pons *et al.* 1994; Anten *et al*. 1995; Peng *et al*. 1995; for analysis of single species and Field & Mooney 1986; Reich *et al.* 1999 for analysis across species; Fig. 2.12a). Similar holds true when both parameters are expressed on a dry mass basis (e.g. Reich *et al.* 1991; Nakamura *et al*. 1999 for analysis of single species and Mooney *et al*. 1981; Field & Mooney 1986; Reich *et al.* 1992, 1997, 1999; Ellsworth *et al.* 2004 for analysis across species).

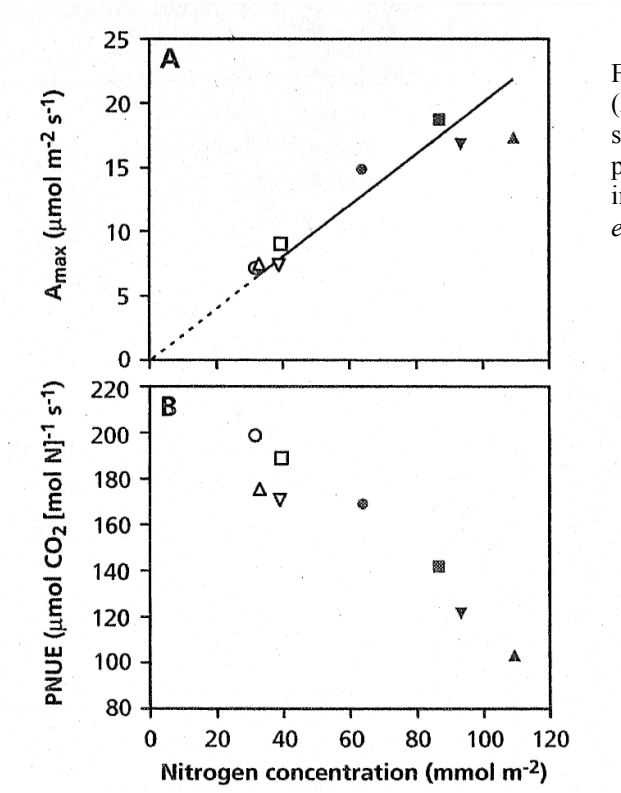


Figure 2.12: The light-saturated rate of photosynthesis (A_{max}) of four grass species grown at high (closed symbols) and low (open symbols) N supply (**A**) and their photosynthetic N use efficiency (PNUE) at growth irradiance (**B**) as a function of leaf N content. From Pons *et al.* (1994).

It has been reported that the relation between photosynthesis and leaf N is curvilinear when a sufficiently broad range of leaf N concentrations are examined (e.g. Takano & Tsunoda 1971; Evans 1983; Sinclair & Horie 1989; Vos *et al.* 2005), but Sage & Pearcy (1987a) argue that when the measurements are conducted on plants of similar age, growth conditions and variety, and when N storage forms are accounted for, A_{max} versus N_L is usually linear across the entire range of N_L.

The overall strength and generality of the A_{max}-N_L relationship is impressive, with independent surveys finding similar regression relations (Field & Mooney 1986; Reich *et al*. 1992, 1997).

Despite this apparent similarity in the relation between photosynthesis and leaf N content across many species, individual species of contrasting ecological characteristics may vary in the form of and conformity to this relationship (Evans 1989; Peterson *et al.* 1999).

So far few studies have analysed how the A_{max}-N_L relationship changes with environmental conditions, if it alters the form of the relationship or if it simply alters N_L while keeping the A_{max}-N_L relation constant. Some evidence indicates the latter possibility (Seemann *et al.* 1987).

The photosynthetic rate per unit N (photosynthetic N use efficiency, PNUE) at a given growth irradiance is highest in leaves with low N concentrations, as these leaves have a higher degree of utilization of the photosynthetic apparatus (Lambers *et al.* 2008; Fig. 2.12b).

As much of the leaf N is allocated to photosynthetic compounds (see 2.2.1.3) it is not surprising that both the chlorophyll (Chl) content and the Rubisco content of leaves also increase linearly with N_L (e.g. Evans 1983; Makino *et al.* 1984a; Lawlor *et al*. 1989; Makino *et al*. 1992; Theobald *et al.* 1998; Fig. 2.13).

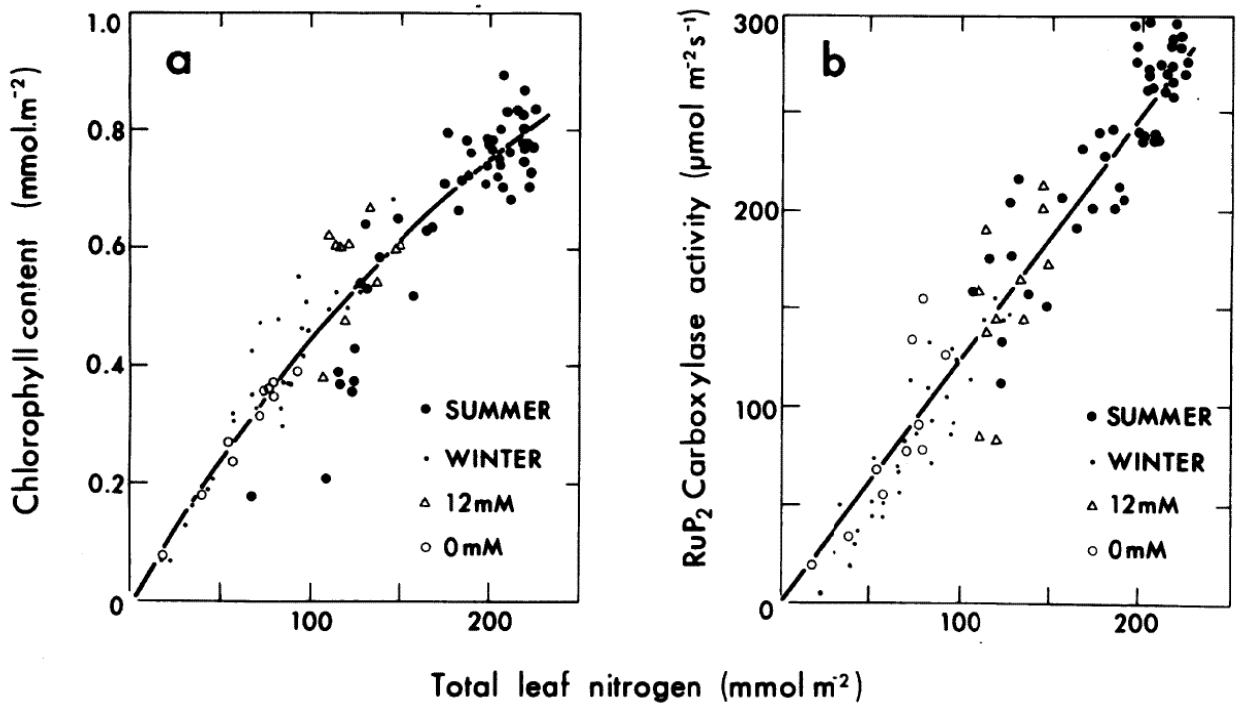


Figure 2.13: Chlorophyll content (a) and Rubisco content (b) versus total leaf N in *Triticum aestivum* grown in summer or winter and with different N supplies (12 mM N or 0 mM nitrate). From Evans (1983).

Several other parameters related to photosynthesis, e.g. stomatal conductance (g_s), are also strongly correlated with leaf N content, as they scale with A_{max} (Schulze *et al*. 1994; Wright *et al*. 2004).

As always in front of such a strong correlation, one has to pose the question, whether the A_{max}-N_L relation depicts a simple correlation or a cause. This assessment is important to understand the ecological controls on photosynthetic capacity. Field & Mooney (1986) propose three hypothesis for possible functional relationships that are consistent with the correlation: (i) N levels may determine A_{max} and photosynthesis may be directly limited by N; (ii) N levels may be controlled in response to A_{max}; (iii) or both N and A_{max} may be regulated by some other factor or factors,

suggesting that the correlation is not due to a functional relationship. Lambers *et al.* (2008) support yet another, fourth hypothesis: (iv) that A_{max} is controlled in response to N levels, but without N imposing a direct limitation for photosynthesis.

To evaluate these different possibilities, in the next section I will analyse the limitations imposed on photosynthesis by the different processes involved in photosynthesis.

2.2.1.2 Does N control the photosynthetic rate?

The mechanistic photosynthetic model of Farquhar *et al*. (1980a) - which has been extensively used and is supported by a large amount of experimental data (e.g. von Caemmerer & Farquhar 1981) - distinguishes between two processes that limit photosynthesis: At low intercellular CO_2 concentrations (C_i), photosynthesis is limited by the rate of carboxylation of Ribulose-1,5-*bis*phosphate (RuBP), which is reflected by the maximum Rubisco activity per unit leaf area, while the substrate RuBP is present at a level that is saturating for the enzyme Rubisco. In this phase there is an almost linear response of photosynthesis to C_i. At high C_i instead, when the rate of RuBP carboxylation is increased sufficiently, photosynthesis becomes limited by the rate of RuBP regeneration. This rate reflects the capacity of electron transport and the rate at which electron transport regenerates NADPH and/or photophosphorylation regenerates ATP, which in turn depends on absorbed irradiance. In this region the rate of RuBP regeneration is virtually independent of C_i, yet the assimilation rate still increases with C_i as RuBP is increasingly diverted from oxygenation to carboxylation (Farquhar *et al*. 1980a; Fig. 2.14).

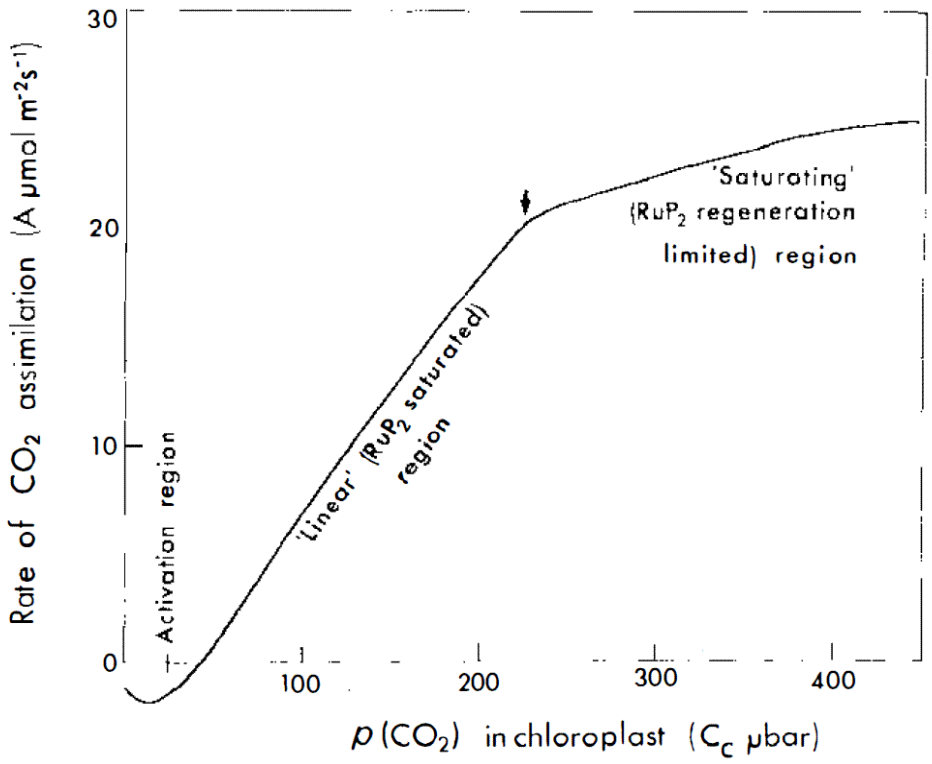


Figure 2.14: Rate of CO_2 assimilation versus CO_2 partial pressure in the chloroplast according to the model of Farquhar *et al.* (1980a). The transition from RuBP (abbreviated as RuP_2 in the graph) saturation to RuBP limitation is indicated by an arrow. From Farquhar & Sharkey (1982).

Sometimes a third limiting state has been suggested, in which the chloroplast reactions have a higher capacity than the capacity of the leaf to use the products of photosynthesis (triosephosphates). In this condition photosynthesis is limited by the capacity of starch and sucrose synthesis to utilize triosephosphates and subsequently to regenerate inorganic phosphate (P_i) for photophosphorylation (Sharkey 1985; Harley & Sharkey 1991). This state of so called triosephosphate usage (TPU) limitation does not respond to increasing CO_2 and is relevant at high internal C_i (Fig. 2.15).

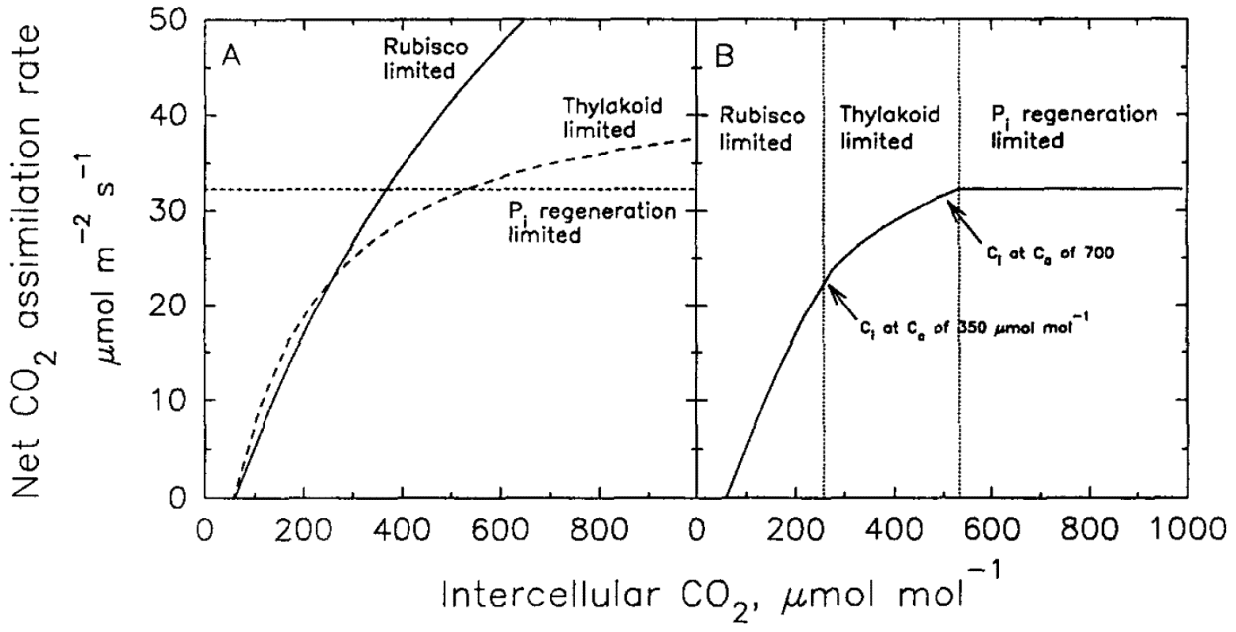


Figure 2.15: Rate of CO_2 assimilation versus CO_2 partial pressure in the chloroplast according to the model of Farquhar et al. (1980a), as extended by Sharkey (1985). In **A** photosynthesis is modelled assuming that either Rubisco capacity is limiting at all C_i (solid line), RuBP regeneration capacity is limiting at all C_i (long-dashed line) or the TPU is limiting at all C_i (short-dashed line). In **B**, the corresponding A/C_i response that would be exhibited by a leaf assuming the combined biochemical limitations as in A is depicted. From Sage (1994).

If the photosynthetic rate is limited mainly by either Rubisco or by thylakoid protein, this could suggest that N limits photosynthesis directly and that the A_{max}-N_L relationship depicts a causal connection, as the amount of protein in leaves depends on N supply. However, the concepts described so far encounter several problems.

The models described above assume that photosynthesis under the prevailing conditions is limited by single reactions, and that the surplus capacity of the other reactions is downregulated to match the single limiting step (Stitt & Schulze 1994). Under high light and low CO_2 the activity of Rubisco constitutes this limiting step, while under low light and high CO_2 the regeneration of RuBP and thus the capacity of electron transport becomes limiting and at moderately low temperatures and high CO_2 it is the rate of regeneration of P_i through utilization of photosynthates that is limiting (Farquhar *et al*. 1980a; Sharkey 1985; Woodrow & Berry 1988).

However, the assumption that photosynthesis is controlled by single limiting factors does not align with the highly interactive and complex regulation of photosynthesis (see 2.2.3) (Stitt 1991). In addition, it is suggested that the overall efficiency of N use (i.e. the C gain per unit N, NUE) is maximized if the amounts of the N compounds involved in the different photosynthetic processes

are balanced so as to co-limit photosynthesis (Field & Mooney 1986). Under current growth conditions in fact the control on photosynthesis seems to be shared between Rubisco and factors which determine the rate of RuBP regeneration, as at current ambient CO_2 concentrations the C_i in plants is close to the point of transition between the linear and curvilinear region of their A/C_i response curve (Stitt 1991; Wullschleger 1993). In transgenic tobacco plants grown with adequate N supply, one third of the Rubisco activity could be removed before it began to exert any strong control on photosynthesis (Quick *et al*. 1991), suggesting that Rubisco is not the only or major controlling factor for photosynthesis under growth conditions. While in another experiment, tobacco plants under growth irradiance compensated for reduced Rubisco protein content through an increase in the activation state of Rubisco, and Rubisco thus exerted only a small co-limitation on photosynthesis (Stitt & Schulze 1994). The processes of the light reaction, i.e. the biochemical factors affecting RuBP regeneration, in addition do exert a significant control, as many plants are not light-saturated under growth conditions (Stitt 1991).

Although the contribution of the stomata was certainly overestimated in early studies (discussed in Farquhar & Sharkey 1982), several studies indicated a significant control of CO_2 diffusion through the boundary layer and stomata on photosynthesis under growth conditions (Woodrow *et al.* 1990; Stitt *et al*. 1991). Many quantitative studies of the limitation to the photosynthetic rate in fact have evaluated the process of CO_2 diffusion to the stroma, which can restrict the supply of CO_2 for the carboxylation of RuBP (Farquhar & Sharkey 1982). Generally it is believed that photosynthesis is co-limited by CO_2 diffusion and photosynthetic capacity (Lambers *et al.* 2008).

The synthesis of end products and regeneration of P_i instead did not seem to exert any strong control on photosynthesis under current conditions (Stitt *et al.* 1991). It has been questioned whether stromal P_i concentration altogether restricts the rate of photophosphorylation and ultimately the rate of CO_2 fixation, as the ATP synthase is apparently, under most conditions, saturated with P_i (Woodrow & Berry 1988). To summarize, those analysis that looked at plants under conditions that resembled those under which the plants actually grow (i.e. the growth conditions), suggest that in plants under current conditions the control on photosynthesis is normally shared between several processes, namely CO_2 diffusion, Rubisco content and activity and the processes of the light reaction.

So can we now conclude that, as photosynthesis seems to be limited by either or a combination of the enzymatic processes of the dark and light reaction, both of which are dependent on N-compounds, the A_{max}-N_L relationship depicts a direct limitation of photosynthesis by N? It has been suggested that the generality of the A_{max}-N relationship indicates a direct limitation of photosynthesis by a nitrogenous compound, mainly Rubisco (Chapin *et al.* 1987). However it could still be, that the apparent limitation of photosynthesis by the enzymatic processes of the dark and light reaction – irrespective of whether one single limiting step prevails under certain conditions or whether the limitation is shared between several different processes – just depicts a downregulation or simply a decrease in these photosynthetic components, driven by yet another, primarily limiting factor.

So far I have always assumed that leaf N content increases with increasing N supply. Although this pattern could be expected considering that with increasing leaf N the PNUE decreases (see Fig. 2.12b) and thus if the amount of total leaf N is limited for a plant, low N_L contents would increase the efficiency of N use (Takano & Tsunoda 1971; Hikosaka & Terashima 1995), it is not always the case. In several studies instead the leaf N increased only little with an increased N supply (Gulmon & Chu 1981; Evans 1983; Sage & Pearcy 1987b; McDonald 1989). Considering that there should exist an optimal N_L for each leaf according to its light environment, with a smaller or greater N_L than this optimum implying potential costs for the leaf (with a lower value the leaf could not use the incident light for photosynthesis; with a higher value the cost of high N would be high, but the

excess N could not be used because of light limitation; see 2.1.6), this observation is not totally surprising. From this point of view it would be sensible for the plant to adjust the development of the canopy according to its N supply instead of changing the leaf N content. Grindlay (1997) suggests that under a limiting N supply the plant is faced with a "joint optimization problem", it should try to optimize both leaf N content and leaf area. In fact experiments suggest that in numerous species the primary effect of a growth limiting N availability primarily was a decrease in leaf area development (Novoa & Loomis 1981; Hirose 1984; Waring *et al*. 1985; Sage & Pearcy 1987b; Chapin *et al.* 1988b; Gastal *et al.* 1992). And in several plant species high N availability had no significant effect on net or gross photosynthesis rate per unit leaf area in leaves receiving similar irradiance (Thomas & Thorne 1975; Pearman *et al*. 1978; Waring *et al.* 1985). In clover (*Trifolium subterraneum*) instead the rates of photosynthesis per unit leaf area and total leaf area per plant decreased to about the same extent at low N (Bouma 1970). At the same time several results also showed a significant decrease in the rate of photosynthesis with N deficiency (Lawlor *et al.* 1987b; Freeden *et al*. 1991; Gastal & Belanger 1993; Muchow & Sinclair 1994). While under some field conditions the photosynthetic rate on a leaf area basis in the flag leaf of wheat at saturating light intensities even increased under low N (Pearman *et al.* 1977, 1979).

Thus, studies with different species and different experimental conditions lead to quite different results regarding the response of the photosynthetic rate to a change in the N supply. Instead the response of the development of the canopy to low N availability seems much more uniform across species and across experimental conditions; leaf area always declines with a decline in N supply (Thomas & Thorne 1975; Pearman *et al.* 1977; Evans 1983; Green 1987; Sage & Pearcy 1987b; Garcia *et al.* 1988; Gastal *et al.* 1992; Gastal & Belanger 1993).

Grindlay (1997) concludes from a review of the literature that over a range that is typical for field conditions plants do not change their leaf N content significantly and that they respond to a limited N availability mainly through a decline in leaf area development. This would imply that under low N not photosynthesis is limiting, but that the N supply mainly limits growth and the use of photosynthates for canopy expansion (see 2.2.3.2). This hypothesis is corroborated by the observation that a low N supply often leads to the accumulation of carbohydrates (McDonald *et al*. 1986; Lawlor *et al.* 1987b; Logan e*t al.* 1999). An exception could be plants grown under severe N shortage (Grindlay 1997).

If this hypothesis applies, the relation between A_{max} and N_L would be an indirect one. Plants would adapt their leaf N content primarily according to the irradiance received by the leaf. A small decrease in the leaf N content as a response to a low N supply would be the effect of a downregulation of the synthesis or activity of proteins involved in photosynthesis, as the use of photosynthates for growth becomes limited by the availability of N.

Similarly the hypothesis that the expansion of the canopy is limited by a low N availability and not by the production of carbohydrates in photosynthesis, would lead to an alternative explanation for the linear relationship between the N content of the whole plant (%N) and the relative growth rate (RGR) (described in Greenwood *et al.* 1991). While Greenwood *et al.* (1991) suggest that this relationship is due to a decrease in radiation interception under suboptimal N supply due to Rubisco shortage limiting photosynthesis, Grindlay (1997) suggests that the limitation of canopy expansion under limited N leads to a reduction in radiation interception and in the production of dry matter and following from this to a decrease in RGR, while the accumulation of photosynthates, through the restriction on leaf area development, leads to a reduction of %N in the dry matter of plants. Thus, as the A_{max}-N_L relationship, also the relation between %N and RGR would be indirect.

Several authors agree with the hypothesis that under low N supply not photosynthesis is ultimately limiting but the utilization of photosynthates for growth (McDonald *et al.* 1986; Gastal & Belanger 1993; Grindlay 1997; Lawlor *et al.* 2008). However, as discussed above, there does not emerge a

consistent picture from the experimental evidence about the response of leaf N content and photosynthesis rate on a leaf area basis to a variation in the N supply. Thus possibly different species follow different strategies, for example with species evolved in N-poor habitats decreasing N_L under low N supply in order to enhance NUE, while species evolved in N-rich habitats may maintain a more constant N_L, near the optimal value for a given irradiance even under N limitation. For species growing in dense, mixed stands instead, a large canopy may bring competitive advantage and they may thus reduce photosynthesis rate per unit leaf area under low N supply, so that they can invest more N in leaf growth, in order to minimize the reduction in canopy expansion.

At the same time the different responses observed in different experiments could also be attributed to different experimental conditions. Grindlay (1997) noted that extreme N shortage and N starvation could lead to a reduction in the photosynthesis rate because of a remobilization of N within the plant as a stress response. Plants under field conditions rarely experience N stress, as they can maintain their N supply through soil exploration by their roots (Ingestad 1982). Plants in the field could thus have high N_L even without fertilizer application, while in solution culture plants are markedly N deficient if not enough nutrients are added (Grindlay 1997).

2.2.1.3 Use of N in the photosynthetic apparatus

N is an important component of several compounds involved in the different parts of photosynthesis, namely soluble proteins involved in the Calvin cycle (especially Rubisco) as well as several N-compounds involved in the light-driven electron transport including Chl, pigment-protein complexes involved in light capture, membrane-bound proteins involved in photosynthetic electron transport (primarily the cytochrome b/f and ferredoxin NADP reductase complexes) and the ATP-synthesizing enzyme (Field & Mooney 1986). Approximately 75% of leaf N in C3 plants is invested in chloroplasts (Chapin *et al.* 1987) and most of it, i.e. between 50 and 80% of total leaf N, is allocated to photosynthetic proteins (Evans 1989; Makino & Osmond 1991). It is the amount and activity of these proteins that determines the photosynthetic potential of the leaf. Consequently the photosynthetic capacity is determined by the amount of protein (which is equivalent to organic N) per unit leaf area (Evans 1996). The proportion of leaf N invested in photosynthetic components is fairly constant for a given species. By contrast the N content per unit leaf area (see 2.1.6.1) and the allocation of N between the different photosynthetic processes varies with environmental factors (Kumar *et al.* 2002).

Evans (1989) divided N used in photosynthesis into two components: (i) soluble protein, dominated by Rubisco, and (ii) thylakoid protein, including proteins involved in light capture and electron transfer. This division is useful as the soluble and thylakoid protein functionally represent the dark and light reactions of photosynthesis respectively, which can be transposed into the photosynthetic model of Farquhar *et al.* (1980a). The two rate-limiting processes of this model, i.e. RuBP carboxylation and RuBP regeneration (see 2.2.1.2), can be described by the contents and activities of the soluble and thylakoid protein fractions respectively (Evans 1989).

Rubisco has a rather low turnover number, a low catalytic activity and a rather poor affinity for CO_2 and is therefore present at high concentrations in the leaf accounting for up to 50% of the total leaf protein (Woodrow & Berry 1988). Most of the remaining soluble protein of leaves is made up of other chloroplast enzymes of the Calvin cycle, photorespiratory enzymes in the mitochondria and peroxisomes, carbonic anhydrase and ribosomes (Evans 1989).

The majority (60-85%) of the thylakoid N associated with light capture and photosynthetic electron transport is found in the pigment-protein/reaction centre complexes (Chapin *et al.* 1987; Evans 1989). Although Chl makes up only a relatively small proportion of total leaf N (ca. 1.7% in a sun

leaf, Chapin *et al.* 1987) it is to a first approximation proportional to total thylakoid N, with 50 mol thylakoid N mol^{-1} Chl (Evans 1989).

Light and nutrition are the two major features of the environment affecting the development of photosynthesis. The changes that occur in the photosynthetic apparatus under varying environmental conditions have been given the unifying term "photosynthetic acclimation" (Evans 1996). I will now look at the changes observed in photosynthetic N partitioning under different light and N environments.

Acclimation to light

The acclimation of leaf photosynthesis to growth irradiance is not only determined by a change in the absolute N content (see 2.1.6.1) but also by changes in the partitioning of N within the leaf between the various pools involved in photosynthesis (Evans 1989; Hikosaka & Terashima 1995; Evans & Poorter 2001). At low irradiances, photosynthetic rate depends on the proportion of incident light absorbed by the leaf (i.e. the rate of RuBP regeneration becomes the predominant limitation on photosynthesis) which is closely related to the Chl content of the leaf (Evans 1996). The most important features of high-light grown leaves in comparison with low-light grown leaves are: (i) less Chl per unit N; (ii) a higher Chl a/b ratio; (iii) an increased cytochrome f content per unit Chl; (iv) a slightly greater ratio of electron transport capacity to Rubisco activity (Evans 1996; Evans & Poorter 2001). These findings are interpreted as follows: at low light (i) the proportion of N in thylakoid proteins generally increases (Evans 1989); the composition of thylakoid N changes (Terashima & Evans 1988), (ii) with an increase in the proportion of Chl in the light-harvesting complex and a concomitant reduction in the number of photosystem II (PSII) reaction centres, and thus with (iii) more N being allocated to light capture than to electron transport; and finally (iv) the proportion of leaf N in Rubisco, depending on the species, remains unchanged or decreases (Evans 1989). Acclimation to low growth irradiance thus primarily involves re-allocation of N from soluble protein and electron-transport components into pigment-protein complexes (Terashima & Evans 1988; Evans 1989; Pons & Pearcy 1994; Hikosaka & Terashima 1995; Hikosaka & Terashima 1996; Niinemets *et al.* 1998; Evans & Poorter 2001). In common bean (*Phaseolus vulgaris*) for example the proportion of leaf N in Rubisco increased from 6% in low light to 20% in high light (Seemann *et al*. 1987).

The increase in photosynthesis at high irradiances, which is achieved by higher investment in soluble proteins, is thus offset by less photosynthesis at low irradiances, where a higher investment in thylakoid transport would be more beneficial. Thus the ratio of soluble to thylakoid protein that maximizes daily photosynthesis is dependent on the light environment. It has therefore been suggested that plants allocate their N among the different photosynthetic components in order to optimize photosynthetic gain under a given light availability (Hikosaka & Terashima 1995, 1996). Yet in several species the changes in N partitioning associated with different growth irradiances were far from optimal (Lauerer *et al*. 1993; Stitt & Schulze 1994; Makino *et al*. 1997a).

Despite these changes in the relative proportion of soluble and thylakoid protein, the ratio between Rubisco activity - which reflects the photosynthetic response at low C_i - and Hill activity - which is the rate of O_2 evolution, representing the rate of whole-chain electron transport and thus reflecting the photosynthetic response at high C_i (see 2.2.1.2) – is generally unaffected by light (Evans 1996). This suggests that the processes of light and dark reaction are coordinated and that the changes in photosynthesis partitioning observed under different irradiances are undertaken in order to achieve a balance between the capacities of the two processes.

Acclimation to N supply

Generally the proportion of total leaf N in Rubisco is not constant, but increases with increasing leaf N (Natr 1975; Evans 1989). In spinach (*Spinacia oleracea*) for example it increases from 10 to 19% (Terashima & Evans 1988). Yet the Rubisco concentration does not always change to a greater extent than other nitrogenous compounds. Several studies have also observed that the Rubisco proportion remained constant with N treatment (e.g. van Caemmerer & Farquhar 1981; Evans 1983; Lawlor *et al*. 1987a; Makino *et al.* 1992).

When the proportion of total N in Rubisco increases with increasing leaf N, the *in vitro* capacity of Rubisco increases relative to the electron transport/photophosphorylation capacity, yet the *in vivo* capacities of the different photosynthetic components remain largely balanced (Evans & Terashima 1988; Terashima & Evans 1988). Interestingly in these cases the photosynthetic rate increases curvilinearly with the amount of Rubisco (e.g. Makino *et al.* 1988; Lawlor *et al*. 1989), suggesting that the measured *in vitro* Rubisco activities at the higher enzyme contents exceed the rates of CO_2 assimilation. One possible explanation for this is that Rubisco is less efficient at these high enzyme and high N levels, probably due to a higher internal CO_2 transfer resistance at higher N levels (probably because of an increase in the volume of chloroplasts) resulting in lower CO_2 concentrations at the site of carboxylation (Evans & Terashima 1988; Makino *et al.* 1988; Terashima & Evans 1988). An over-investment of N in Rubisco at non-limiting N supply provides a reserve of N, enabling the leaf at the same time to better exploit short periods of intense illumination (Millard 1988; Stitt & Schulze 1994; see 2.1.6.3).

In the C4 plant maize (*Zea mays*), N deficiency decreases the proportion of N in Rubisco and in several enzymes of the C4 cycle, but with the relative decrease in C4 enzymes being significantly greater than the decrease in Rubisco (Sugiyama *et al.* 1984; Khamis *et al.* 1990; Sugiharto *et al*. 1990).

The composition of the thylakoid membranes instead is largely unaffected by N levels (Evans 1989), with thylakoid N in spinach representing 24% of total leaf N irrespective of growth conditions (Terashima & Evans 1988). N nutrition thus affected the amount of thylakoids per unit leaf area but not the photosynthetic properties of the thylakoid membranes (Evans & Terashima 1988; Terashima & Evans 1988).

In marine phytoplankton N limitation seems to have a strong effect on photochemical energy conversion, resulting from a loss of PSII reaction centre proteins (Kolber *et al*. 1988; Berges *et al.* 1996). It seems that while PSII is strongly affected by N shortage, there is no apparent effect of N starvation on photosystem I (PSI) (Berges *et al.* 1996). In *Chlamydomonas* cells high N (Plumley *et al.* 1989) and in NR-deficient tobacco mutants the accumulation of nitrate (Lauerer 1996, as cited in Stitt & Krapp 1999) decreased the Chl a/b ratio, suggesting an increase in the amount or size of PSII antennas. Yet in wheat (*Triticum aestivum*) the Chl a/b ratio usually does not change with increasing N supply (Lawlor *et al*. 1987b; Theobald *et al*. 1998).

N deficiency decreases the point at which light saturates photosynthesis, thus increasing the likelihood of photoinhibitory damage (Kumar *et al.* 2002). Green algae have in fact been shown to be more susceptible to photoinhibition under N limitation (Kolber *et al*. 1988), while higher plants seem to avoid photoinhibitory damage through increased zeaxanthin contents and increased thermal dissipation (Foyer *et al*. 1994a; Khamis *et al*. 1999).

The combined changes in photosynthetic N partitioning that occur in response to changing N content or growth irradiance are illustrated in Fig. 2.16 (from Evans 1989).

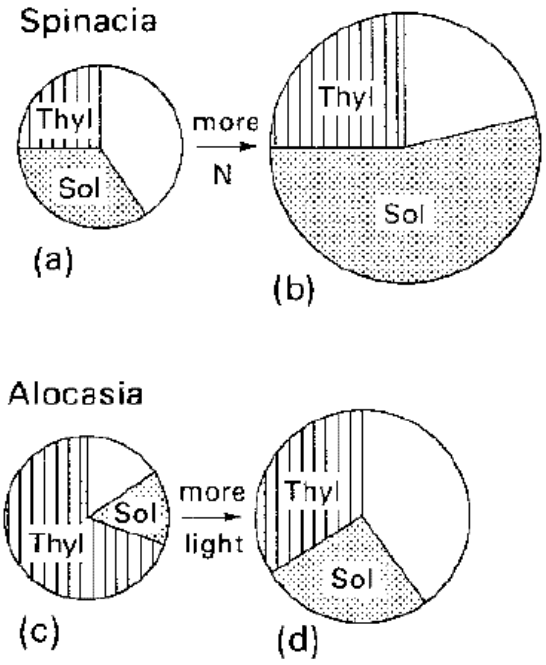


Figure 2.16: Patterns of N partitioning of leaves supplied with different amounts of N (a,b) or grown at different irradiances (c, d) using the examples of spinach and *Alocasia*. The relative area of each pair of circles is proportional to the N content of leaves, being 75mmol N m^{-2} (a), 200 mmol N m^{-2} (b), 57 mmol N m^{-2} (c) and 95 mmol N m^{-2} (d). The irradiances received are 20 mmol quanta m^{-2} s^{-1} (c) and full sunlight (d).

In spinach thylakoid N was constant at 24% of total leaf N, irrespective of N supply, while the soluble protein fraction increased from 33 to 58% at the expense of the "other" fraction (a, b). While the proportion of "other leaf N" declined, the absolute amount remained relatively constant.

In the shade plant *Alocasia* with transfer to high light the proportion of N in thylakoids declined from 71% to 29%, while soluble protein increased from 14 to 21%. From Evans (1989).

Acclimation to CO_2

It is also suggested that N partitioning among photosynthetic components is important in the acclimation of photosynthesis to elevated CO_2 (Hikosaka & Hirose 1998). The CO_2 concentration determines which one of the two capacities that control photosynthesis according to the model of Farquhar *et al.* (1980a), i.e. the rate of carboxylation or the rate of regeneration of RuBP, actually limits photosynthesis. Under current concentrations of CO_2 these two processes are considered to co-limit photosynthesis (Wullschleger 1993). It is assumed that under elevated atmospheric CO_2 conditions, photosynthesis may be limited more by RuBP regeneration, as the rate of RuBP consumption increases relative to the rate of RuBP production (Sage 1994). In addition, under high CO_2 the rate of regeneration of P_i from the use of photosynthates for carbohydrate synthesis, can limit photosynthesis (Harley & Sharkey 1991). From theoretical considerations (Woodrow 1994; Hikosaka & Hirose 1998) and experimental observations (Hogan *et al*. 1991; Webber *et al*. 1994) it has therefore been proposed that under elevated CO_2 the amount of Rubisco is reduced and N is reallocated to proteins of the light reaction, so that both protein fractions, i.e. the soluble and thylakoid N, again co-limit photosynthesis, leading to efficient use of N. In addition plants should also increase the investment in processes supporting the use of triosephosphates for synthesis of non-phosphorylated end-products in order to regenerate P_i (Sage 1994).

However several species do hardly change N partitioning among the photosynthetic components with increasing atmospheric CO_2 concentration (Sage 1994; Nakano *et al.* 1997) and these plants thus do not optimize their N partitioning according to external CO_2 levels. And even if a redistribution of leaf N from Rubisco to thylakoid N is observed, it often is still less than the predicted optimum (Nakano *et al*. 1997; Theobald e*t al.* 1998). It is thus yet not clear how the photosynthetic apparatus will respond to elevated CO_2 concentrations. It appears that the degree of acclimatisation is strongly dependent on N supply, development stage and leaf position in the canopy, with the response being most pronounced in lower shaded leaves and at later development stages (Adam *et al.* 2000).

Short-term exposure to elevated CO_2 concentrations leads to an increase in photosynthesis per unit leaf area and plant mass is also enhanced during a subsequent long-term exposure to elevated CO_2. However growth at elevated CO_2 often leads to a decrease in total leaf N content, a net decrease in the amounts of Rubisco and other photosynthetic components and frequently also to a gradual decrease of the initial stimulation of photosynthesis (for reviews see Stitt 1991; Bowes 1993; Sage 1994). The lower N content under elevated CO_2 may be explained by greater N sinks elsewhere in the plant due to greater growth and due to accumulation of carbohydrates (Kumar *et al.* 2002). The gradual inhibition of photosynthesis observed in many plants during acclimation to enhanced CO_2 could be due to an inadequate demand for carbohydrates in the remainder of the plant as the rate of photosynthesis exceeds the capacity of the sinks to utilize the photosynthates for growth (Stitt 1991). The lower N content of plant tissues under elevated CO_2 might also be due to the fact that under high CO_2 concentrations plants take up more C per N (i.e. have higher N productivity) because of an increase in the carboxylation rate (with simultaneous reduction of the oxygenation rate) of the Rubisco enzyme (Kattge 2002).

2.2.1.4 Conclusion

Light-saturated photosynthesis (A_{max}) often shows a positive linear response to leaf N content (N_L). This relationship has been observed in numerous studies across numerous species, yet so far not much is known about the physiological basis of the relationship and about the response of the relationship to environmental conditions. Some studies depict a curvilinear relation between A_{max} and N_L, possibly because of "luxury consumption" of N in non-photosynthetic components, e.g. structural tissue or storage compounds, at high leaf N contents.

Although photosynthesis is controlled by the contents and activities of N compounds, especially Rubisco and/or thylakoid N, photosynthesis is often believed not to be limited by suboptimal N availability. Instead a suboptimal N supply appears to limit the expansion of the canopy and thus the use of photosynthates for growth. If the contents and activities of photosynthetic N compounds are downregulated as a result of a low N supply, this depicts a feedback regulation - as the photosynthates can no longer be sufficiently used for growth - and not a primary response to N limitation. If instead leaf N contents are decreased and adjusted to light availability (see 2.1.6.2), this neither depicts a limitation of photosynthesis by N, but it is an optimization of N resources and a reallocation of photosynthetic N that cannot further be used because of light limitation of photosynthesis under the current conditions. Thus the A_{max}-N_L relationship seems to be an indirect relationship, caused through the regulation of photosynthetic proteins according to the need for and the potential of photosynthesis under the prevailing conditions.

A large proportion of leaf N is allocated to the photosynthetic apparatus, as the majority of the processes of photosynthesis depend on nitrogenous compounds. From optimization theory it is predicted that the partitioning of N between the different components of photosynthesis should be adjusted according to light availability, N supply and ambient CO_2 concentrations. Yet the actual partitioning of N in plants often differs substantially from the theoretical optimum.

The photosynthetic proteins can be broadly divided into soluble proteins, dominated by Rubisco and representing the capacity to carboxylate RuBP, and thylakoid proteins, which represent the capacity to regenerate RuBP in the light reactions. Yet sometimes this division is too coarse, as changes in the photosynthetic apparatus happen within e.g. the thylakoid protein fraction. Under low irradiance for example the proportion of light harvesting protein complexes increases, while the proportion of proteins involved in electron transport, such as the cytochrome b/f and ferredoxin NADP reductase complexes as well as PSII reaction centres, decreases.

Although there have been observed considerable differences in the response of the photosynthetic apparatus to differences in the N supply between different species and between different experimental trials, in general it seems that in higher plants the composition of the photosynthetic apparatus is often largely unaffected by N levels. If however – as sometimes observed in C3 plants - the proportion of Rubisco is reduced under low N supply, the balance between the light and the dark reactions is still maintained. The excess investment in Rubisco under high N may be due to an increased internal CO_2 diffusion resistance and/or due to a use of Rubisco as N store. C4 plants instead seem to differ slightly from this pattern. Under N limitation the decrease in the proteins involved in the C4 cycle is far more pronounced than the decrease in Rubisco protein, resulting in an increased proportion of total soluble protein present as Rubisco.

In general it can be said that if the partitioning of N within the photosynthetic apparatus is acclimatized to the prevailing environmental conditions and the proportion of a single or a number of photosynthetic protein fractions is changed, this change does not disrupt but in fact maintain the balance between the activities of the different photosynthetic processes.

2.2.2 Respiration

Several plant processes rely on C skeletons, energy (i.e. ATP) and reducing power (i.e. NAD(P)H) from respiration of substrates, including (i) biosynthesis of new structural biomass, (ii) translocation of photosynthates from source to sinks, (iii) uptake of ions from the soil solution, (iv) assimilation of N (including N_2) and S into organic compounds, (v) protein turnover, and (vi) cellular ion-gradient maintenance (Amthor 2000). Approximately half of all photosynthates produced per day are respired in the same period, with the exact fraction depending on species and environmental conditions (Lambers *et al.* 2007).

Respiration has often been broadly divided into respiration associated with biosynthesis and related processes such as transport of substrates (growth respiration, R_G) and respiration needed to maintain existing biomass in a functional state (maintenance respiration, R_M). Experimental evidence indicates that the respiratory cost of maintenance in herbaceous plants is about equal to the respiratory cost of growth over a growing season (Amthor 1984).

The production of biomass requires the respiration of carbohydrates to generate metabolic energy (ATP and NAD(P)H) and to provide C skeletons, as plant tissue is in general more reduced than the primary carbohydrates from which it is produced (Lambers *et al*. 2008). In photosynthetically active leaves some of the energy for biosynthesis may come directly from photosynthesis. In heterotrophic tissue such as roots and in leaves in the dark instead, respiration provides the required energy (Lambers *et al*. 2008). In barley (*Hordeum vulgare*) – where a high proportion of nitrate assimilation occurs in roots – around 5% of the total energy from root respiration is required for absorption of nitrate, 15% for reduction of nitrate and 3% for the assimilation of reduced N. Thus in total 23% of the energy from root respiration is used in the assimilation of nitrate, compared with only 14% for the assimilation of ammonium (Bloom *et al*. 1992). In part due to this large cost of N assimilation, root respiration has been shown to increase linearly with root N content (Ryan *et al*. 1996; Atkinson *et al.* 2007; see Fig. 2.17c). The respiratory cost of growth has been estimated based on (i) the biochemical composition of plant tissue, (ii) the elemental composition of plant tissue, and (iii) the heat of combustion (Lambers *et al.* 2008).

Leaf dark respiration (R_d) – which is distinguished from photorespiration, associated with the oxygenating activity of Rubisco - increases linearly with leaf N content, but to a smaller degree than photosynthesis (Terashima & Evans 1988; Makino & Osmond 1991; Ryan *et al*. 1996; see Fig.

2.17a, b). This suggests that with increasing leaf N content the ratio of respiration to photosynthesis decreases. In fact the ratio of mitochondrial respiratory enzyme activities to leaf N content decreased with increasing leaf N content in pea and wheat (Makino & Osmond 1991). The correlation between R_d and leaf N content tends to be quite general among different species (Reich *et al.* 1998; Tjoelker *et al*. 1999). Dark respiration rates tend to increase with increasing T and decrease with increasing atmospheric CO_2 concentrations, yet these changes can be explained by underlying changes in leaf N contents (Tjoelker *et al.* 1999).

The cost for the maintenance of biomass has been estimated to lie in the range from 35 to 80% of the photosynthates produced per day (Amthor 2000). As maintenance respiration involves mainly protein turnover and other processes that are likely to be related to protein content, R_M is closely related to tissue N concentrations and often increases linearly with increasing plant N content (Irving & Silsbury 1987; Li & Jones 1992). R_M has therefore been successfully estimated from tissue N concentrations (Ryan 1991).

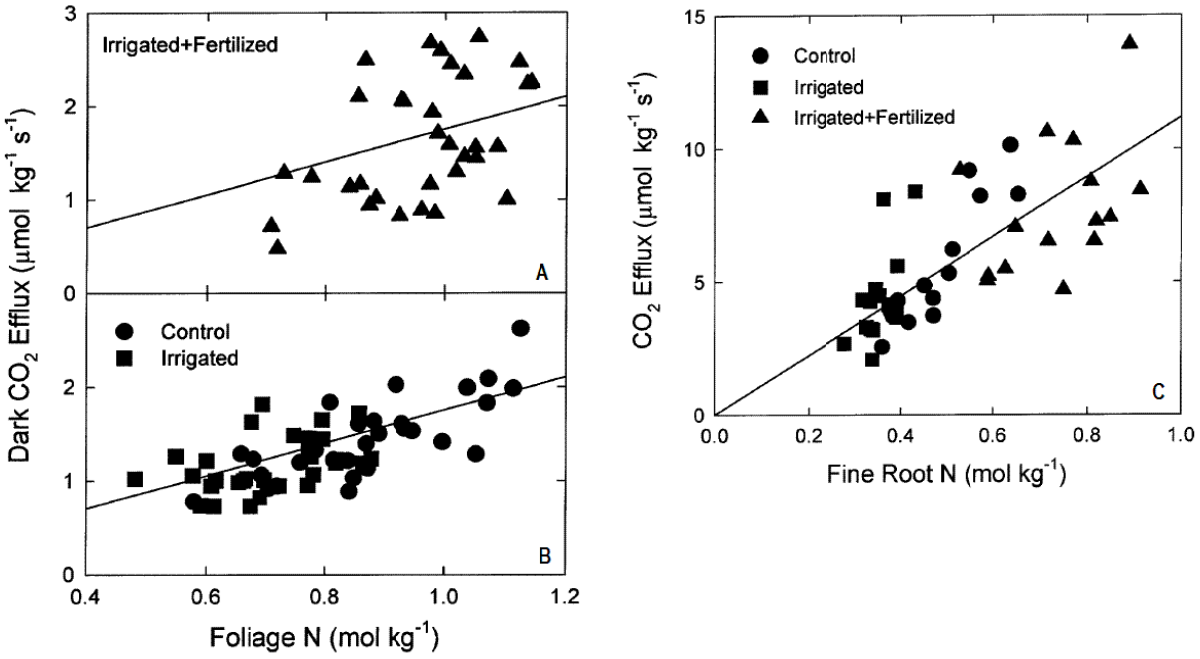


Figure 2.17: CO_2 efflux of fully expanded leaves at night (A, B) and of fine roots (C), corrected to 15°C, in relation to tissue N concentration for *Pinus radiata* growin in three treatments. From Ryan *et al.* (1996).

In plants grown at a low N supply, the rate of root respiration is lower than that of plants grown with adequate N supply (Atkinson *et al*. 2007). This is not surprising as under higher N supply, growth, N uptake rates and root N concentrations are increased. However the specific costs of growth, maintenance and N uptake (i.e. the respiration rate per unit biomass produced, per unit biomass to be maintained and per unit N taken up) increases in plants grown at a limiting N supply (Lambers *et al*. 2008). Consequently the fraction of photoassimilates that is respired increases with decreasing N supply to the plant (van der Werf *et al.* 1992; see Fig. 2.18). In the two monocotyledonous species *Dactylis glomerata* and *Holcus lanatus* the C assimilated per day that was lost through total plant and root respiration was 30 and 14% respectively at a near-optimum N supply (Fig. 2.18a), while at a limiting N supply the loss was 71 and 52% respectively (Fig. 2.18b). This is due to lower rates of photosynthesis per unit leaf weight and greater allocation of biomass to

non-photosynthesizing organs, rather than to a higher specific respiratory activity (van der Werf *et al.* 1992).

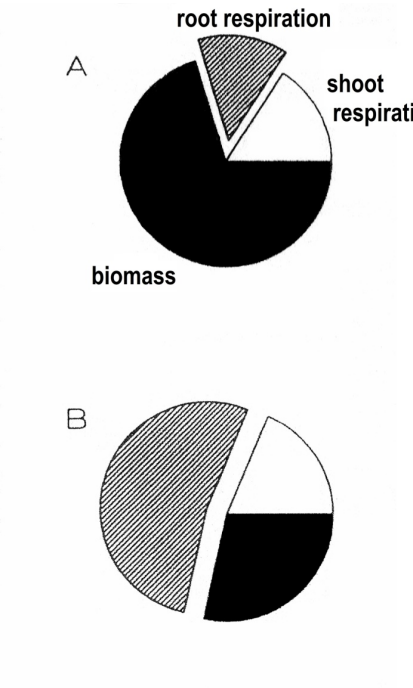


Figure 2.18: Average C budgets of two monocotyledonous species grown at optimum (**A**) and limiting nitrate supply (**B**). The black section of the pie refers to C invested in growth; the white section refers to C used in shoot respiration and the loose section refers to C used in root respiration. From van der Werf et al. (1992).

2.2.3 Regulation of C metabolism by N signals

It is often believed that in leaves the pathways of C and N assimilation compete for energy and C skeletons (see 2.1.3). Yet several lines of evidence suggest that these pathways are highly coordinated and that they exercise reciprocal control on each other, thus preventing undesirable competition and accumulation of toxic intermediates (Foyer *et al*. 1994a). The regulation of N assimilation by C assimilation has already been discussed (see 2.1.5.2). Here I will look at how C assimilation is coordinated with N assimilation.

2.2.3.1 Photosynthesis

Photosynthetic activity is mainly determined by the amount and composition of the photosynthetic apparatus in leaves (Paul & Foyer 2001). The identity and direction of the changes in the photosynthetic components induced by environmental conditions have been discussed above (see 2.2.1.2), here the focus will be on how these changes are regulated.

Regulation by N signals

Nitrate has been shown to affect the expression of several genes in photosynthesis (see Tab. 2.1; Sugiyama & Sakakibara 2002), including the light harvesting Chl a,b-binding protein (*Cab*) in *Chlamydomonas* (Plumley & Schmidt 1989), the small nuclear-encoded subunit of Rubisco (*rbcS*) in *Chlamydomonas* (Plumley & Schmidt 1989) and maize (*Zea mays*) (Sugiyama & Sakakibara 2002), several proteins involved in the C4 cycle (PEPCase, PPDK, CA) in maize (Sugiharto & Sugiyama 1992) as well as the enzymes AlaAT (Son *et al.* 1992) and AspAT (Taniguchi *et al.* 1995) involved in the C4 pathway in millet (*Panicum miliaceum*).

Table 2.1: Plant photosynthesis genes regulated by N availability. From Sugiyama & Sakakibara (2002).

Protein (gene)	Nitrogen (effect)	Other factors (effect)
PEPCase (*C4Ppc1*)	Nit, Am, Gln (+)	CK, Light (+); Suc (-)
PPDK (*C4Ppdk*)	Nit, Am, Gln (+)	CK, Light (+); Suc (-)
CA (*C4Ca*)	Nit, Am, Gln (+)	CK, Light (+)
AlaAT (*AlaAT-2*)	Nit, Am (+)	
AspAT (*cAspAT, mAspAT*)	Nit, Am (+)	
Rubisco (*rbcS*)	Nit, Am, Gln (+)	CK, Light (+); Suc (-)
LHCP (*Cab*)	Nit, Am, Gln (+)	CK, Light (+); Suc (-)

PEPCase, phosphoenolpyruvate carboxylase; PPDK, pyruvate orthophosphate dikinase; CA, carbonic anhydrase; AlaAT, alanine aminotransferase; AspAT, aspartate aminotransferase; Rubisco, ribulose-1,5-bisphosphate carboxylase/oxygenase; LHCP, light-harvesting chlorophyll a,b-binding protein; Nit, nitrate; Am, ammonia; Gln, Glutamine; CK, cytokinin; Suc, sucrose.

The mechanisms involved in the regulation of genes encoding C4 enzymes by inorganic N have been extensively studied in maize. The expression of the C4-type PEPCase enzyme has been shown to be regulated by N availability both at the transcriptional and post-transcriptional level (Sugiharto *et al.* 1990; Suzuki *et al.* 1994). The signals mediating this response to the N status have been proposed to be cytokinins - regulating the transcription of the *C4Ppc1* gene - and glutamine and/or its metabolites - regulating the mRNA level by controlling the stability of mRNAs (Suzuki *et al.* 1994; Sugiharto *et al.* 1992 a, b).

The pattern observed for the nitrate regulation of PEPCase in maize seems to also apply to other nitrate-responsive photosynthesis genes: instead of a direct effect of nitrate, the nitrate-response of gene expression of photosynthetic proteins seems to be mediated by other signals derived from the N status, e.g. phytohormones and downstream N metabolites. So far no responses of photosynthesis genes to nitrate itself have been reported (Stitt 1999; Wang *et al.* 2000; Wang *et al.* 2003).

Glutamine levels have been shown to respond sensitively to nitrate supply (Scheible *et al.* 1997b) and thus provide a promising candidate for regulating photosynthesis according to the N status of the plant. Tobacco (*Nicotiana tabacum*) mutants overexpressing the GS enzyme have been shown to have increased photosynthetic rates despite N starvation (Fuentes *et al*. 2001), supporting the important role of glutamine in regulating photosynthesis. Yet Rubisco transcript abundance shows no direct response to glutamine (Stitt *et al*. 1995, as cited in Paul & Foyer 2001). Thus the extent of and the mechanisms involved in the regulation of photosynthesis by glutamine remains largely to be elucidated.

Cytokinins are another promising candidate for an important role in signalling the N status for photosynthetic regulation, as cytokinin levels in the root respond strongly to N supply (Samuelson et al. 1992). Cytokinins are suggested to be a root-to-leaf signal involved in the expression of inorganic N-responsive genes (Sugiyama & Skakakibara 2002). In addition to the C4 PEPCase (see above), cytokinins have also been shown to increase the transcript levels of several other photosynthetic genes including Rubisco (Lerbs *et al.* 1984; Flores & Tobin 1989), carbonic

anhydrase (Sugiharto *et al.* 1992a) and light harvesting Chl a,b-binding protein (Flores & Tobin 1989). The exact role of cytokinins has often been reported to be a post-transcriptional increase in mRNA stability (Flores & Tobin 1989; Suzuki *et al.* 1994).

Sink regulation

Several of the N-responsive photosynthesis genes - e.g. *rbcS* and *Cab* in C3 (Krapp *et al.* 1993) and C4 plants (Sheen 1990), PEPCase and PPDK in C4 plants (Sheen 1990) - and also several other photosynthesis genes - e.g. thylakoid ATPase (Krapp *et al.* 1993), Plastocyanin (Dijkwel *et al*. 1996) – are repressed by sugars such as sucrose and glucose (see Koch 1996 and Pego *et al*. 2000 for reviews). From this evidence a concept of C-mediated feedback or sink-regulated inhibition of photosynthesis has been suggested (Krapp *et al*. 1993; Jang & Sheen 1994), stating that the so-called sink regulation, i.e. the control of photosynthesis in source tissue by carbohydrate demand in sink tissue, is mediated by the mechanism of sugar repression of photosynthesis genes. It has been proposed that the reduction of photosynthesis resulting from N deficiencies might also be mediated by carbohydrates, with higher carbohydrate levels associated with low N availability resulting in feedback inhibition of photosynthesis (Thorsteinsson *et al.* 1987; Arp 1991; Paul & Driscoll 1997).

Yet a simple hypothesis that sugars alone mediate sink regulation of photosynthesis is too simplistic. In fact there seems to be a strong interaction between N status and sugar-mediated repression of photosynthesis genes (Paul & Driscoll 1997; Stitt & Krapp 1999). In tobacco plants the decrease of Rubisco activity, transcript and protein levels under N-limitation was enhanced by addition of sucrose, while N-sufficient plants grown with additional sucrose maintained high Rubisco levels (Paul & Sitt 1993). These results indicate that N-limited plants are increasingly susceptible to sugar repression and that sugars only repress Calvin cycle enzymes in specific circumstances (Stitt & Krapp 1999). Thus it seems that sugar repression of photosynthesis is controlled by the whole plant source-sink balance, which in turn depends more crucially on the C:N balance rather than on the carbohydrate status alone (Paul & Driscoll 1997; Paul & Foyer 2001).

Sink regulation of photosynthesis can override the direct short-term controls of photosynthesis by light and CO_2 (Paul & Foyer 2001).

2.2.3.2 Organic acid metabolism

Organic acids are required for nitrate assimilation, especially 2-oxoglutarat which acts as the acceptor for ammonium in the GOGAT pathway (see 2.1.3.2). In fact nitrate leads to a marked increase in several transcripts encoding key proteins of organic acid metabolism, namely phosphoenolpyruvate carboxyylase (PEPCase), cytosolic pyruvate kinase (PK), citrat synthase (CS) and NADP-isocitrate dehydrogenase (NADP-ICDH) as well as to the accumulation of the organic acids malate, citrate, isocitrate 2-oxoglutarate (Scheible *et al.* 1997b; see Fig. 2.19).

The PEPCase – which has already been discussed in the context of photosynthesis in C4 plants (see 2.2.3.1) – is also important in C3 plants, where it replenishes the TCA cycle intermediates consumed in amino acid biosynthesis (Schuller *et al.* 1990; see Fig. 2.19). PEPCase is considered to be an important cross point between the C and N metabolism by delivering oxalacetate to the citric cycle or to aspartate synthesis (Miller & Cramer 2004).

Like the C4 PEPCase also the PEPCase involved in the anapleurotic pathway is upregulated by nitrate (Scheible *et al.* 1997b). Yet unlike the C4 PEPCase the expression of the anapleurotic PEPCase gene is directly enhanced by nitrate (Scheible *et al.* 1997b; Wang *et al.* 2000; Wang *et al.* 2003). Nitrate also enhances expression of the PEPCase kinase, which controls the phosphorylation

state and thus the activity of PEPCase enzyme (Wang *et al*. 2003). Yet as the C4 PEPCase, also the anapleurotic PEPCase is controlled by glutamine. Glutamine increases PEPCase activity through induction of gene expression and changes in the phosphorylation state of the protein (Manh *et al*. 1994; Murchie *et al*. 2000).

This induction of enzymes and proteins favouring increased C flux through the anapleurotic pathway under high N status of the plant thus enables the provision of C skeletons for N assimilation instead of usage of C for carbohydrate synthesis.

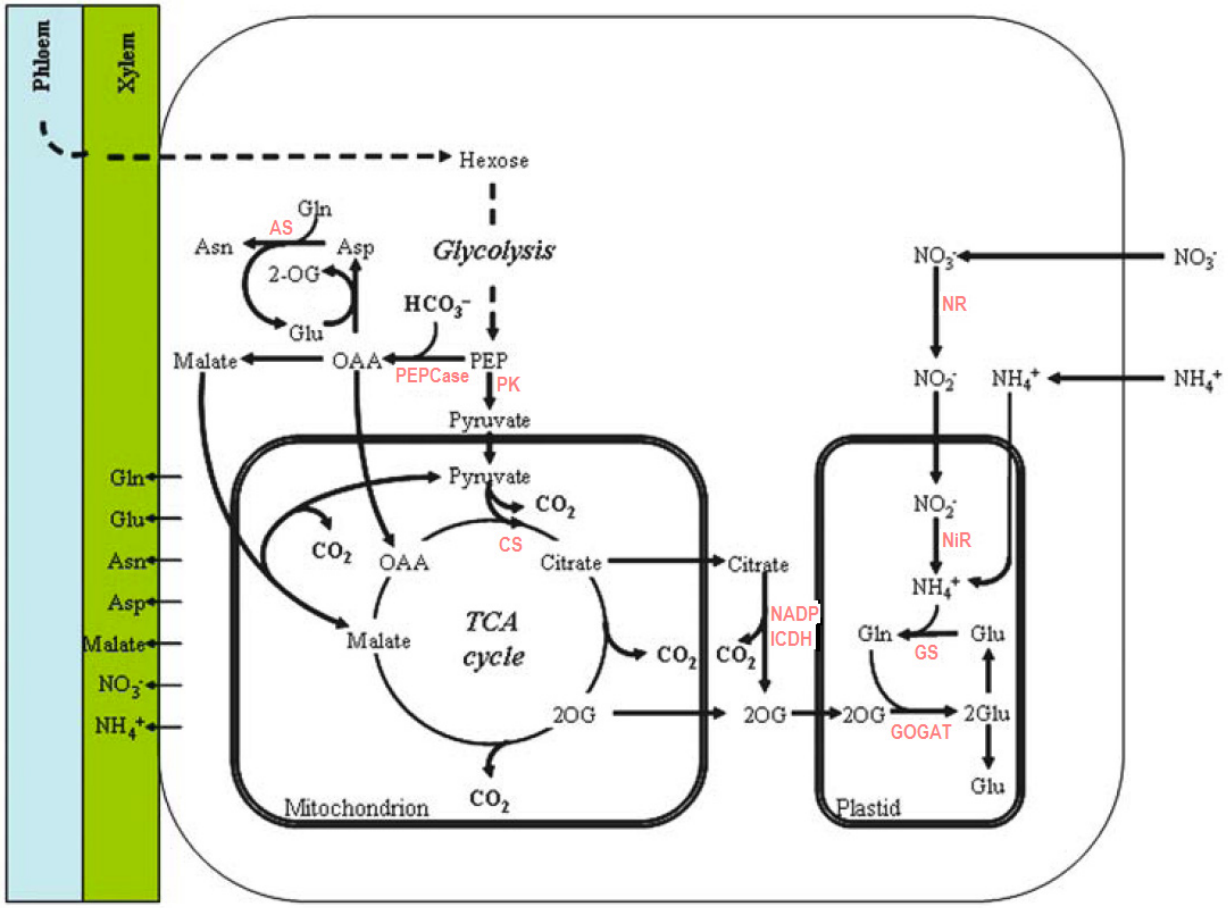


Figure 2.19: Simplified scheme of pathways of primary N and C metabolism in roots linked by shared intermediates. The C available in the root is mainly delivered from the shoot in the phloem and mainly in the form of carbohydrates (mostly scurose). Sucrose is then metabolised in glycolysis to yield reductant. The C products of glycolysis (malate and pyruvate) are then available to the mitochondria. C enters the TCA cycle through pyruvate and through oxalacetate (OAA) or malate, which may be derived from carboxylation of phosphoenolpyruvate (PEP) by the enzyme PEPCase. OAA may also be transaminated to yield aspartate (Asp) and asparagine (Asn). The TCA cycle provides citrate and 2-oxoglutarate (2OG) for synthesis of glutamate (Glu) and glutamine (Gln). Key enzymes of the two pathways that are regulated directly by nitrate are depicted in pink: asparagine synthase (AS), phosphoenolpyruvate carboxylase (PEPCase), pyruvate kinase (PK), citrate synthase (CS), NADP-dependent isocitrate dehydrogenase (NADP-ICDH), glutamine:oxoglutarate aminotransferase (GOGAT), glutamine synthetase (GS), nitrite reductase (NiR) and nitrate reductase (NR). From Miller & Cramer (2004).

2.2.3.3 Starch synthesis

Nitrate limitation typically leads to a large increase in the starch content of plants (e.g. Lawlor *et al.* 1987c; Stitt & Schulze 1994; Robinson 1996; Nakano *et al.* 1997; Logan *et al*. 1999). Matching this observation, the gene *AGPS*, encoding the regulatory subunit of the ADP-glucose pyrophosphorylase (AGPase), a key enzyme in the starch synthesis pathway, is repressed by nitrate (Scheible *et al.* 1997b; see Fig. 2.20). The transcriptional regulation of starch synthesis in direct response to nitrate permits coordinate changes in carbohydrate allocation and N assimilation and utilization, without this requiring large changes in the sugar pools (Stitt & Krapp 1999).

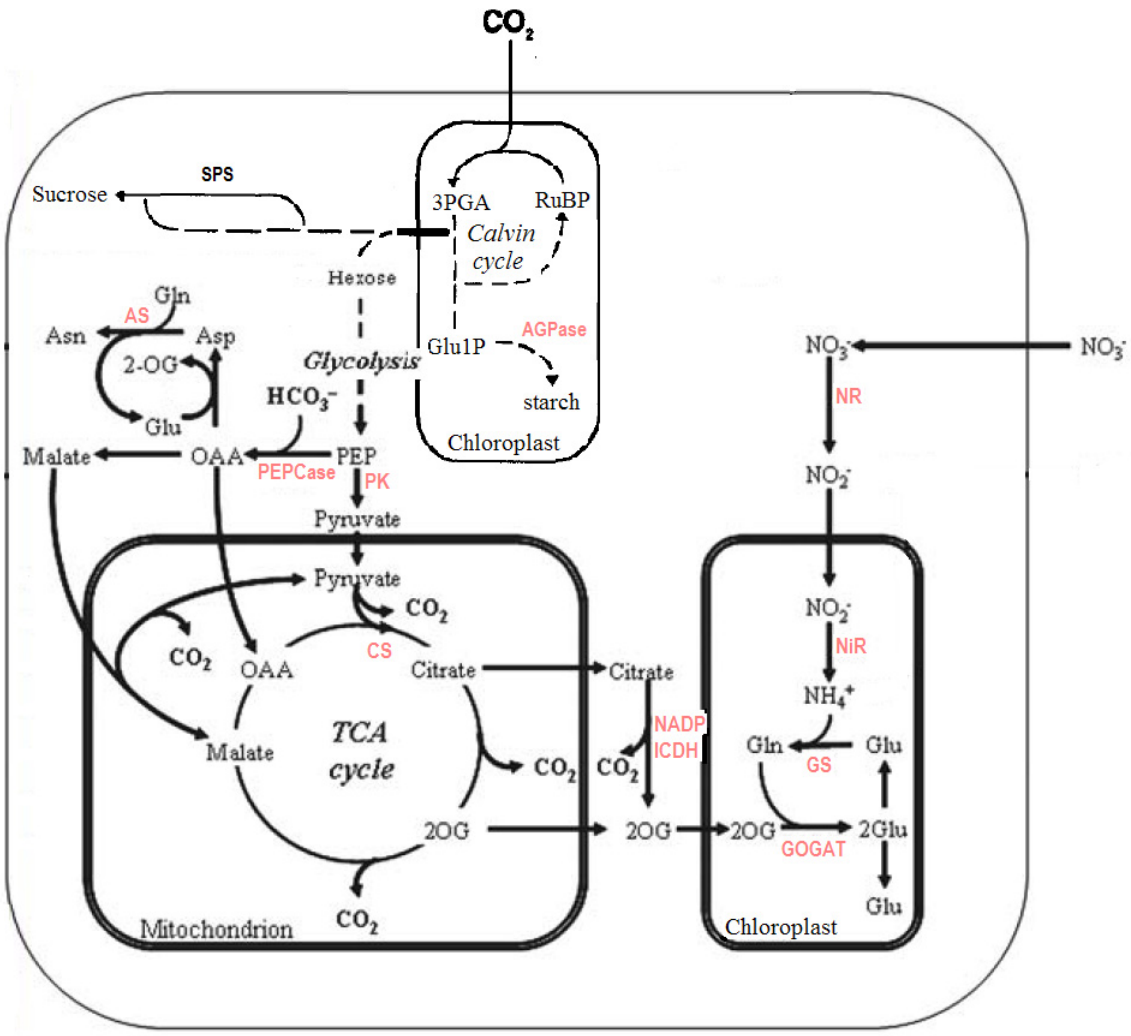


Figure 2.20: Simplified scheme of pathways of primary N and C metabolism in leaves linked by shared intermediates. The C in leaves is assimilated through carboxylation of Ribulose-1,5-biphosphate (RuBP) to form two molecules of glycerate-3-phosphate (3PGA). A triosephosphate leaves the Calvin cycle and is further transformed to be used in central metabolic pathways, e.g. in the synthesis of starch through the intermediate glucose 1-phosphate (Glu1P), in the synthesis of sucrose catalyzed among others by the enzyme sucrose phosphate synthase (SPS) and in the synthesis of other carbohydrates. C enters organic acid metabolism through conversion of glucose to phosphoenolpyruvate (PEP) via glycolysis. Processes of the TCA cycle and of N assimilation are as those described for roots (see Fig. ..). Key enzymes of the two pathways that are regulated directly by nitrate are depicted in pink: ADP-glucose pyrophosphorylase (AGPase), asparagine synthase (AS), phosphoenolpyruvate carboxylase (PEPCase), pyruvate kinase (PK), citrate synthase (CS), NADP-dependent isocitrate dehydrogenase (NADP-ICDH), glutamine:oxoglutarate aminotransferase (GOGAT), glutamine synthetase (GS), nitrite reductase (NiR) and nitrate reductase (NR). Adapted from Scheible *et al*. (1997b) and Miller & Cramer (2004).

Interestingly the enzyme sucrose phosphate synthase (SPS) is not repressed by nitrate, instead gene transcription and SPS activity are unaltered or even increased after nitrate addition (Scheible *et al.* 1997b), indicating that sucrose production continues under high nitrate. It is thus not surprising that sucrose contents often behave in an opposite way to starch contents, with no change (Logan *et al.* 1999) or a decrease (Pearman *et al.* 1978; Thorsteinsson *et al.* 1987; Nakano *et al*. 1997) in sucrose contents under reduced N availability, even when other, monosaccharidous sugars increase (Thorsteinsson *et al.* 1987; Logan *et al*. 1999).

Yet sugars themselves also regulate starch synthesis, through enhancement of transcription of *AGPS* (Koch 1996), a post-translational redox modification of AGPase (Tiessen *et al.* 2002) and an allosteric activation of AGPase (Sowokinos 1981) at high sugar levels. The accumulation of starch under nitrate-deficiency, despite the often lower sucrose levels under these conditions, thus suggests that in plants the direct effects of N on starch synthesis may be so powerful that they can replace or even override the regulation by sugars (Stitt & Krapp 1999).

2.2.3.4 Respiration

N uptake and assimilation is dependent on ATP, NAD(P)H, C skeletons and reduced ferredoxin, all of which derive in non-photosynthetical tissue from respiration and the breakdown of carbohydrates.

The regulation of the TCA cycle by N signals has already been discussed in the section 2.2.3.2 “organic acid metabolism”. Nitrate in roots leads to the induction of several more genes relevant in respiratory metabolism. The oxidative pentose phosphate pathway produces NADPH, which is needed to support generation of reduced ferredoxin in roots, by converting glucose-6-phosphate into ribulose-5-phosphate. Two key genes of this pathway are induced as a primary response to nitrate, namely the genes encoding glucose-6-phosphate dehydrogenase (G6PDH) and 6-phosphogluconate dehydrogenase (6PGDH) (Wang *et al.* 2000). Ferredoxin itself and ferredoxin:NADP oxidoreductase are also directly enhanced by nitrate in roots (Stitt 1999).

The expression of several enzymes related to glycolysis is also induced by nitrate, including the genes encoding the glycolytic enzymes PGM and glucose-6-phosphate isomerase (G6PI) as well as several genes involved in the synthesis of trehalose-6-P (Wang *et al.* 2003).

The rate of respiration is not only regulated by environmental factors, such as temperature, and by energy requirements (e.g. ATP for N uptake), but also by the demand for reducing equivalents and intermediates of carbohydrate decomposition (Marschner 1995). The variable demand for C skeletons, NADH and ATP can in part be met by the “alternative pathway”. This pathway is an alternative to the oxygenation of NADH in the phosphorylating electron transport chain over the cytochromes; instead electrons are transferred directly from a flavoprotein to oxygen. As a consequence less ATP is synthesized per molecule NADH oxidized. This “alternative pathway” is thus less efficient than the cytochrome pathway, but it is important if plants for example have a higher demand for C sekeletons than for reducing equivalents and ATP (Marschner 1995). In roots supplied with ammonium for example the proportion of the “alternative pathway” is very high, while in roots supplied with nitrate it is negligible (Barneix *et al*. 1984b). This result is in line with the higher demand of nitrate assimilation for energy and reducing equivalents and the in contrast higher demand of ammonium assimilation for C sekeletons (see 2.1.3).

2.2.3.5 Conclusion

Photosynthetic genes seem not to be regulated directly by nitrate. Glutamine instead seems to be a direct regulator of PEPCase gene products in maize and it could be potentially involved also in the regulation of other photosynthetic enzymes. The supply of photosynthates and phytohormones, especially cytokinins, interact with N supply to control the expression of photosynthesis genes (Fig. 2.21). Plants are more susceptible to sugar repression of photosynthesis under N-limiting conditions, suggesting that sink regulation of photosynthesis is controlled through signal transduction pathways coordinated by the plant C:N balance.

Several enzymes involved in respiratory processes are directly nitrate regulated. This enables the plant cell to provide energy, reducing equivalents and C skeletons for the uptake and assimilation of N.

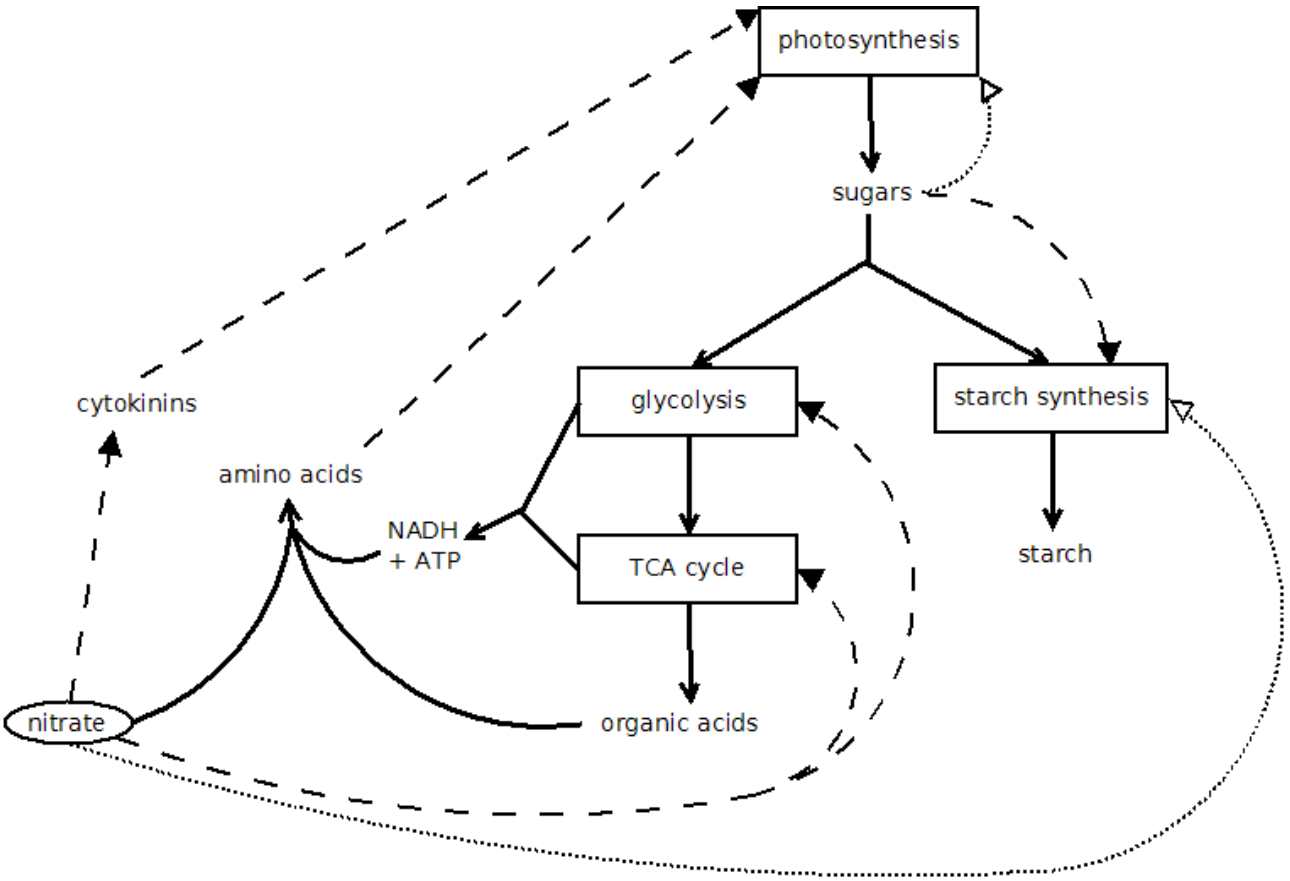


Figure 2.21: A model summarizing the regulation of processes of the primary C metabolism by signals derived from N. Solid lines denote the movement of molecules or electrons. Dotted lines (····) with white arrow indicate a negative feedback (i.e. downregulation), broken lines (- - -) with black arrows indicate a positive feedback (i.e. upregulation).

In the presence of nitrate, carbohydrate synthesis is decreased and more C enters the organic acid metabolism. Products of the organic acid metabolism are necessitated as C skeletons for N assimilation and the synthesis of amino acids. This redirection is achieved through upregulation of several key enzymes involved in organic acid metabolism and a simultaneous decrease of starch

biosynthesis. This mechanism ensures C flux to amino acid synthesis in the presence of nitrate.

The production of NAD(P)H, ATP and C skeletons in respiration is tightly regulated according to the demand of plant cells for these respiratory products. Under ammonium nutrition for example more C skeletons are needed than NADH or ATP and more NADH is thus oxidized over the "alternative pathway".

Figure 2.21 shows a model of the combined regulation of different C processes by N and C signals.

2.2.4 C allocation

One of the most commonly observed responses under limited N availability is a diversion of resource allocation from shoot growth to root growth (Ericsson 1995). Investment in plant parts that acquire the limiting resource is favoured, at the expense of allocation to plant parts that have a high requirement for the limiting resource (Lambers *et al.* 2008). Nitrate availability directly triggers this response, affecting both root and shoot morphogenesis (Krapp *et al*. 2002; van der Werf & Nagel 1996). On the other hand changing the availability of ammonium does not trigger similar phenotypic responses (Krapp *et al.* 2002). Nitrate is thus required for plants to adapt their morphology in order to better exploit low N resources under limited N availability (Krapp *et al.* 2002). Experiments with NR-deficient tobacco (*Nicotiana tabacum*) mutants further suggest that nitrate plays a direct signalling role in the partitioning of biomass between roots and leaves (Scheible *et al.* 1997a).

2.2.4.1 Root growth

The size and architecture of the root system is an important variable for plants to adapt to a varying N supply. Thus it is not surprising that root growth and development are regulated by N availability.

While high nitrate concentrations elicit a systematic repression of lateral root growth (Scheible *et al.* 1997a; Zhang *et al.* 1999), localized sources of nitrate enhance lateral root growth, so that the lateral roots colonize the nutrient-rich patch (Robinson 1994; Scheible *et al.* 1997a; Zhang & Forde 1998; Zhang *et al.* 1999; Forde 2002). In barley (*Hordeum vulgare*) this lateral root growth was also induced by ammonium and inorganic phosphate (Drew 1975), while in *Arabidopsis* it was not stimulated by other N sources (Zhang *et al.* 1999). Localized nitrate increases the rate of lateral root elongation, based on an increase in the cell production in the lateral root meristem (Drew 1975; Zhang *et al*. 1999), and in barley it also increases the rate of lateral initiation (Drew 1975). The signal for this increased lateral root growth comes from nitrate itself and not from a downstream N metabolite (Scheible *et al.* 1997a; Zhang & Forde 1998; Zhang *et al.* 1999).

Two genes have been identified that probably are involved in a signal transduction pathway stimulating the lateral root growth under localized nitrate. The first, ANR1, is induced by nitrate and encodes for a nitrate-specific transcription factor (Zhang & Forde 1998; Forde 2002). When the expression of ANR1 is repressed, roots no longer respond to a localized nitrate supply, implicating a central role of the gene product of ANR1 in this mechanism (Zhang & Forde 1998). The second, AXR4, is a gene that was first identified as an auxin-sensitivity gene with an important role in root gravitropism (Forde 2002). Mutants deficient in AXR4 failed to respond to a localized nitrate treatment (Zhang *et al.* 1999), suggesting involvement of the phytohormone auxin in the nitrate-stimulated lateral-root expansion. However, as the exact role of the AXR4 gene product is

unknown, it is unclear how the nitrate and auxin response pathways might interact (Zhang & Forde 2000).

The inhibitory effect of nitrate on lateral root growth is distinct in a number of ways to its stimulating effect: (i) It is systematic rather than localized to the zone exposed to the nitrate treatment; (ii) the extent of the inhibitory effect is related not only to the size of the external nitrate supply, but also to the extent of the rooting system exposed to the nitrate supply, indicating that it depends on the total amount of nitrate absorbed by the root system rather than the external nitrate concentration *per se*; (iii) while the stimulating effect acts specifically on the elongation of mature lateral roots (see above), the inhibitory effect acts only on immature lateral roots during a discrete phase just after their emergence from the primary root (Zhang & Forde 2000). The initiation of lateral roots is not inhibited by high nitrate, but the development is suppressed at a stage just after emergence through the epidermis, probably just before or during the process of activation of the lateral root meristem (Zhang *et al.* 1999). The inhibition of root growth by high nitrate concentrations seems to be mediated by nitrate accumulation in the shoot (Scheible *et al.* 1997a). Auxine, as an important plant growth regulator likely playing a key role in shoot-to-root communication, could act as a long-range signal, mediating the nitrate signal of the shoot to the root and thus regulating root branching. Yet so far the evidence that this occurs is still largely absent (Forde 2002). Absisic acid has also been suggested as a signal regulating root growth in relation to N status (Signora *et al.* 2001). While changes in C allocation could not account for the nitrate effect on root branching in tobacco (Scheible *et al.* 1997b), there is evidence that in *Arabidopsis* (Crookshanks *et al.* 1998) and wheat (Bingham *et al.* 1998) increased lateral root densities are correlated with increased supplies of carbohydrates to the roots. Yet whether this correlation was due to a C limitation of the root or whether the carbohydrates were acting as signals to regulate lateral root initiation is not clear (Forde 2002). It has been suggested that long-distance signalling by sugars controls the biomass partitioning between roots and shoots in relation to nutrient supply (Farrar 1996; van der Werf & Nagel 1996). Farrar (1996) proposes that under low N availability leaf growth is reduced (see below), so that products of photosynthesis accumulate. From the increased level of carbohydrates in the leaves follows that more photosynthates are available for translocation to the roots. In the roots then the carbohydrates may act as signals and probably regulate root branching (Forde 2002).

The two opposing effects of nitrate provide a regulatory system that enables root branching to respond to both the plant N status and the local availability of nitrate (Fig. 2.22). In this way the intensity of the response to a localized nitrate source (i.e. the foraging response) can be adjusted according to the plants N demand, so that resource allocation within the plant as a whole can be optimized (Zhang & Forde 2000). Both effects described are specific to lateral roots, while the growth of the primary root is largely insensitive to nitrate supply (Forde 2002). Figure 2.22 summarizes the different components of nitrate regulation of lateral root growth.

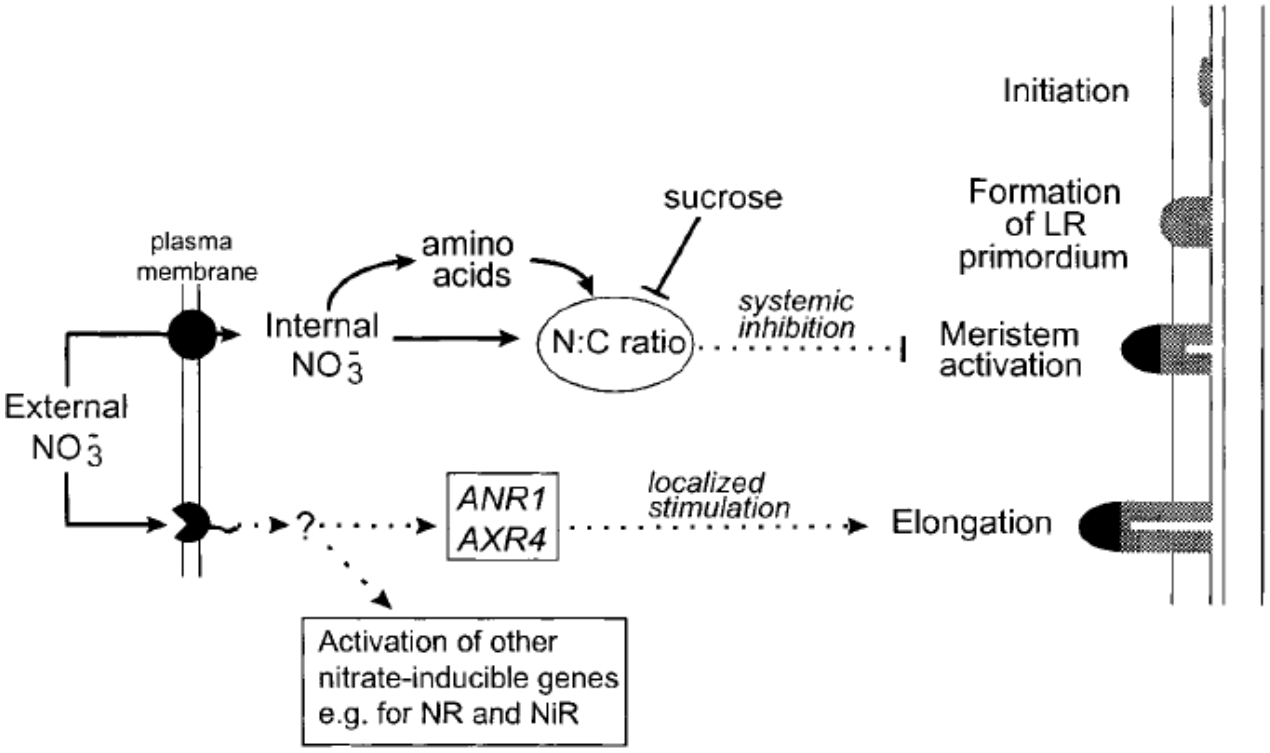


Figure 2.22: A model of the dual-pathway for regulation of lateral root growth and development by nitrate in *Arabidopsis*. Dotted lines (····) indicate signalling steps, solid arrows indicate transport or metabolic steps. The localized stimulatory effect depends on the external nitrate concentration and acts on the mature lateral root tip to increase meristematic activity. As the ANR1 gene is rapidly induced by nitrate, the putative nitrate sensor and the mechanism for transcriptional activation of ANR1 are likely to be shared with other nitrate-inducible genes, e.g. those encoding NR and NiR. The systematic inhibitory effect depends on the internal N status of the plant and acts on a critical stage of lateral root development prior to activation of the lateral root meristem. From Zhang & Forde (2000).

N allocation priority under limited N availability is similar to that of biomass allocation. Vessey & Layzell (1987) showed that only N in excess of the requirements of the root was exported to the shoot under N deficiency.

2.2.4.2 Leaf growth

McDonald *et al.* (1986) have shown that growth and N accumulation in leaves of birch (*Betula pendula*) ceased immediately after a step-decrease in N supply, while stems and especially roots continued to grow and to take up N. Decreased leaf expansion rates at a low N supply have been observed in many experiments (e.g. Bouma 1970; Chapin *et al.* 1988b; Gastal *et al.* 1992). Such growth responses can be very rapid, suggesting an efficient mechanism for root-to-shoot signalling (Forde 2002). The reduction of leaf expansion under deprivation of nitrate has been shown to be both due to a decrease in cell division and cell size (Roggatz *et al.* 1999; Walch-Liu *et al.* 2000). Several lines of evidence suggest that cytokinins – which are important regulators of cell division and growth and which are considered to be synthesized in roots – could provide the long-range signals that regulate leaf morphogenesis in accordance with changes in the nitrate supply (Kuiper *et al*. 1989; Beck 1996; van der Werf & Nagel 1996; Walch-Liu *et al.* 2000; Forde 2002). In addition of promoting leaf cell division and leaf cell expansion and thus enhancing leaf expansion, cytokinins also increase the photosynthetic capacity and delay leaf senescence (Lambers *et al.* 2008;

see 2.2.3.1). Yet the mechanisms of this regulation and the identity of the key regulatory steps are still largely unknown (Forde 2002). In the shoot however a possible signal transduction pathway for the perception and response to the cytokinin signal has emerged. In leaves of maize (Sakakibara *et al*. 1998) and *Arabidopsis* (D'Agostino *et al*. 2000) a group of type-A response regulator genes have been identified that are induced in the primary response to cytokinins. Their expression can be induced in leaves either by direct application of cytokinins or by resupplying nitrate to N-starved plants (Taniguchi *et al.* 1998). Yet that these response regulators have any role in regulating leaf expansion has not yet been demonstrated, but potentially they could be a component of a nitrate cytokinin signalling pathway (see Fig. 2.23, Forde 2002).

In addition to phytohormones also a decrease in root hydraulic activity, leading to a decrease in water availability in expanding leaves, might be involved in the decreased leaf expansion under N limitation (Radin & Boyer 1982). Yet in tomato (*Lycopersicon esculentum*), changes in water transport to the shoot could not explain reduced leaf elongation rate, as leaf water content and water potential were unaffected by N at the time that leaf elongation began to decline (Chapin *et al.* 1988b).

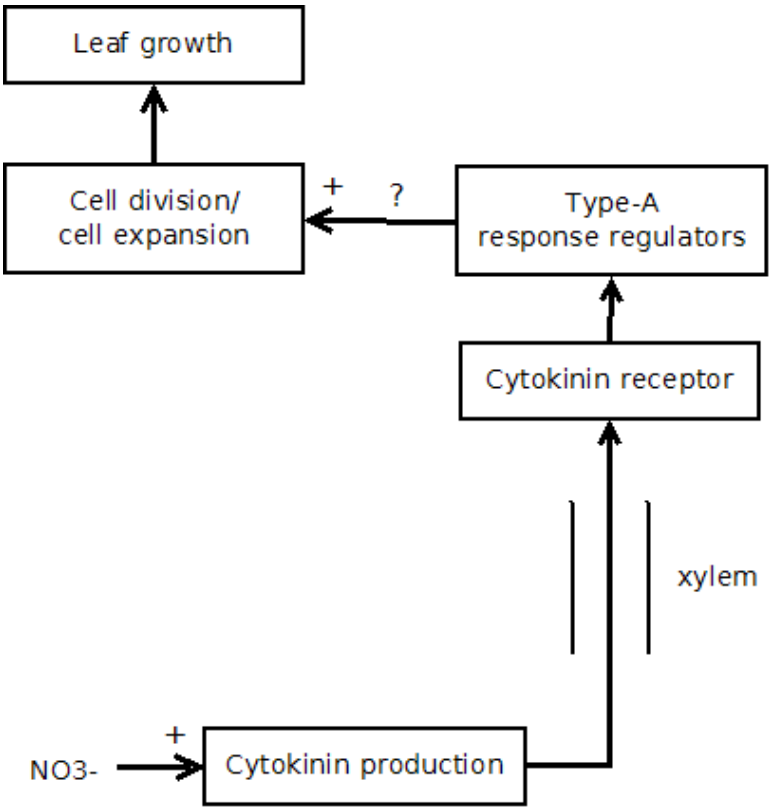


Figure 2.23: A model for a long-range signaling pathway for NO_3^- regulation of leaf expansion. See text for explanations. Redrawn from Forde (2002).

In addition to the decrease in leaf area, a reduced N availability also has effects on the anatomy of leaves. N shortage invariably enhances the proportion of leaf tissue occupied by sclerenchymatic cells, predominantly due to an increase in the number of these cells. The area occupied by veinal tissue doubles, whereas that occupied by epidermal cells remains more or less constant, despite a substantial decrease in the size of the epidermal cells (van Arendonk *et al.* 1997). Under low soil N

availability plants adapt by producing leaves that are thicker, have a higher leaf mass density, a lower SLA and a longer leaf life span (Reich *et al*. 1997). The decrease in SLA observed in many plant species under limited N (e.g. Waring *et al.* 1985; Sage & Pearcy 1987b) is at least partly due to accumulation of non-structural carbohydrates or of secondary compounds like lignin or other phenolics (Lambers & Poorter 1992). It is not known how these changes are mediated (Lambers *et al.* 2008). The function of the anatomical changes seems to be a better protection of leaves from herbivores and desiccation (Lambers & Poorter 1992).

From the results described so far, Lambers *et al.* (2008) (based on information in van der Werf & Nagel 1996) have developed an integrated physiological model, involving signals from cytokinins and sucrose, trying to explain the patterns of root and shoot allocation observed under N limitation: Roots supplied with high N (Fig. 2.24, left) thereafter produce large amounts of cytokinins, which are exported via the xylem to the leaves. Here these plant hormones enhance photosynthetic capacity and leaf expansion. This consumption of carbohydrates for leaf growth reduces the fraction of photosynthates that is translocated to the roots, resulting in a low root growth rate. Instead roots supplied with low N (Fig. 2.24, right) produce only small amounts of cytokinins. The leaves thus sense small cytokinin concentrations, so that their photosynthetic capacity and the leaf expansion rate are reduced. As only a small fraction of the photosynthates from the leaves are consumed in leaf growth, a large proportion is translocated to the roots. The high level of sugars in the leaves suppresses genes encoding photosynthetic enzymes (see 2.2.1), while the high level of sugars in the roots enhances root growth.

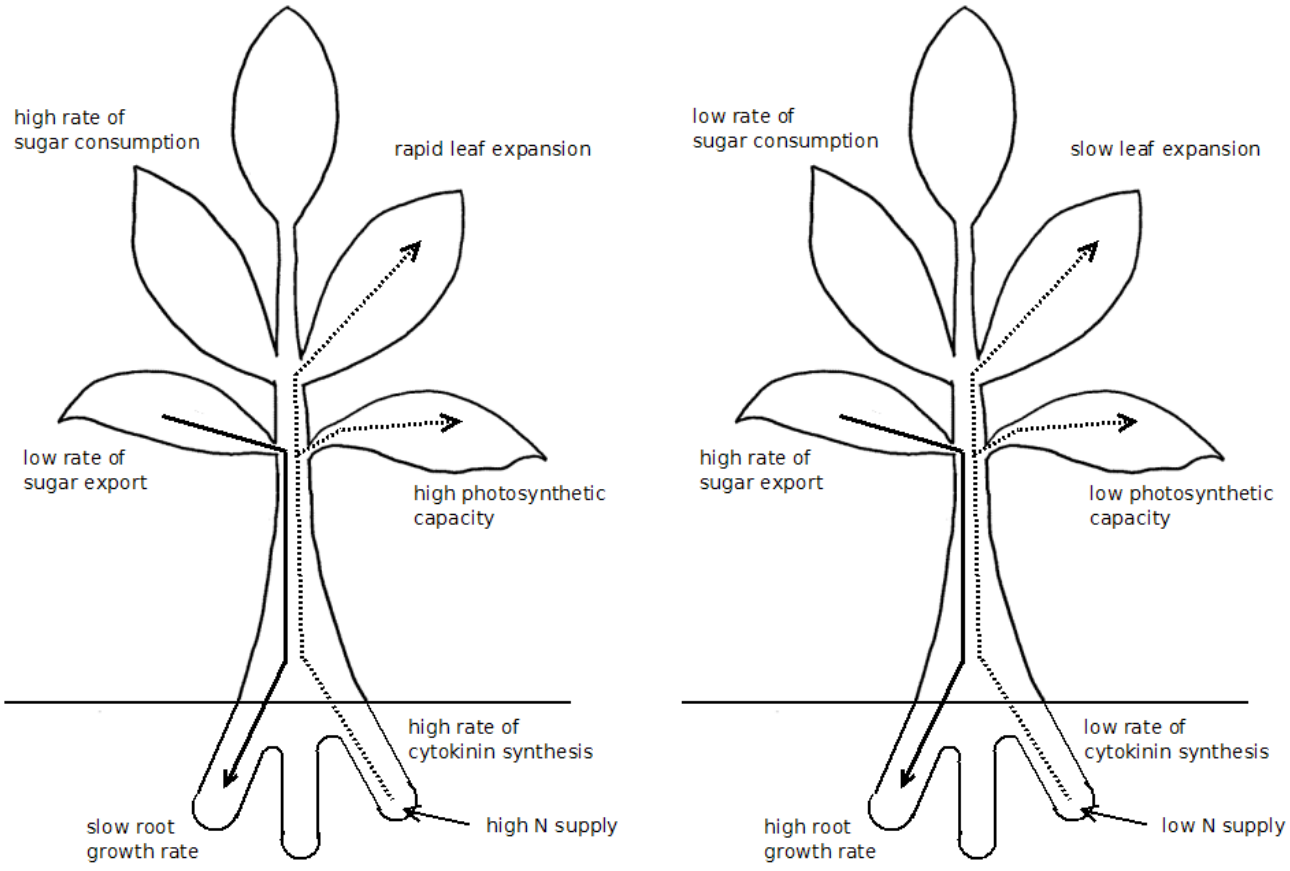


Figure 2.24: A hypothetical model to account for the effects of N supply on plant growth and biomass allocation between roots and shoot. The broken line (- - -) represents cytokinin export from root, the solid line represents sugar export from leaves. See text for explanations. Redrawn from Lambers *et al.* (2008).

2.2.4.3 Conclusion

Shoot and root growth is controlled by N availability and by signals derived from N supply. While high nitrate triggers a systematic repression of lateral root growth, probably mediated by the whole plant N:C balance (possibly through amino acids, sugars and/or phytohormones, especially auxine), localized nitrate resources trigger an increase in lateral root elongation as a direct response to nitrate, probably involving an auxine-related signal tranduction pathway.

Leaf expansion instead is reduced under low N supply. Cytokinins are assumed to play an important role in the regulation of leaf expansion by the N supply. A hypothetical model has been proposed, involving a signal from cytokinin at high N supply, stimulating leaf growth, while under low N supply a sugar-mediated feedback regulation, resulting from low leaf expansion and accumulation of carbohydrates that cannot be used for growth, stimulates root growth.

2.3 Synthesis of information on N and C metabolism

The N metabolism is regulated by the plant according to the internal C and N status as well as according to external environmental factors. In addition, processes of the C metabolism exert a control on N metabolism (see Fig. 2.25, red arrows) through the provision of energy, reducing equivalents and C skeletons. Summarizing, the different processes in N metabolism are regulated and controlled as follows:

I. **N uptake** is regulated according to the external N supply as well as the internal C:N status, mediated by concentrations of nitrate (or ammonium respectively), amino acids and carbohydrates. Environmental factors (like temperature and water status) do not regulate N uptake directly but control it by influencing the growth of the plant and thus the C:N status of the plant, as well as by influencing the soil N supply. N uptake is an active process and therefore dependent on energy from respiration.

II. **N fixation** is – as N uptake – regulated according to the internal C:N status, mediated by the concentrations of nitrate, amino acids and carbohydrates. Yet unlike N uptake, temperature and water status control N fixation directly. N fixation is dependent on the provision of reducing equivalents and energy from respiration.

III. **N assimilation** is regulated by the C:N status of the plant, which here is mediated by the concentrations of amino acids and carbohydrates. Nitrate instead does not signal the N status, but as a substrate of N assimilation it signals the potential for this process. N assimilation is dependent on the provision of energy, reducing equivalents and C skeletons from respiration.

IV. **N allocation** is a much more diffuse process, compared to N uptake, N fixation and N assimilation. N allocation is regulated through the processes of storage and remobilization, protein synthesis and protein degradation. Storage and remobilization are controlled by the C:N status of the plant as well as by developmental needs. The synthesis and degradation of proteins for different processes is controlled by the demand for the respective processes. In shaded leaves for example the demand for photosynthetic proteins decreases, as photosynthesis is limited by light availability, so that photosynthetic proteins are degraded and their N translocated to leaves where photosynthetic proteins are needed because of higher irradiance.

The N content of the whole plant declines with increasing age, as proportionally more C than N is taken up with increasing age, due to (i) an increase in N-poor structural tissue, and (ii) a degradation and remobilization of previously used N from old tissue for new growth. Thus the often limiting resource N undergoes strong recycling in the plant, while the resource C, which is not as limiting as N, is hardly recycled.

As Evans (1975), noted: “Neither the rate nor the extent of production need bear a close relation to photosynthetic rate, or be determined by it (…). The processes that follow photosynthesis, such as respiration and translocation, or other limitations on the capacity of plants to grow and utilize photosynthates can be major determinants of productivity”. Generally it seems that photosynthesis is not a factor limiting growth under suboptimal N supply, but instead the utilization of photosynthates for growth. Yet this is only a hypothesis emerging from the review of the literature, but not a generally shared paradigm.

N influences primary C metabolism through direct regulation (Fig. 2.25, broken black lines) as well as through a control through its nutritional role, as a major component of proteins (Fig. 2.25, dark blue arrows). The following regulations and controls can be summarized:

- N is a major component of the **photosynthetic apparatus**. Under current conditions

photosynthesis seems to be co-limited by CO_2 diffusion and the amount and activities of nitrogenous compounds, namely Rubisco and thylakoid protein. Because of the large proportion of leaf N involved in photosynthesis, the photosynthetic rate is correlated with leaf N content. Photosynthetic partitioning is coordinated to maintain a balance between light and dark reactions and the proportion of the different photosynthetic components seems to get adapted to environmental conditions.

- N supply controls **leaf expansion** through root-to-shoot signals by phytohormones. At a low N supply the utilization of photosynthates in leaf growth is inhibited through a limited availability of N compounds that can be utilized for growth, as well as through a downregulation of growth by N-derived phytohormonal signals. As a secondary response the accumulation of photosynthates as well as the phytohormonal signals downregulate photosynthesis.
- N supply controls **root growth** through shoot-to-root signals - likely by phytohormones, probably also involving signals (or at least some kind of control) by sugars - that mediate the C:N status of the shoot to the root. At a low N supply photosynthates accumulate, as they cannot be utilized in leaf growth, they are translocated to the roots, where they enhance lateral root growth.
- A localized N supply increases **lateral root elongation** in the N rich patch through a signalling pathway involving the phytohormone auxine.
- Some processes involved in **respiration** are regulated directly by N, as the processes of N uptake, N assimilation and N fixation require energy, reducing equivalents and C skeletons. N for example redirects C flow from the synthesis of starch to the synthesis of organic acids – thus providing C skeletons for N assimilation. Yet also respiratory processes that are not regulated directly by N are correlated with tissue N concentrations, as tissue N concentrations reflect the metabolic activity of tissues, which again control the rate of both growth and maintenance respiration.

Cytokinins seem to play a major role in the coordination of C and N metabolism. They not only respond to root N supply and thus constitute a signal mediating the response of growth and photosynthesis to N availability (see Fig. 2.24) but they probably also mediate – through the dependence of their inflow on transpiration rate – the increase in photosynthetic proteins in leaves high up in the canopy with higher light availability and thus the differential allocation of N within the canopy (see Fig. 2.9).

The points mentioned so far are more or less accepted, yet some questions remain much more controversial. From the review of the literature conducted so far therefore several questions emerge:

I. Do nitrate and ammonium affect crop growth differently?

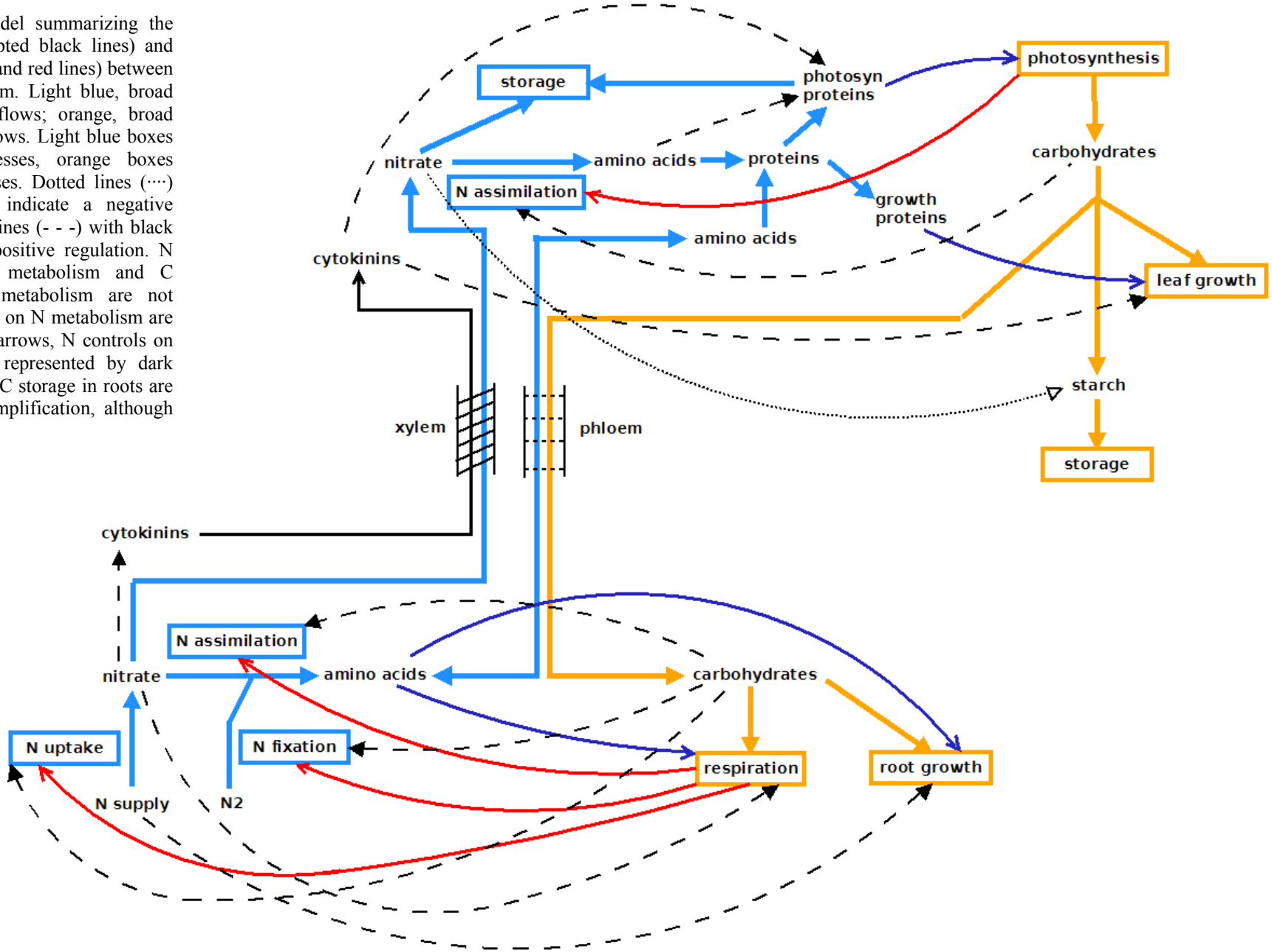
II. Does the leaf N content change significantly with a varying N supply or is it maintained more or less constant and changes mainly according to growth irradiance?

III. Does the photosynthetic rate on a leaf area basis decline under suboptimal N supply or can the reduction in growth be explained mainly by a decrease in leaf area expansion?

IV. Does the Rubisco content decline more strongly than the Chl content under decreased N availability and does thus the composition of the photosynthetic apparatus change with N supply?

V. Do irradiance and CO_2 supply affect the composition of the photosynthetic apparatus and does the plant optimize photosynthetic N partitioning according to light and CO_2 availability?

Figure 2.25: A model summarizing the regulations (interrupted black lines) and controls (dark blue and red lines) between C and N metabolism. Light blue, broad lines represent N flows; orange, broad lines represent C flows. Light blue boxes represent N processes, orange boxes represent C processes. Dotted lines (····) with white arrow indicate a negative regulation, broken lines (- - -) with black arrows indicate a positive regulation. N regulations on N metabolism and C regulations in C metabolism are not depicted. C controls on N metabolism are represented by red arrows, N controls on C metabolism are represented by dark blue arrows. N and C storage in roots are not depicted for simplification, although they do occur.



Chapter 3
Crop responses to N limitation: a meta-analysis

Under suboptimal nutrient conditions, a plant has two possibilities: it can increase the allocation of existing resources to the capture of the resource in short supply and/or it can increase the efficiency of the use of the resource in short supply. Under N limitation the first strategy would involve an increase in the N uptake capacity through an increase e.g. in root biomass. The second strategy on the other hand would involve an increase in nitrogen use efficiency (NUE), e.g. through the maintenance of an optimal leaf N content, through the depletion of N stores or through remobilization of N from old leaves to younger, more productive leaves. The mechanisms of different responses of plants to N supply have been discussed in the preceding section. However, several uncertainties emerged from the review of the literature as to the direction, magnitude and proportion of several N-mediated and N-dependent plant processes. For example, it appeared that the main effect of the N supply on growth is mediated by its influence on light absorption through its impact on the development of the canopy and not through its impact on energy conversion, i.e. photosynthetic rate. However, different studies and different experiments yielded varying results as to the proportion of the response of leaf area, leaf N content and photosynthetic rate to N limitation. Thus, no consistent picture about the relative importance of different strategies in the response of crops to N limitation emerged. In the following section, I therefore conduct a quantitative review in the form of a meta-analysis to examine the relative effect of N limitation on crop physiology and growth processes.

3.1. Meta-analysis as a tool for quantitative analysis of ecological effects

Meta-analysis is a statistical method for reviewing and synthesizing research findings across studies (Gurevitch & Hedges 1999). In recent years this quantitative data synthesis - which has been developed in other disciplines, especially the medical and sociological sciences - has been increasingly applied in ecological studies. In ecology it proved to be a useful tool as it allows to draw conclusions from a large body of experimental data and to reach generalizations concerning ecological questions. Meta-analysis has been extensively used to assess the effect of an elevated CO_2 concentration on plant growth (e.g. Curtis 1996; Cotrufo *et al.* 1998; Curtis & Wang 1998; Medlyn *et al.* 1999; Peterson *et al*. 1999; Wand *et al.* 1999; Ainsworth *et al.* 2002; Jablonski et al. 2002); it has also been applied to assess several other ecological effects, e.g. the effect of ozone depletion on plants (e.g. Searles *et al.* 2001; Newsham & Robinson 2009), the effect of warming on plants (e.g. Arft et al. 1999; Rustad *et al.* 2001), the effect of competition (e.g. Goldberg et al. 1999; Gurevitch *et al.* 2000; Gómez-Aparicio 2009) and herbivory (e.g. Hawkes & Sullivan 2001). However, the effect of N limitation on plants to my knowledge has not yet been analysed by the means of a meta-analysis. This might be due to several difficulties associated with an application of meta-analysis to N experiments, which will be further discussed below. Still meta-analysis promised to provide a very useful tool to draw conclusions from the – as was discussed in chapter 2– often contradicting and heterogeneous results in the experimental literature regarding the effect of N limitation on physiological processes like photosynthesis or N allocation.

3.1.1 A short introduction to meta-analysis

Meta-analysis generally aims at examining the relationship between an explanatory and a response variable, thus it analyses “the effect of X on Y” (DeCoster 2004). From each experiment the “effect size”, which represents an estimate of the magnitude of the response to the manipulation, is calculated and then the effect sizes from different experiments and studies are compared and further analysed (Gurevitch & Hedges 1999; Rosenberg *et al.* 2000). According to Osenberg *et al.* (1999) one can distinguish between three related but distinct goals of a meta-analysis: (i) the construction of an aggregated and more powerful test of a null hypothesis, (ii) the estimation of the magnitude of response (which might take the form of parameter estimation), and (iii) the subsequent examination of the relationship between these estimates and environmental and biological variables. The choice of a certain metric of effect size is a crucial step in conducting a meta-analysis and it strongly depends on the questions asked (Osenberg *et al.* 1999; Rosenberg *et al.* 2000). The Hedges' *d* index is such an effect size metric, that has often been used in ecological meta-analysis, yet it's validity for ecological questions has been disputed (Osenberg *et al.* 1999). If one primarily wants to quantify the magnitude of a response rather than test a null-hypothesis, other metrics should be used (Osenberg *et al.* 1999). Another important effect size metric is the natural logarithm of the response ratio (Hedges *et al*. 1999):

$$L = lnR = \ln\left(\frac{X_e}{X_c}\right) \quad (3.1)$$

where R is the response ratio, which is calculated as the ratio of the experimental mean (X_e) to the control mean (X_c). The use of the natural logarithm linearizes the metric and provides a more normal sampling distribution in small samples (Hedges *et al.* 1999). Unlike the Hedges' d index that estimates the standardized mean difference, the log response ratio estimates the effect as a proportionate change resulting from experimental manipulation (Rosenberg *et al.* 2000). An effect size that is significantly different from zero indicates an experimental effect. Values above zero indicate that the experiment has a positive effect on the response variable, while values below zero indicate a negative effect. The variance of L is calculated as

$$v_L = \frac{(SD_e)^2}{n_e \times {X_e}^2} + \frac{(SD_c)^2}{n_c \times {X_c}^2} \quad (3.2)$$

with SD_e and SD_c as the standard deviations and n_e and n_c as the sample sizes of the experimental and control group respectively (Hedges *et al.* 1999; Rosenberg *et al.* 2000).

A meta-analysis also requires the choice of an appropriate model and statistical test to calculate the total variances of the effect sizes, and to statistically summarize the effect sizes across studies (Gurevitch & Hedges 1999; Rosenberg *et al.* 2000). This choice depends on the statistical properties of the effect size metric. Usually, when summarizing results from independent studies some information about sample sizes and some sort of variance estimate, mainly the standard deviation, for each effect size value is required, as weighted means are used (Gurevitch & Hedges 1999; see equation 3.2). The use of weighted means allows giving greater weight to experiments whose results have greater precision and thus it increases the precision of the cumulative effect size. Typically, each effect size is weighted by the inverse of its variance. In mixed-effects models, this variance has two sources: (i) the within-study variance (i.e. experimental error, which is quantified by v_i for each experiment i; see equation 3.2) and (ii) the between-study variance (i.e. variance among studies in their true effect sizes), which can be derived from the Q statistic (Gurevitch & Hedges 1999; Hedges *et al.* 1999). The cumulative effect size, calculated as the weighted mean of the k individual effect sizes i, represents the overall magnitude of the effect present in the studies that were included in the analysis (Rosenberg *et al.* 2000). This value is considered to be significantly different from zero (i.e. the explanatory variable shows a significant effect on the response variable) if its confidence interval does not overlap zero.

If the dataset has some underlying structure and studies can be categorized into more than one group – for example different plant species or different experimental facilities -, a categorical meta-analysis should be conducted (Rosenberg *et al.* 2000). For such data in addition to the overall effect size, one can calculate the cumulative effect size for each group, which again is considered significantly different from zero if its confidence interval does not bracket zero (Rosenberg *et al.* 2000). Curtis & Wang (1998) describe a method by which one can test whether the effect sizes within a categorical group are homogenous and whether there are significant differences in the mean responses between categorical groups. The total heterogeneity (Q_T) of a sample can be partitioned into the within-class heterogeneity (Q_W) and the between-class heterogeneity (Q_B). The Q statistics has a χ^2 distribution with k-1 degrees of freedom, where (when examining Q_W) k is the number of studies in the group or (when examining Q_B) k is the number of groups (Rosenberg *et al.* 2000). Curtis & Wang (1998) divided their dataset according to categorical variables (e.g. "CO_2 exposure period" and "pot size"), each represented by several

categorical groups (e.g. short, medium and long exposure). In a first step, the Q_B was estimated across all data for each categorical variable. If the Q_B yielded by comparing categorical groups of a categorical variable was greater than the critical value of the χ^2 distribution, the authors concluded that the categorical groups (i.e. levels of the categorical variable) were significantly different from each other. In a second step, the dataset was then subdivided according to levels of those categorical variables that revealed a significant Q_B and the first step was repeated. Thus if the categorical variable "exposure period" revealed a significant Q_B, the dataset was subdivided into the sub-groups of this category and the Q_B in each sub-group for the other categorical variable "pot size" was estimated. The analysis was repeated until the number of categorical variables exhibiting significant Q_B had been reduced to one or zero, suggesting that no further partitioning of the dataset was justified. At this point, the cumulative mean effect size for each significant category was calculated. To identify which categorical groups within a categorical variable differ from each other, the confidence intervals were compared: mean effect sizes were considered to be significantly different from each other if their 95% confidence intervals did not overlap (Curtis & Wang 1998).

3.1.2 Methods used in a meta-analysis of the N limitation effect

An effect size in a meta-analysis typically compares the performance of a "control" group with that of an "experimental" group. With some ecological effects, the choice of the control group is straightforward; for example in a meta-analysis of the CO_2 effect, the control typically is the ambient CO_2 concentration (typically CO_2 concentrations of < 400 ppm[6]) and the experimental group is the elevated CO_2 concentration (typically lying between 600 and 800 ppm; e.g. Curtis 1996; Medlyn *et al.* 1999). Yet with other ecological effects the definition of a control is not as clear and one has to decide which group is comparable as a control across studies. In a study about competition for example, Gurevitch *et al.* (2000) chose competitors at natural densities as the control group, while competitors at manipulated densities represented the experimental group.

If one wants to analyse the effect of N limitation on plant growth thus the control group would need to be represented by a non-limiting N supply. Yet it is not simple to define a certain N supply as non-limiting and to compare N supplies across studies, as the experimental conditions and the sources and doses of N given to plants vary strongly across studies. Unlike the CO_2 supply, which is always reported in similar and comparable units - as μmol mol^{-1} or ppm – and which quantity can be easily experimentally controlled, the N supply is not reported in comparable units and is not available to the plant in a single form.

Yet one important advantage of meta-analysis is the very possibility to compare effects that are measured with different units in each study, as the effect size standardizes them to a uniform scale. Still a control group that is comparable across studies needs to be defined, thus N supplies needed to be somehow comparable. For this reason I decided to restrict the meta-analysis to experiments conducted under controlled conditions, i.e. only to crops grown in pots on a defined medium. Field experiments were thus excluded, as they are conducted on mediums, i.e. soils,

[6] parts per million of air volume

that vary strongly in their properties and in most studies reporting field experiments with crops these soil properties are not stated. If for example in a field experiment a quantity of 200 kg N ha^{-1} is applied, this allows no conclusion as to the magnitude of this N supply, if no information on the initial N content of the soil, on soil properties like texture, or on the extent of N leaching are given. On a highly fertile loamy soil, 200 kg N ha^{-1} might be accompanied with high soil N concentrations, while on a sandy soil with a high proportion of N leaching, the same 200 kg N ha^{-1} might not be associated with comparably high N concentrations in the soil solution. Although even in a glasshouse or growth chamber a true control of the N supply, similar to the control of a constant temperature or constant light conditions, is not achieved in the majority of experiments (Ingestad 1982; Ingestad & Agren 1992); however, the N conditions in pot experiments can at least be described as "semi-controlled", as no N is lost from the system, and pot experiments are thus more comparable than field experiments.

As the aim of this thesis is to deduce a conceptual model for the integration of plant N processes in a model of the managed planetary land surface from crop physiology perspective, the meta-analysis was further restricted to important crops. Important crops were defined as the 10 primary crops with the largest area harvested and the 10 primary crops with the highest production worldwide, based on information from the Food Agricultural Organization (FAO) for the year 2007 (FAOSTAT 2007; Tab. 3.1).

Table 3.1: The 10 worldwide most important crops and associated crop species regarding the total area harvested and the total amount produced in the year 2007, based on information from FAOSTAT (2007). The specific crop species from each FAO crop category involving more than one species that was included in the meta-analysis is highlighted in bold.

	Area harvested (Ha)		**Production (tonnes)**	
	Category	Species	Category	Species
1	wheat	***Triticum aestivum**, T. durum*	sugar cane	*Saccharum officinarum*
2	maize	*Zea mays*	maize	*Zea mays*
3	rice	*Oryza sativa*	rice	*Oryza sativa*
4	soybean	*Glycine max*	wheat	***Triticum aestivum**, T. durum*
5	barley	***Hordeum vulgare**, H. disticum, H. hexastichon*	potatoes	*Solanum tuberosum*
6	sorghum	***Sorghum bicolor**, S. vulgare, S. guineense, S. dura*	sugar beet	*Beta vulgaris*
7	millet	*Echinochloa frumentacea, Eragrostis abyssinica, Paspalum scrobiculatum, **Pennisetum glaucum**, Setaria italica, Panicum miliaceum, Eleusine coracana*	soybean	*Glycine max*
8	cotton	*Gossypium hirsutum*	cassava	*Manihot esculenta*
9	rapeseed	*Brassica napus*	oil palm fruit	*Elaeis guineensis*
10	beans, dry	***Phaseolus vulgaris**, P. lunatus, P. angularis, P. aureus, Vigna angularis, V. mungo, V. radiata, V. unguiculata*	barley	***Hordeum vulgare**, H. disticum, H. hexastichon*

Only FAO categories were considered that referred to a single or a restricted number of crop species, thus the category "vegetables fresh nes[7]", which is the 7th most produced crop according to FAO statistics and represents all vegetables pooled together that are not listed singularly, was excluded; instead the category "millet", which refers to seven different species according to FAO definition (see Tab. 3.1), was included in the analysis. From each FAO crop category one representative species (i.e. the one quantitatively most important) was chosen, e.g. the FAO category "wheat" refers to *Triticum aestivum* and *Triticum durum*, but only *T. aestivum* was used in the analysis. Using the method described, 15 species were included in this analysis (Tab. 3.2).

Table 3.2: List of the 15 crop species included in the meta-analysis and their associated characteristics, i.e. their crop type (following FAO definitions), their photosynthetic pathway and whether they are a leguminous species.

Crop species	Crop type	Photosyn. pathway	Leguminous
Triticum aestivum	cereal	C3	no
Zea mays	cereal	C4	no
Oryza sativa	cereal	C3	no
Hordeum vulgare	cereal	C3	no
Sorghum bicolor	cereal	C4	no
Pennisetum glaucum	cereal	C4	no
Gossypium hirsutum	fibre crop	C3	no
Glycine max	oilseed and oleaginous fruits	C3	yes
Brassica napus	oilseed and oleaginous fruits	C3	no
Elaeis guineensis	oilseed and oleaginous fruits	C3	no
Phaseolus vulgaris	pulses	C3	yes
Saccharum officinarum	sugar crop	C4	no
Beta vulgaris	sugar crop	C3	no
Solanum tuberosum	edible roots and tubers	C3	no
Manihot esculenta	edible roots and tubers	C3	no

[7] "nes" refers to "not elsewhere specified"

Data compilation

A literature search was conducted for experimental research published in peer-reviewed journals on crop growth with a varying N supply, using web-based search engines (mainly Google Scholar and PubMed) and by searching the reference lists of published articles. In order to be included in the analysis, studies had to meet the following criteria:

I. The study organism was a crop species of interest (see above, Tab. 3.2).

II. Experiments were conducted with at least two different N rates and these were supplied for at least 4 consecutive days or for at least 1/3 of the entire growth period of the plant.

III. At least one parameter of interest concerning the physiology or growth of crops was measured (see below, Tab. 3.3).

IV. The study was conducted under controlled conditions, i.e. in a glasshouse or growth chamber and with plants grown in pots with a defined and comparable amount of N available (see discussion above).

V. The maximum N rate supplied to the plants could be categorized as non-limiting for growth and could thus be taken as control treatment (see below).

VI. For any variable measured, the mean (X), standard deviation (SD) or standard error (SE) and sample size (n) were reported as numerical or graphical data or were available by personal communication, since weighted meta-analysis was used. If the study did not report this information, it was attempted to contact the author(s).

Experiments in which other variables (apart from N supply) were varied, were only included in the analysis, if they could be attributed to categorical variables (see below), e.g. to the general category stress or to a varying growth CO_2 concentration. Not included for example were experiments where spikelets were excised (e.g. Guitman *et al.* 1991) or where N/P ratios were varied (e.g. Adalsteinsson & Jensen 1988). Split root experiments (i.e. localized variations in N supply) were not included as the different treatments in such experiments are not independent.

Because of the great variety in N application methods and doses it was difficult to compare the N rates given in different experiments and to ascertain a non-limiting N supply that could be used as a control treatment. In most cases, the authors did not state whether the maximum N rate in their experimental setup was assumed to be non-limiting for plant growth.

Therefore, a tentative definition of a minimum non-limiting N dose was used. A certain N supply was thus assumed to be non-limiting either if this was stated explicitly in the paper or was communicated by the author, if this could be concluded from growth results or if the N supply was above the following minimum non-limiting N rates:

- Full-strength Hewitt's solution - which is often used in N experiments (e.g. Wong 1979; Evans 1983) - contains 12 mM N (Hewitt & Smith 1975), while full-strength Hoagland's solution (used e.g. in Fricke *et al.* 1997) contains 15 mM N (Hoagland & Arnon 1950). Yet these concentrations are relatively high (Leggett & Frere 1971) and other complete nutrient solutions have considerably lower concentrations in the range from 5 mM (e.g. Carvajal *et al.* 1996) to 10 mM (e.g. Chapin *et al.* 1988a, b). In addition, two authors (F. Pugnaire and J. Morgan) conveyed through personal communication that they considered the half-strength Hoagland's solution used in their experiments to not limit growth. Therefore a value of **8 mM N** for a nutrient solution, supplied or renewed at least once a

week, was chosen as the minimum non-limiting N concentration.

- An application of 12.5 mM N in a nutrient solution twice a week resulted in a total application of 21 g N m^{-2} and was shown to be adequate for 60 days of growth of wheat (Theobald *et al.* 1998). Therefore the minimum N dose on an area basis that was assumed to be non-limiting was **25 g N m^{-2}** (equivalent to 250 kg N ha^{-1}).
- In Mitchell *et al.* (1993) a total amount of 1.4 g N supplied to a 5-l-pot was shown to be non-limiting for 200 days of growth of wheat. For potato instead an author (P. van der Putten) communicated that a total amount of 12 g N supplied to a 20-l-pot, i.e. corresponding to 4 g N per 5-l-pot, was non-limiting for growth. Therefore a medium value of **2.5 g N** supplied to a 5-l-pot was assumed as the minimum non-limiting N supply on a pot basis.

The literature review was not intended to be comprehensive, because of the large amount of experimental studies with a varying N supply. A database of 318 papers was compiled. 85 studies from this database met the first four criteria (see above), but unfortunately many of the studies did not meet all of the requirements needed to quantify the relative magnitude of N limitation. In total 58 of the 85 short-listed studies could not be included in the analysis (see Appendix B, Tab. 1), as either (i) criterion V was not met and the maximum N supply was clearly not non-limiting for growth (e.g. Bloom & Chapin 1981) or it was unclear whether it was non-limiting (e.g. van den Boogaard *et al.* 1995), (ii) as criterion VI was not met, i.e. sample size and/or errors were not reported in the paper and could not be gathered from the authors, as the authors could not be contacted (e.g. Wong *et al.* 1985), they did not reply to the inquiry (e.g. Shangguan *et al.* 2004) or the data was stored in outdated formats and could not be extracted and reported (e.g. Robinson *et al.* 1991, 1994) or finally (iii) as the reported parameter could not be included in the analysis because the studies that reported this parameters were too few and the sample size (see below) was not high enough (e.g. transpiration rate from Radin 1990).

If data was only reported in graphical form and could not be gathered from the authors, the freeware program DataThief III (Tummers *et al.* 2008) was used to extract data from figures. If the data was presented in graphs sometimes the error was not shown, if it was smaller than the size of the symbol (e.g. Devienne *et al.* 1994b; Vos & van der Putten 1998). In these cases, the outmost margin of the symbol was taken as the error value. The difficulty to extract information on n, SD or SE was the main reason for exclusion of studies (see Appendix B, Tab. 1). Many studies did not report any error measures at all; several more did report errors, but not the associated sample sizes. In total 27 studies from 10 different journals spanning the period 1979-1999 met the criteria described above (Tab. 3.4) and could be included in the analysis. A database was compiled with information on 33 response variables, 23 of which can be reported here because of a large enough sample size (i.e. number of effect sizes, $k > 6$; Tab. 3.3).

Table 3.3: List of general categories of response variables that were analyzed in the meta-analysis and their respective definitions.

General category	Parameter	Definition
C allocation	LA	leaf area per plant
	W_T	total biomass per plant
	W_S	shoot biomass per plant
	W_R	root biomass per plant
	RSR	root:shoot ratio, either reported as such or calculated as inverse of shoot:root ratio or from absolute shoot and root biomass
	SLA	specific leaf area, either reported as such or calculated as inverse of specific leaf mass
	Sug_L	total leaf sugar content on mass or area basis, either reported as such or calculated as sum of sucrose, glucose and malate contents
	$Stch_L$	leaf starch content on mass or area basis
	NSC_L	leaf total non structural carbohydrates on mass or area basis, either reported as such or calculated as sum of sugars and starch
	RGR	relative growth rate, i.e. weight increase per unit weight per time
N allocation	N_L	leaf N content on mass or area basis
	N_G	grain N content on mass basis
	N_T	whole plant N content on mass basis
	Nit_L	leaf nitrate content on mass or area basis
	Nit_R	root nitrate content on mass basis
	Nit_T	whole plant nitrate content on mass basis
	AA_L	leaf free amino acid content on mass basis
	$Prot_L$	leaf soluble protein content on mass or area basis
Photosynthesis	Chl	leaf chlorophyll content on mass or area basis
	Rub	Rubisco activity on area basis
	A	leaf net photosynthesis on a mass or an area basis
	g_s	stomatal conductance
N uptake	N_{up}	N uptake rate on mass basis

Table 3.4: Characteristics of studies included in the meta-analysis. Response variable abbreviations and definitions are described in Table 3.4. In the column describing the maximum N rate applied the basis for the categorization of this rate as non-limiting is stated in parenthesis; "complete" refers to a complete nutrient solution, "growth" refers to a categorization as non-limiting based on growth results and "author" indicates that this information was communicated by the author(s) of the study. The column "contact" states whether the author(s) of the study were contacted and if yes, whether they could send the data (yes/yes), whether the data was too old and could not be extracted from outdated formats (yes/no d) or whether contacted authors did not reply (yes/no r).

Reference	Journal	Variables	Species	Experimental set up	Maximum N supply	N rates	Contact
Barneix *et al.* (1992)	Physiol. Plant.	N_G	*Triticum aestivum*	pots in glasshouse + growth chamber	16 mM N (complete)	4	yes/no d
Biemond & Vos (1992)	Ann. Bot.	W_T, W_S	*Solanum tuberosum*	pots in glasshouse	16 g N pot^{-1}	3	yes/no d
Caputo & Barneix (1997)	Physiol. Plant.	W_S, Sug_L, N_T, Nit_T, AA_L	*Triticum aestivum*	pots in glasshouse	20 mM N	6	yes/no d
Carvajal *et al*. (1996)	Planta	g_s	*Triticum aestivum*	nutrient rafts	5 mM N (complete)	2	no
Chapin *et al.* (1988a)	Planta	Nup	*Hordeum vulgare*	hydroponically	10 mM N (complete)	2	no
Chapin *et al*. (1988b)	Planta	W_T, W_S, W_R, SLA, RSR, Nit_L, Nit_R, AA_L, A, g_s	*Hordeum vulgare*	hydroponically	10 mM N (complete)	2	no
Cramer & Lewis (1993)	Ann. Bot.	W_T, RSR, A, g_s	*Triticum aestivum, Zea mays*	hydroponically	12 mM N	2	no
Devienne *et al.* (1994)	Journ. Exp. Bot.	W_T, W_S, W_R, RSR, N_T, Nit_R, Nit_T	*Triticum aestivum*	pots in growth chamber	5 mM N (growth)	5	yes/no r
Evans (1983)	Plant Physiol.	LA, N_L, A, Chl, Rub	*Triticum aestivum*	pots in glasshouse	12 mM N (complete)	2-5	no
Fricke *et al.* (1997)	Planta	RGR	*Hordeum vulgare*	hydroponically	15 mM N (complete)	3	no
Guitman *et al.* (1991)	Physiol. Plant.	W_T, Sug_L, N_L, N_G, N_T, AA_L, $Prot_L$, Chl	*Triticum aestivum*	pots in glasshouse	16 mM N	2	yes/no d
Khamis & Lamaze (1990)	Physiol. Plant.	W_T, W_S, W_R, RSR, Sug_L, $Stch_L$, NSC_L, Nit_L, Nit_R, Nit_T, AA_L, $Prot_L$, A, Chl	*Zea mays*	pots in glasshouse	3 mM N (growth)	4	yes/no r
Khamis *et al.* (1992)	Physiol. Plant.	W_S, Nit_L, A, Rub	*Zea mays*	pots in growth chamber	12 mM N	4	yes/no r
King *et al*. (1993)	Plant Physiol.	Nup	*Hordeum vulgare*	hydroponically	10 mM N	3	no
Mitchell *et al.* (1993)	Plant Cell Environ.	W_T	*Triticum aestivum*	pots in glasshouse	47.5 mM N	2	yes/no r
Morgan (1984)	Plant Physiol.	g_s	*Triticum aestivum*	hydroponically	7.5 mM N (author)	2	yes/no d
Nakamura *et al.* (1999)	Photosynthetica	LA, W_T, SLA, N_L, A	*Glycine max*	pots in growth cabinet	30 g N per m^2	2	no
Nakano *et al.* (1997)	Plant Physiol.	Sug_L, $Stch_L$, NSC_L, N_L, A, Chl	*Oryza sativa*	hydroponically	8 mM N	3	yes/no d
Pugnaire & Chapin (1992)	Oecologia	W_T, W_S, W_R, SLA, RSR, RGR, N_L, N_G	*Hordeum vulgare*	pots in glasshouse	half strength Hoagland (author)	2	yes

Radin (1983)	Plant Cell Environ.	RGR, N_{up}	*Hordeum vulgare, Gossypium hirsutum*	pots in glasshouse	5 mM N (complete)	2	no
Robinson (1996)	Photosynth. Res.	LA, W_T, W_S, W_R, SLA, RSR, Sug_L, $Stch_L$, NSC_L, $Prot_L$, A, g_s, Chl	*Glycine max*	pots in glasshouse	14.5 mM N (sufficient)	2	no
Siddiqi *et al.* (1989)	Plant Physiol.	Nit_R	*Hordeum vulgare*	hydroponically	10 mM N	5	no
Siddiqi *et al.* (1990)	Plant Physiol.	N_{up}	*Hordeum vulgare*	hydroponically	10 mM N	2	no
Vos & Biemond (1992)	Ann. Bot.	LA, SLA	*Solanum tuberosum*	pots in glasshouse	tot. 16 g N pot^{-1}	3	yes/no d
Vos & van der Putten (1998)	Field Crop Res.	SLA, N_L, Nit_L	*Solanum tuberosum*	pots in glasshouse	tot. 12 g N pot^{-1} (author)	2-5	yes/yes
Wong (1979)	Oecologia	LA, W_T, N_T, $Prot_L$, A, Chl, Rub	*Gossypium hirsutum, Zea mays*	pots in glasshouse	24 mM N	4	no
Wong (1990)	Photosynth. Res.	W_T, SLA, RSR, NSC_L, RGR, N_L	*Gossypium hirsutum*	pots in glasshouse	24 mM N	4	no

A separate meta-analysis was conducted for each response variable. Units of measurement are not important in a meta-analysis since the calculated effect size is dimensionless, but in some cases variables respond differently to a varying N supply depending on the unit they are expressed on (e.g. photosynthesis rate on a leaf area or on a mass basis). Therefore for such variables where data was collected both on a mass and an area basis (i.e. Sug_L, $Stch_L$, NSC_L, N_L, Nit_L, $Prot_L$, Chl, A), it was analyzed whether the category "weight based unit" or "area based unit" had any significant effect on the response variable (see description of categorical analysis below). If the unit showed a significant effect, first one analysis was conducted with all values pooled together and subsequently – if the sample size was high enough – two more separate analyses were conducted with only those values reported on a mass or an area basis respectively.

Each individual record in a meta-analysis needs to be independent. Values from different experiments and different treatments from the same paper were assumed to be independent and were included as separate effect sizes in the analysis. This approach has some flaws as different experiments from a single study might not be totally independent, as for example the same materials, procedures and experimental conditions were applied. This can lead to an underestimation of the overall heterogeneity in effect sizes (Gurevitch & Hedges 1999). Yet excluding multiple results from a single paper would underestimate the effect sizes. Most studies conducting meta-analyses therefore include results from separate experiments reported in a single article (e.g. Curtis & Wang 1998; Medlyn *et al.* 1999; Gurevitch *et al*. 2000; Gómez-Aparicio 2009) and this approach was also applied here.

If an article reported several results from a single experiment, e.g. for different plant organs, from different leaves in a canopy or from different points during the growing season, these results were not included in the same meta-analysis, but could potentially be included in more than one of the meta-analyses if they reported different response variables. For example if the data from a single experiment on N content in young leaves and old leaves were reported, only one of these were included (namely the results for young leaves), as both results corresponded to the same response variable (i.e. N_L). On the contrary, if the N contents of the whole plant and of leaves were reported from the same experiment, both values could be used, as they were analysed in separate meta-analyses (i.e. N_L and N_T). If several measurements were reported for different durations of N limitation, the value following the longest period of varying N supply was used; if several values over the course of the plant growth were reported, in most cases the value from the middle of the growing season was taken, except for response variables like total plant biomass (W_T), where the final value was used.

In total 422 pairs of means distributed across the 23 response variables could be extracted from the 27 studies. It is important to note that the sample size *k* in the meta-analysis does not refer to separate studies but to the number of pairs of means from which a single effect size is calculated.

An especially interesting component of a meta-analysis is the question how categorical variables describing experimental conditions and biological characteristics of the study species influence the response variables. Each record for each response variable was therefore classified according to categories concerning experimental conditions (e.g. the magnitude of the N supply, the N source, the growth medium; see Tab. 3.5) and biological plant characteristics (crop species, crop type, photosynthetic pathway, leguminous, development stage; see Tab. 3.2).

As not many studies involved any variable interacting with N, apart from CO_2 growth level (Tab. 3.7), all other experimental manipulations were included into a single "stress" category (Tab.

3.5). This procedure unfortunately does not distinguish between the effects of temperature, light or water stress, yet it has been applied in several other meta-analytic studies (e.g. Curtis 1996; Curtis & Wang 1998; Jablonski *et al*. 2002).

Table 3.5: List of experimental categorical variables and their classes with the respective definition.

Category	Class	Definition
N rate	very low	< 10% of control (i.e. non-limiting) N rate
	low	10-29% of control N rate
	medium	30-50% of control N rate
	high	> 50% of control N rate
N source	nitrate	only nitrate as N source
	ammonium	only ammonium as N source
	nit + amm	mixture of nitrate and ammonium sources
duration of N limitation	< 1/2	less or up to half of the entire growth period
	> 1/2	more than half of the entire growth period
	entire	entire growth period
frequency N application	< 1	more than once per day
	1-2	every 1-2 days
	3-7	every 3-7 days
	> 7	less than every 7 days
pH control	yes	pH monitored and held constant
	no	pH not monitored
growth medium	soil	any type of soil (e.g. peat, loam, garden soil) or a mixture of soil with other substrates
	sand	sand without any other substrate; sandy soil is categorized as soil
	inert medium	growth on a solid, inert potting medium (e.g. arcillite, perlite, vermiculite)
	hydroponic	defined as growth in mineral nutrient solutions, with no solid medium for the roots (following Gericke 1937)
pot size	small	0.3-2.4 l
	medium	2.5-9 l
	big	> 9 l
stress	none	no intentional stress component (apart of course from N stress)
	stress	low water availability, high temperature or low light intensity
growth CO_2 level	ambient	< 360 ppm
	elevated	> 640 ppm

The analysis of the category "development stage" was considered to be of special interest, as it is important information for the modelling of plant processes, whether the plant response to N limitation differs between different development stages. Yet most publications did not report the development stage of the plants during measurements. In order to be able to attribute development classes to the different experimental values, development stages and their respective lengths needed to be defined for the different crop species. Therefore, each development stage was defined by a literature-based value of days after emergence (DAE). If possible, more than one value from different literature sources for each stage and each species was gathered and the average of these values used for the definition of classes (Tab. 3.6). The reproductive stage was assumed to start at anthesis, except in barley, where no flower is visible and thus anthesis is defined by head emergence (Anderson *et al.* 1985) and in rice, where the reproductive stage begins with the elongation of internodes (Miller & Street 2009). The seedling stage could only be defined for barley, rice, wheat, and maize. For barley, rice and wheat the end of the seedling stage is defined by the beginning of tillering (Zadok *et al.* 1974); in maize on the other hand it is defined as the emergence of the first fully expanded leaf (Ritchie *et al.* 1993).

As an example I illustrate the method used for the definition of DAE to reach a certain development stage for soybean. Three papers were found that reported the amount of days that soybean plants needed to reach a certain growth stage: Egli (1997) reported that two early cultivars needed 95 and 91 days and two late cultivars needed 140 and 144 days to reach maturity. Soybean plants in Zhang *et al.* (1997) instead needed 92 days and those in Quebedeaux *et al.* (1975) needed 83 days to reach physiological maturity. Thus, a value of 108 days (average of the six values reported) was taken for soybean to reach maturity. The date of anthesis instead was only reported by Zhang *et al.* (1997), where it was reported as being 46 days, and by Quebedeaux *et al.* (1975), where plants needed 35 days to reach flowering. Thus, the value for anthesis and for the beginning of the reproductive stage was taken as 41 DAE. None of the papers reported any values for the day of transition from seedling to vegetative stage, so the early development stage for soybean could not be further subdivided.

The method used for attributing development stages is very tentative, as the time to reach a certain development stage varies strongly within a species, depending on the variety and on environmental conditions. However a better and more accurate classification was not possible due to the lack of information that was reported in the original publications.

Table 3.6: Definition of time range (DAE, days after emergence) of development classes for different crop species.

Species	DAE	Development class	Reference
Glycine max	0-41	seedling/vegetative	Quebedeaux *et al.* (1975), Egli (1997), Zhang *et al.* (1997)
	41-108	reproductive	
	108	maturity	
Gossypium hirsutum	0-61	seedling/vegetative	Hutmacher (2002), Ritchie *et al.* (2007)
	61-135	reproductive	
	135	maturity	
Hordeum vulgare	0-15	seedling	Zadok *et al.* (1974), Anderson *et al.* (1985b)
	15-45	vegetative	
	45-85	reproductive	
	85	maturity	
Oryza sativa	0-28	seedling	Hill & Williams (1997), Miller & Street (2009)
	28-54	vegetative	
	54-116	reproductive	
	116	maturity	
Solanum tuberosum	0-37	seedling/vegetative	Ali *et al.* (2003), Worthington & Hutchinson (2005)
	37-87	reproductive	
	87	maturity	
Triticum aestivum	0-18	seedling	Zadok *et al.* (1974), Anderson *et al.* (1985a)
	18-59	vegetative	
	59-90	reproductive	
	90	maturity	
Zea mays	0-4	seedling	Ritchie *et al.* (1993), Leakey *et al.* (2004)
	5-50	vegetative	
	50-103	reproductive	
	103	maturity	

Table 3.7: Characteristics of studies included in the meta-analysis: interactions with other factors and attribution of categorical variables. Classes and definitions of categorical variables are described in Tab. 3.5 and Tab. 3.6

Reference	Interactions	Duration	N source	Frequency	pH control	Pot size	Medium	Dev stage
Barneix *et al.* (1992)	light intensity	< ½ – entire growth	nit + amm	1-2	no	medium	soil	maturity
Biemond & Vos (1992)		entire growth	?	>7	no	big	sand	maturity
Caputo & Barneix (1997)		entire growth	nit	1-2	no	small	sand	seedling
Carvajal *et al.* (1996)		< ½	nit	>7	no	?	?	seedling
Chapin *et al.* (1988a)		< ½	nit	3-7	no	small	hydroponic	vegetative
Chapin *et al.* (1988b)		< ½	nit	3-7	no	small	hydroponic	vegetative
Cramer & Lewis (1993)	N source	entire growth	nit/ amm	3-7	yes	big	hydroponic	vegetative
Devienne *et al.* (1994)		entire growth	nit	1-2	no	big	hydroponic	vegetative
Evans (1983)		entire growth	nit	3-7	no	medium	soil	-
Fricke *et al.* (1997)		entire growth	nit + amm	<1	no	medium	hydroponic	maturity
Guitman *et al.* (1991)		entire growth	nit + amm	3-7	no	medium	soil	reproductive/ maturity
Khamis & Lamaze (1990)		< ½ - > ½	nit	<1	yes	small	sand	vegetative
Khamis *et al.* (1992)		> ½	nit	<1	no	medium	sand	vegetative
King *et al.* (1993)		< ½	nit	1-2	yes	big	hydroponic	seedling
Mitchell *et al.* (1993)	CO_2 level, temperature	entire growth	nit	>7	no	medium	inert medium	maturity
Morgan (1984)	water supply	entire growth	nit + amm	3-7	yes	medium	hydroponic	vegetative
Nakamura *et al.* (1999)	CO_2 level	entire growth	amm	?	no	small	soil	reproductive
Nakano *et al.* (1997)	CO_2 level	< ½	nit + amm	3-7	yes	medium	hydroponic	vegetative
Pugnaire & Chapin (1992)	water supply	entire growth	nit	1-2	no	small	inert medium	maturity
Radin (1983)		entire growth	nit	?	no	?	soil	vegetative
Robinson (1996)		entire growth	nit + amm	1-2	yes	medium	inert medium	vegetative
Siddiqi *et al.* (1989)		> ½	nit	<1	no	big	hydroponic	seedling
Siddiqi *et al.* (1990)		> ½	nit	<1	no	big	hydroponic	seedling
Vos & Biemond (1992)		entire growth	?	>7	no	big	sand	maturity
Vos & van der Putten (1998)		entire growth	nit + amm	>7	no	big	soil	vegetative/ reproductive
Wong (1979)	CO_2 level	entire growth	nit	1-2	no	medium	soil	vegetative
Wong (1990)	CO_2 level	entire growth	nit	1-2	no	medium	soil	vegetative

Statistical analysis

All analyses were carried out using MetaWin 2.0 (Rosenberg *et al.* 2000). The natural logarithm of the response ratio *R* (Curtis & Wang 1998; Hedges *et al.* 1999; see 3.1.1) was chosen as the effect size metric and is reported as mean percent change ([*R*-1]x100) under N limitation (Ainsworth *et al.* 2002). A weighted analysis was used, as generally recommended (Gurevitch & Hedges 1999). Because of the assumption of fixed effects of categories on the effect of N limitation and of random variation among experiments within categories in the effect of N limitation, a mixed-effect model was used (Gurevitch & Hedges 1999). The effect size was weighted by the reciprocal of the mixed-model variance, which is the sum of the variance of the natural logarithm of the response ratio and the pooled between-experiment variance (Hedges *et al.* 1999; see 3.1.1). The confidence intervals around the weighted-mean effect sizes were bootstrapped using resampling tests generated from 999 iterations, as the sample size of most parameters was not large enough for the assumptions of parametric meta-analysis tests (Adams *et al.* 1997; Gurevitch & Hedges 1999; Rosenberg *et al*. 2000).

Cumulative effect sizes of single response variables were considered significantly different from zero (i.e. they showed a significant response to N limitation) if their 95% confidence intervals did not overlap zero. Differences between categories were tested using a procedure analogous to the partitioning of variance in an analysis of variance, as described by Curtis & Wang (1998; see 3.1.1). If the categorical analysis revealed a significant Q_B – which implies that there are differences among cumulative effect sizes for the defined categories (Rosenberg *et al.* 2000) - the mean log ratios for categories within the significant categorical variable were calculated. Means of two different categories (e.g. development stages) were considered significantly different from each other if their 95% confidence intervals did not overlap. This procedure differs slightly from that described by Curtis & Wang (1998) as the sample sizes were too small for a further partitioning of the dataset for the calculation of cumulative effect sizes. Still, where possible, i.e. if categories were represented by at least two separate pairs of means (i.e. if $k \geq 2$), in a second step the dataset was subdivided according to the levels of those categorical variables that revealed a significant Q_B and the first step was repeated, as proposed by Curtis & Wang (1998). This procedure allowed testing for the effect of the other categorical variables within a subgroup, or level of a certain categorical variable (Curtis & Wang 1998).

3.2 Effect of N limitation on crop physiology and growth

3.2.1 C allocation

Despite the small sample size, most response variables describing C allocation showed a significant response to N limitation (Fig. 3.1).

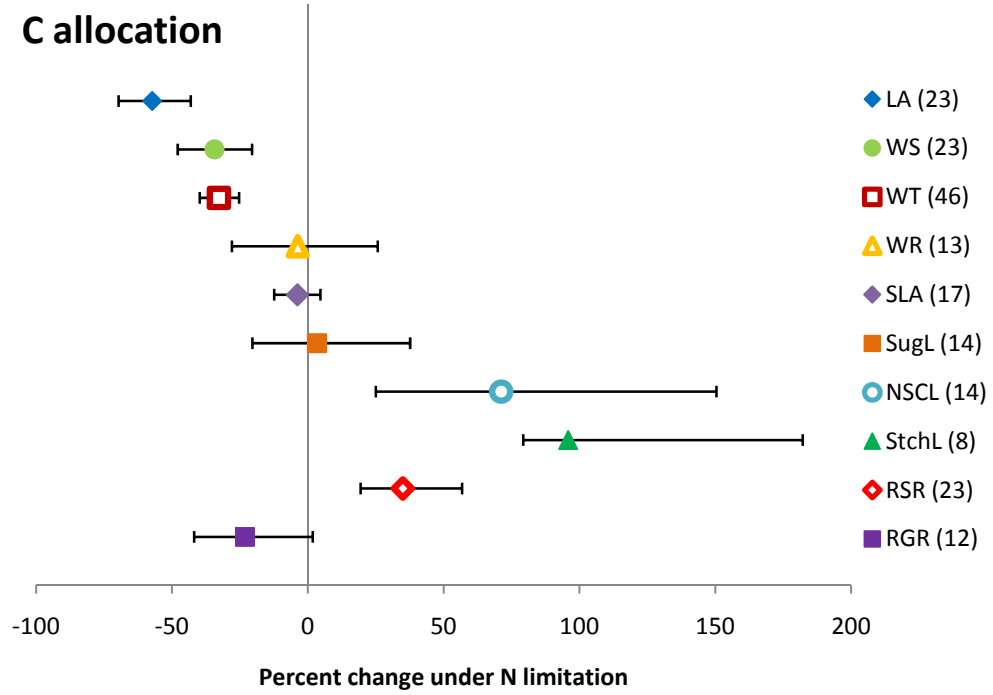


Figure 3.1: The response of parameters describing C allocation to a limiting N supply. Symbols show means surrounded by 95% confidence intervals. Sample sizes (*k*) for each point are in parenthesis. Abbreviations: LA: leaf area; W_S: shoot biomass; W_T: whole plant biomass; W_R: root biomass; SLA: specific leaf area; Sug_L: leaf sugar content; NSC_L: leaf non-structural carbohydrates; $Stch_L$: leaf starch content; RSR: root-shoot ratio; RGR: relative growth rate.

Leaf area (LA) was - among the parameters describing C allocation - the one with the largest decrease (-57%, $k = 23$) under N limitation (Fig. 3.1). The single effect sizes for leaf area varied across a relatively broad range (between -2% and -94%), but all showed a negative effect (see Appendix C, Tab. 2). Several experimental and biological categories affected the response of LA to N limitation (Tab. 3.8; Tab. 3.9). Wheat (*Triticum aestivum*) for example showed a more than fivefold stronger decrease in leaf area under N limitation than soybean (*Glycine max*) (Fig. 3.3). LA also decreased stronger under N limitation if plants received a very low N supply, if they were fed with nitrate as an N source, if they were supplied with N every 3 to 7 days or if experiments involved a non-leguminous species (Fig. 3.2).

Table 3.8: Between-group heterogeneity (Q_B) for N limitation effect sizes across experimental categorical variables for different response variables. The response variables are those as described in Tab. 3.3. Each response variable was represented by *k* effect sizes. In addition to the categorical variables as described in Tab. 3.5 also the Q_B of the study and of the unit (if the parameter was reported on both area and weight basis) were analysed. A minus (-) marks categories for which no analysis of heterogeneity could be conducted as there were not enough different classes of that category present in the dataset.

Variable	*k*	Study	Unit	N rate	N source	Duration	Freq	pH	Medium	Pot size	Stress	$[CO_2]$
LA	23	**22.44*****	-	**9.29****	**9.13****	-	**14.58*****	-	0.09	5.0615	-	1.58
W_T	46	8.55	-	**14.89*****	2.71	-	7.41	**7.81****	2.87	2.19	-	1.17
W_S	23	5.47	-	4.42	-	0.001	2.07	1.16	0.57	1.51	-	-
W_R	13	**19.74*****	-	0.14	-	3.68	3.68	**4.83***	3.82	0.47	-	-
SLA	17	**7.08***	-	0.87	1.83	-	**3.90***	-	0.5	**12.74****	-	0.2
RSR	23	5.78	-	**31.95*****	1.07	2.23	1.85	0.47	2.96	4.79	-	1.31
Sug_L	14	3.65	1.32	0.05	**4.68***	1.3	**7.96***	0.8	3.22	**4.68***	-	1.54
$Stch_L$	8	0.001	0.56	2.78	0.56	-	0.001	-	0.001	0.56	-	2.73
NSC_L	14	3.59	2.83	0.19	2.83	3.15	4.03	2.86	3.59	0.01	-	**4.50***
RGR	12	5.1	-	**9.97****	0.6	-	0.92	-	3.4	2.51	-	**5.03***
N_L (all)	24	8.29	**5.61***	**25.91*****	5.82	0.05	2.63	0.05	0.39	5.21	-	0.38
N_L (area)	18	2.41	-	**108.01*****	0.05	1.04	1.98	1.04	1.16	1.83	-	0.99
N_G	10	-	-	2.13	-	**3.96***	-	-	0.03	-	1.83	-
N_T	25	**62.31*****	-	**11.60****	-	-	-	-	**56.46*****	**56.46*****	-	**13.12*****
Nit_T	15	**43.67*****	-	3.83	-	**13.47*****	**13.47*****	**13.47*****	**42.64*****	**42.64*****	-	-
Nit_R	14	**64.29*****	-	5.07	-	**48.17*****	**33.05*****	**33.39*****	**33.39*****	**92.47*****	-	-
Nit_L	9	**32.35*****	1.19	**25.31*****	1.19	0.87	1.03	**18.12*****	1.03	**10.82****	-	-
AA_L	10	1.76	-	**17.15*****	-	1.16	**7.66***	**3.90***	-	-	-	-
$Prot_L$	17	0.0065	0.13	**15.45*****	1.37	0.07	0.05	0.002	0.02	0.07	-	0.001
A (all)	37	**35.35*****	3.09	**16.92*****	4.15	3.82	2.35	1.74	2.63	**8.14***	-	**12.33*****
A (area)	34	**30.47*****	-	**15.51*****	3.82	**16.75*****	0.77	0.47	1.21	**9.08***	-	**10.13****
g_s	9	3.61	-	1.09	0.42	0.91	**5.46***	0.91	**5.46***	0.19	-	-
Chl (all)	25	6.71	3.15	**19.78*****	1.88	1.01	**7.12***	1.16	**7.3***	**7.36****	-	2.39
Chl (area)	21	0.65	-	**16.08*****	0.24	0.64	0.03	0.24	0.53	-	-	1.42
Rub	19	**7.01***	-	1.9	-	2.56	**7.01***	-	2.56	-	-	3.64
N_{up}	7	**72.17*****	-	-	-	**35.44*****	**55.19*****	**4.37***	3.57	-	-	-

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

Table 3.9: Between-group heterogeneity (Q_B) for N limitation effect sizes across biological categorical variables for different response variables. The response variables are those as described in Tab. 3.4. Each response variable was represented by *k* effect sizes. A minus (-) marks categories for which no analysis of heterogeneity could be conducted as there were not enough different classes of that category present in the dataset. The categorical variable "leguminous" was represented by the classes "non-leguminous", "leguminous, nodulating" and leguminous, non-nodulating".

Variable	*k*	Species	Crop type	C3/C4	Leguminous	Dev stage
LA	23	**25.65*** **	**9.10***	0.77	**8.77***	1.1981
W_T	46	3.23	2.4	0.23	0.37	1.04
W_S	23	4.67	1.9	0.01	-	**7.98***
W_R	13	4.15	-	3.13	-	-
SLA	17	**13.00****	**13.00****	-	1.43	**4.51***
RSR	23	2.65	1.18	0.11	-	-
Sug_L	14	1.7	-	0.08	-	2.21
$Stch_L$	8	0.001	-	0.56	-	-
NSC_L	14	3.59	2.86	0.01	-	-
RGR	12	2.32	2.33	-	-	2.45
N_L (all)	24	7.94	7.57	-	**5.85***	1.22
N_L (area)	18	2.41	2.08	-	-	1.83
N_G	10	-	-	-	-	-
N_T	25	**38.98*****	**8.47****	**8.77****	-	0.009
Nit_T	15	**13.47*****	-	**13.47*****	-	**21.58*****
Nit_R	14	**64.98*****	-	**33.39*****	-	0.001
Nit_L	9	1.03	1.19	0.28	-	-
AA_L	10	3.31	-	**3.90***	-	0.48
$Prot_L$	17	2.84	1.78	**4.13***	-	-
A (all)	37	**24.53*****	0.78	0.16	0.65	0.01
A (area)	34	**25.61*****	0.69	0.06	0.87	0.0007
g_s	9	2.64	1.6	0.37	1.6	-
Chl (all)	25	1.32	0.63	0.08	-	-
Chl (area)	21	2.5	1.59	1.59	-	-
Rub	19	5.54	**5.07***	0.66	-	-
N_{up}	7	-	-	-	-	0.45

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

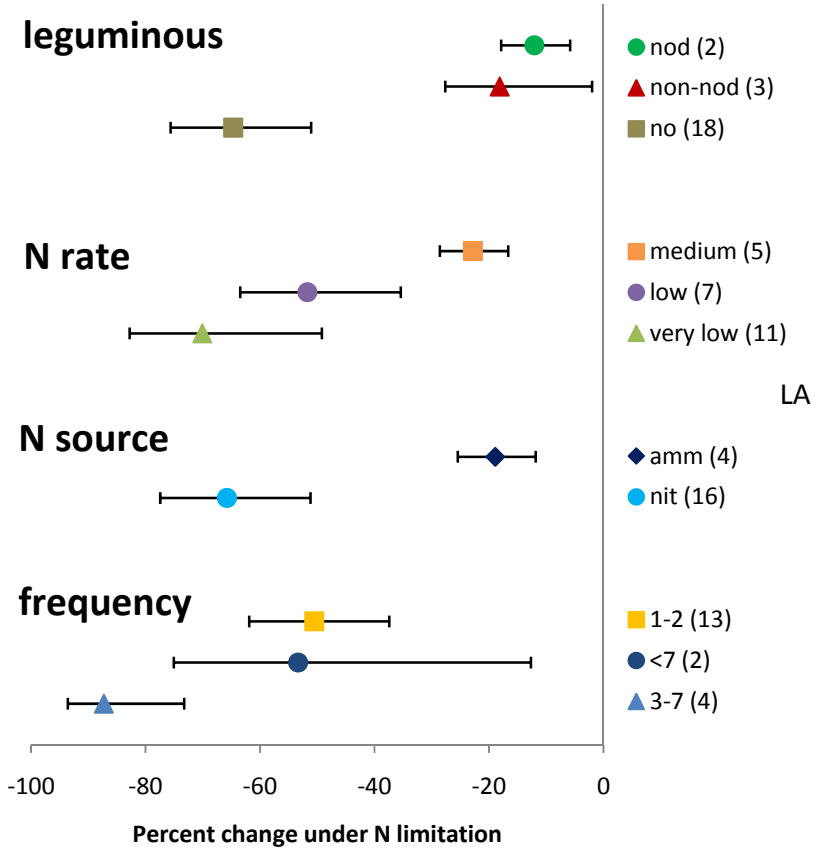


Figure 3.2: The response of leaf area (LA) to N limitation, as influenced by the categorical variables "leguminous", "N rate", "N source" and "frequency of N application". Symbols show means surrounded by 95% confidence intervals. Sample sizes (*k*) for each point are in parenthesis.

Interestingly most of the significant effects that emerged in the analysis across all studies (Tab. 3.8 and 3.9) were also visible if the dataset was subdivided into the classes of the significant categorical variables (Tab. 3.10). The N rate for example had a strong effect on the response of LA to N limitation if all studies were included and as well if only those experiments were analysed that used nitrate as an N source, that applied N every 1-2 days or if the analysis was restricted to experiments that involved cotton (*Gossypium hirsutum*) or maize (*Zea mays*), cereals or fibre crops or only non-leguminous species (Tab. 3.10). Similarly, the N source that crops received influenced the N limitation response of LA across all studies as well as within the class "very low N supply" (Tab. 3.10). Yet the other categories could not be tested for an effect of the N source as not enough different N source classes were present in the sub-datasets for a categorical analysis. If the dataset was subdivided, following the method proposed by Curtis & Wang (1998), many categories could no longer be analysed due to the ever smaller sample size. Not only the significance of Q_B, but also the pattern of response among different classes was similar irrespective of whether the whole dataset or a sub-dataset was analysed. The response of LA in oilseeds for example differed significantly from that in cereals across all studies (Fig. 3.3), as well as if only the class "very low N supply" was examined (Fig. 3.4).

Table 3.10: Between-group heterogeneity (Q_B) for subgroups of significant categorical variables presented in Tab. 3.8 and Tab. 3.9 for the response variables leaf area (LA) and total plant biomass (W_T). Each class for each response variable was represented by *k* effect sizes. A minus (-) marks categories for which no analysis of heterogeneity could be conducted as there were not enough different classes of that category present in the sub-dataset.

Variable		Class	*k*	N rate	N source	Freq	pH	Medium	Pot size	$[CO_2]$	Species	Crop	C3/C4	Leg	Dev
LA	N supply	very low	11	/	**33.20*** **	**206.99*** **	-	-	**33.20*** **	1.37	**213.5*** **	**33.57*** **	0.01	**25.86*** **	**114.6*** **
		low	7	/	-	-	-	-	-	0.02	**15.71*** **	**4.11***	0.16	-	-
		medium	5	/	-	-	-	-	-	0.22	2.2	2.2	0.09	-	-
	N source	nit	16	**49.55****	/	**27.30*** **	-	-	-	2.7	**24.13*** **	0.66	0.66	-	-
		amm	4	-	/	-	-	-	-	0.022	-	-	-	**3.85***	-
	frequency	1-2	13	**16.80*** **	-	/	-	-	-	0.15	0.66	0.66	0.07	-	-
		3-7	4	-	-	/	-	-	-	-	-	-	-	-	-
		>7	2	-	-	/	-	-	-	-	-	-	-	-	-
	species	T. aestivum	4	-	-	-	-	-	-	-	/	-	-	-	-
		G. max	5	-	-	-	-	-	-	0.34	/	-	-	0.12	-
		G. hirsutum	6	**97.77*** **	-	-	-	-	-	0.07	/	-	-	-	-
		Z. mays	6	**90.69*** **	-	-	-	-	-	0.09	/	-	-	-	-
		S. tuberosum	2	-	-	-	-	-	-	-	/	-	-	-	-
	crop type	cereal	10	**17.14*** **	-	**15.72*** **	-	-	-	2.87	**15.72*** **	/	**15.72*** **	-	-
		oilseed	5	-	-	-	-	-	-	0.34	-	/	-	0.12	-
		fibre crop	6	**97.77*** **	-	-	-	-	-	0.07	-	/	-	-	-
		tuber	2	-	-	-	-	-	-	-	-	/	-	-	-
	leguminous	no[1]	18	**45.38*** **	-	**13.13****	-	0.38	0.38	1.71	**12.16****	0.31	0.33	/	0
		nod[2]	2	-	-	-	-	-	-	-	-	-	-	/	-
		non-nod[3]	3	-	-	-	-	-	-	-	-	-	-	/	-
W_T	N supply	very low	16	/	**10.58****	**22.44*** **	-	0.31	**7.52***	2.79	**27.59*** **	**9.83****	0.9	3.25	**10.58****
		low	16	/	-	0.93	3.16	0.63	2.41	0.01	1.37	0.12	0.7	-	**3.93***
		medium	13	/	0.12	2.03	**5.2***	2.37	0.67	0	1.55	0.33	0.01	-	-
	pH control	yes	8	0.17	1.68	0.013	/	0.01	0.01	-	0.26	-	0.03	-	-
		no	38	**9.73****	1.26	0.8	/	1.32	0.28	0.11	2.34	1.14	0.58	0.71	0.7

* P < 0.05, ** P < 0.01, *** P< 0.001

[1]non-leguminous

[2]leguminous, nodulating

[3]leguminous, non-nodulating

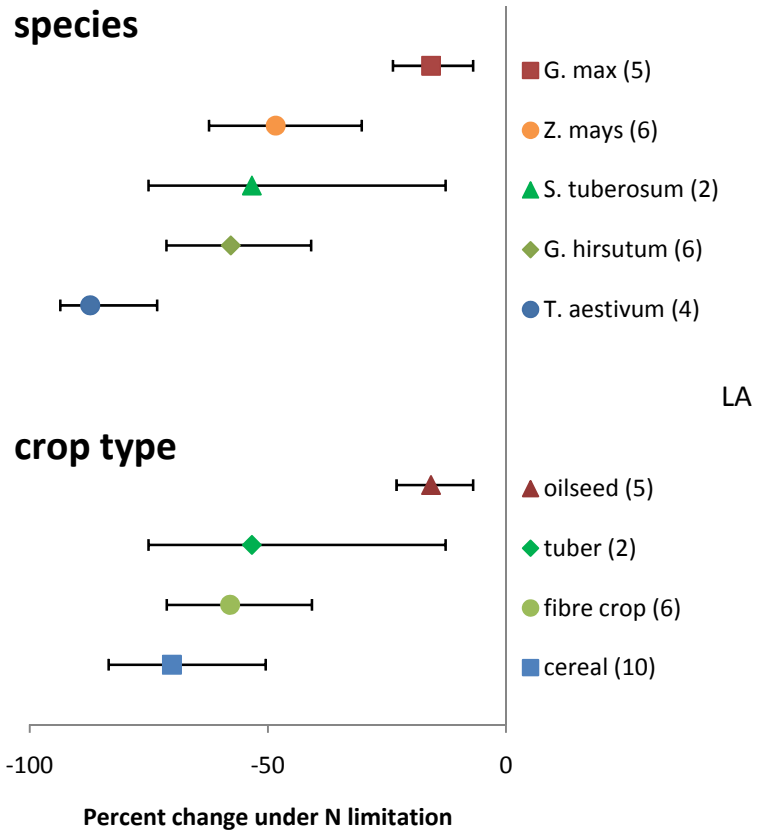


Figure 3.3: The response of leaf area (LA) to N limitation as influenced by the categories "species" and "crop type" across all studies. Symbols show means surrounded by 95% confidence intervals. Sample sizes (*k*) for each point are in parenthesis.

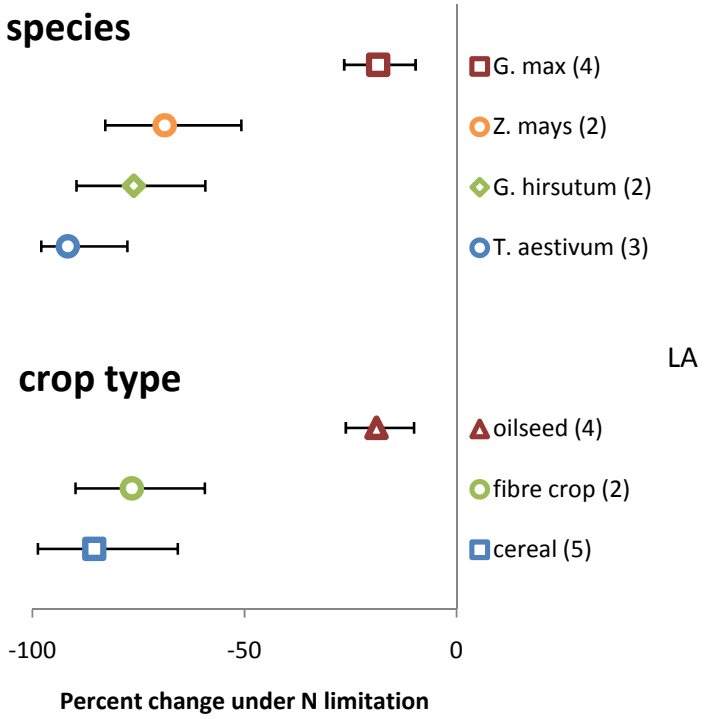


Figure 3.4: The response of leaf area (LA) to N limitation as influenced by the categories "species" and "crop type" within the class "very low N supply". Symbols show means surrounded by 95% confidence intervals. Sample sizes (*k*) for each point are in parenthesis.

While the category "leguminous" did influence LA (Fig. 3.2) the photosynthetic pathway showed no significant effect on the response of LA to N limitation (Tab. 3.9). Yet if the categorical variables "C3/C4" and "leguminous" were combined and the LA dataset was categorized into the classes "C4", "C3-leguminous" and "C3-non-leguminous" this new categorization showed a highly significant effect on N limitation (Q_B = 13.00, p = 0.002), with non-leguminous C3 species decreasing LA by 71% (k = 12), C4 species by 48% (k = 6) and leguminous C3 species by 16% (k = 5) (Fig. 3.5). The difference between non-leguminous C3 species and C4 species however was not significant, as their confidence intervals did overlap (Fig. 3.5). This was also confirmed by the fact that within the class "non-leguminous" the category "C3/C4" did not show a significant Q_B (Tab. 3.10). Thus it seems that LA of leguminous species declines to a lesser extent under a limiting N supply than in non-leguminous species. This relationship holds true even if only experiments with a very low N supply are examined (Tab. 3.10). Species with different photosynthetic pathways on the contrary seem not to differ in the response of LA to N limitation.

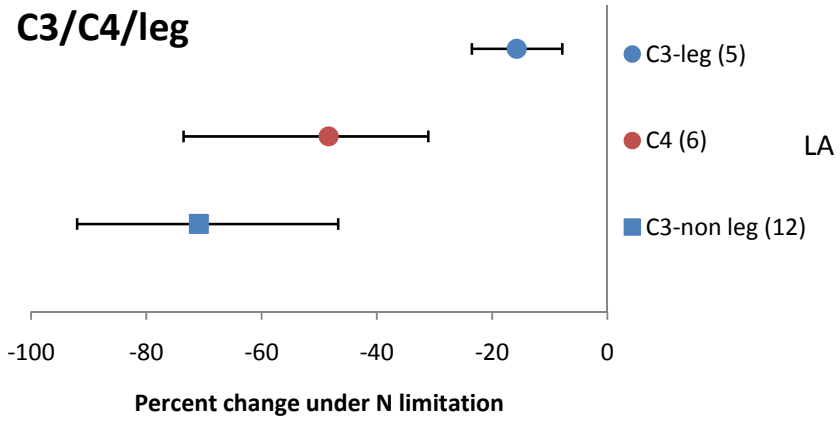


Figure 3.5: The combined effect of the categories "C3/C4" and "leguminous" on the response of leaf area (LA). Symbols show means surrounded by 95% confidence intervals. Sample sizes (k) for each point are in parenthesis.

As mentioned above the form of the N source influenced the response of LA both across all studies as well as when only experiments were considered in which crops received a very low N rate. This is important to note as all response ratios falling into the class "ammonium" received a very low N supply (see Appendix C, Tab. 1). A possible explanation for this effect of the N source on leaf area response could be that crops grown with an adequate N supply but with ammonium as the sole N source had a smaller canopy size due to an adverse effect of ammonium. Under low N supply instead the form of the N source probably did not lead to different responses, as at low concentrations the adverse effect of ammonium is not important (see 2.1.1 for discussion). Thus the decrease in LA under N limitation would be smaller with an ammonium source. However it is generally observed that the ammonium uptake system is much less efficient than the nitrate uptake system at low N concentrations (Gansel *et al*. 2001). This consideration would rather lead to expect an increased reduction in plant growth with ammonium as the sole N source. Besides, the N source did not show any effect on the response of total plant biomass (W_T) to N limitation (Tab. 3.8). In addition all experiments with ammonium as N source

were conducted with soybean, i.e. a leguminous species (see Appendix C, Tab. 1). This could mean that either the apparent ammonium-effect is due to the effect of the leguminous species, or the leguminous-effect is due to the ammonium source or both factors together lead to the small decline of LA in the relevant experiment.

This example demonstrates that the results of the categorical analysis have to be interpreted with reservation, as the meta-analysis was conducted with a very small sample size (k = 23 for LA). In fact a categorical analysis of the category "study" also revealed a strongly significant effect (Tab. 3.8), suggesting that measurements from different studies differed in their response of LA to N limitation. This variance between studies might be explained by differences in the experimental setup or by differences in the biological characteristics of the crop species under examination and might thus be explained by significant categorical variables. Yet it is also possible that the variance was due to some other underlying difference that could not be described by the categorical variables chosen and that probably was not even reported in the corresponding paper (e.g. crops responding differently due to an infection with pathogens). In addition the small sample size lead to parallelisms in the categories that confounded effects, as demonstrated on the example of soybean experiments using an ammonium source. Another example for such a confounding effect was the significant Q_B for the categorical variables "development stage" and "pot size" within the class "very low N rate" (Tab. 3.10), which again resulted from experiments using soybean falling into the class "small pot size" and "reproductive stage" (see Appendix C, Tab. 1). Thus the apparent effect of pot size and development stage on LA presumably was just due to the underlying effect of a leguminous species. Similarly the effect sizes falling into the class ">7" (i.e. N applied at intervals of more than 7 days) described experiments with potatoes (*Solanum tuberosum*) and were the only measurements from plants grown in big pots (see Appendix C, Tab. 1). The apparent smaller decrease in LA under the N application frequency ">7" compared to the N application frequency "3-7" (Fig. 3.2) thus possibly could be due to an effect of the crop species, due to the big size of pots in which potatoes were grown or due to some other difference in the study reporting these measurements.

Because of these difficulties associated with a categorical analysis with small sample sizes I decided not to divide the dataset into classes of significant categorical variables for the remaining response variables as this leads to ever smaller sample sizes and thus to ever more confounding effects.

Growth in terms of biomass increase (W_T) decreased under N limitation (-33%, k = 46), yet the relative growth rate (RGR) declined considerably less (-23%, k = 12) and this response was not significant, as the confidence interval overlapped zero (Fig. 3.1). This no-response of RGR to N limitation observed in the meta-analysis does not agree with the widespread observation that RGR decreases linearly with plant N content (Greenwood *et al.* 1990, 1991). The large variation in the effect sizes of RGR could be attributed to data derived from the study Wong (1990): all N rates in this study, except the very low rate, lead to an increase in RGR (see Appendix C, Tab. 11). Although the control N supply (24 mM) in this study was rather high, the results for W_T from the same study showed that all N rates that were categorized as limiting and thus as experimental N supplies were in fact limiting for plant growth as they lead to a decrease in total biomass (see Appendix C, Tab. 3). Thus it could not be justified to exclude the results from Wong (1990) from the analysis of RGR.

Heterogeneity within the RGR dataset could be described by the categories "N rate" and "$[CO_2]$" (Tab. 3.8). While the RGR did not differ significantly from zero when crops were supplied with

medium or low N rates (Fig. 3.6), a very low N supply lead to a significant decrease in RGR (-41%, $k = 5$). Although the overall responses of W_T and RGR to N limitation thus differed in their magnitude, the response within the "very low N rate" class was similar (-41% for RGR; - 47% for W_T, Fig. 3.7). It seems therefore probable that with a larger dataset the response of RGR to N limitation might be more marked and might show a significant response to N limitation. Growth under elevated CO_2 lead to a non-significant increase in RGR ($k = 3$), while at ambient CO_2 RGR declined by -39% ($k = 9$) under N limitation (Fig. 3.6). Because of the extremely low small sample size for elevated CO_2 this result is of only restricted validity. However it might be interesting to test on a larger dataset whether plants grown under elevated CO_2 have a smaller decrease in RGR due to a growth stimulation resulting from elevated CO_2.

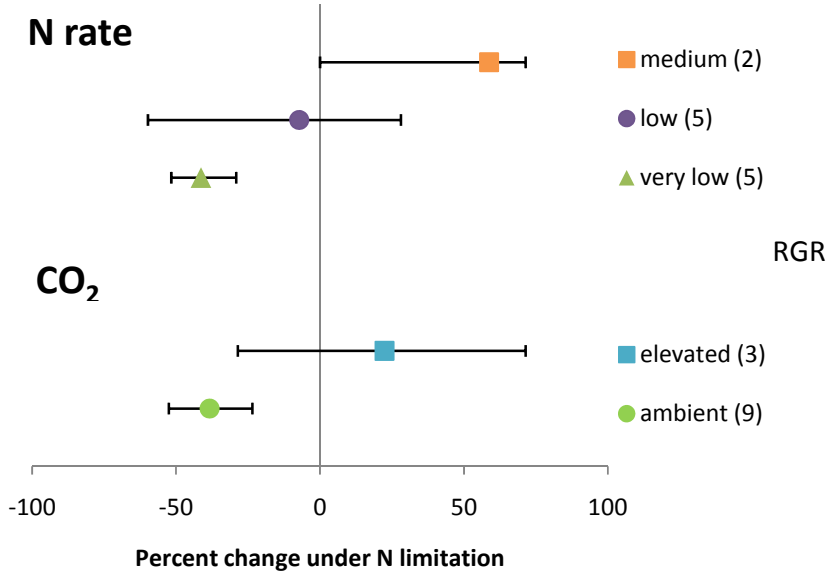


Figure 3.6: The response of the relative growth rate (RGR) to N limitation as influenced by the categorical variables "N rate" and "CO_2 concentration". Symbols show means surrounded by 95% confidence intervals. Sample sizes (k) for each point are in parenthesis.

The effect of the category "pH control" on total biomass (Fig. 3.7) could be attributed to the fact that two out of three studies that controlled the pH of the medium (i.e. Khamis & Lamaze 1990; Cramer & Lewis 1993) were among the few studies that showed an increase in plant biomass with a decrease in the N supply, i.e. a positive effect size (see Appendix C, Tab. 3). It is possible that the maintenance of a constant pH in the growth medium does decrease the adverse effect of a low N supply and plant growth is therefore N limited at lower N concentrations. Yet it is also possible that the studies controlling the pH conducted in general more carefully monitored experiments, possibly leading to a better maintenance of the target N concentration and to more constant N concentrations at the root surface (see discussion of nutrition methods in 3.3).

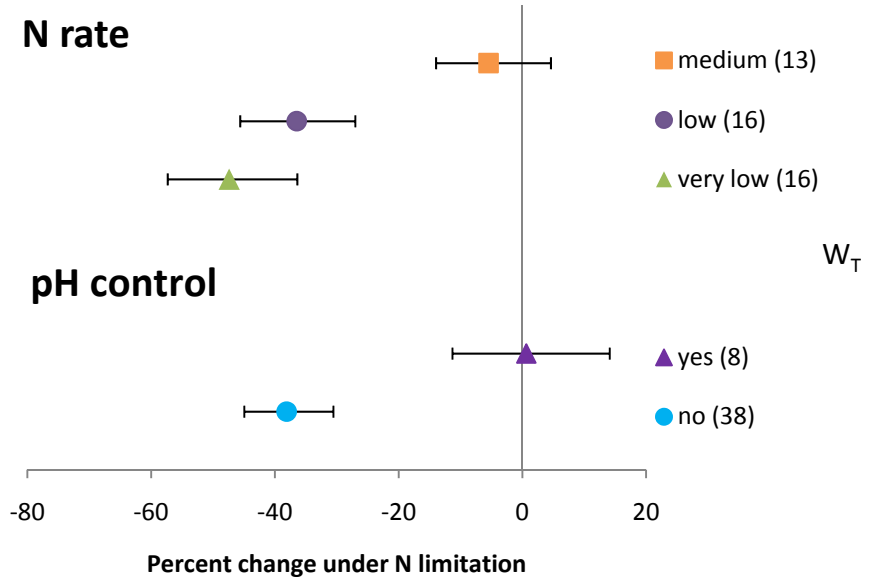


Figure 3.7: The response of total biomass (W_T) to N limitation as influenced by the categorical variables "N rate" and "pH control". Symbols show means surrounded by 95% confidence intervals. Sample sizes (k) for each point are in parenthesis.

For the W_T dataset I made an exception and conducted a categorical analysis within significant classes, as this dataset included a considerably larger number of effect sizes (k = 46). Such an analysis within significant categorical classes is valuable as it allows to identify effects that otherwise might be masked by the stronger effect, e.g. of the N rate. In fact the analysis revealed several significant categorical variables within the "very low N rate" class that did not appear when the analysis was conducted across all data. The categories "species" and "frequency" showed the strongest effect within the "very low N rate" class (Tab. 3.10). The response pattern of the total biomass of different species to N limitation differed slightly from that observed for LA. While soybean still was the species with the smallest decline, the decrease in W_T for wheat supplied with very low N was far less pronounced (-31%, k = 4; Fig. 3.8) than the decline in LA in wheat within the same class (-92%, k = 3; Fig. 3.4). Soybean instead appeared to reduce W_T (-26%, k = 4; Fig. 3.8) to a stronger degree than LA (-19%, k = 4; Fig. 3.4) when supplied with very low N. The crop type classes – as in LA - also differed in their response of W_T to N limitation within the "very low N supply" class (Tab. 3.10); yet the significance level of Q_B for "crop type" – like in LA across all data (Tab. 3.9) - was smaller than that for "species", suggesting that crop species explained variance within the dataset better than the crop type classes.

Interestingly an effect of the N source very similar to that observed for LA (Fig. 3.2) appeared if the analysis was restricted to experiments with a very low N supply (Fig. 3.8). This corroborates the hypothesis outlined above that with ammonium as sole N source the effect of N limitation is less pronounced. However like in the LA dataset the N source effect was confounded by the effect of soybean (see Appendix C, Tab. 3). Similarly the reproductive development stage within the class "very low N supply" was only represented by soybean. Thus again, as with leaf area, the significant effect of the category "development stage" probably was due to the underlying effect of a leguminous species. However the category "leguminous" itself did not have an effect

on the response of W_T to N limitation (Tab. 3.9). Even if the total biomass dataset was categorized into the broader classes leguminous or non-leguminous, these classes did not show a significant Q_B (data not shown). Similarly if the categorical variables "C3/C4" and "leguminous" were combined and the total biomass dataset was categorized into the classes "C4", "C3-leguminous" and C3-non-leguminous" this new categorization showed no significant effect on N limitation (data not shown).

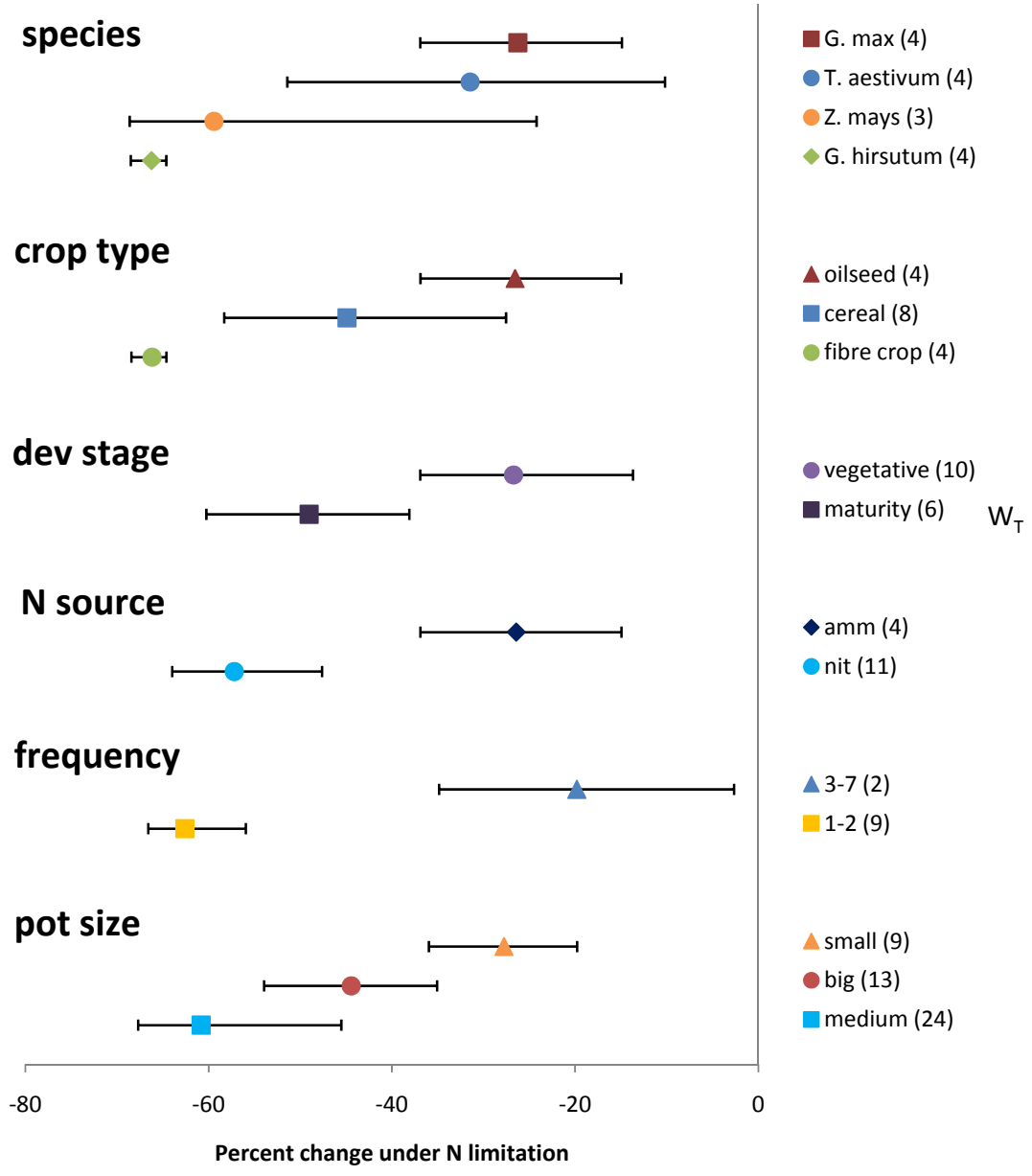


Figure 3.8: The response of total plant biomass (W_T) to N limitation within the "very low N rate" class as influenced by the categorical variables "crop species", "crop type", "development stage", "N source", "frequency of N application" and "pot size". Symbols show means surrounded by 95% confidence intervals. Sample sizes (*k*) for each point are in parenthesis.

Corresponding to the common observation of an increase of C allocation into root organs under a limiting N supply (see 2.2.3), the biomass of the shoot (W_S) declined considerably more (-34%, *k* = 23) than the root biomass (W_R, -4%, *k* = 13), which showed no significant response to N limitation (Fig. 3.1). Concomitantly the root-shoot-ratio (RSR) increased under N limitation (+35%, *k* = 23). The increase in W_R observed for plants grown on a pH controlled medium (Fig. 3.9) – as with W_T - could be attributed to the effect of the study Khamis & Lamaze (1990). Development stage was the only categorical variable that significantly affected the response of W_S to N limitation (Tab. 3.9). The decrease in the shoot biomass under limited N availability was far stronger if plants were grown till maturity (-66%, *k* = 3) than if plants were harvested at the seedling stage (-2%, *k* = 5, n.s.) (Fig. 3.9). The N rate under which plants were grown did not affect the response of root or shoot biomass, but it did show an effect on RSR (Tab. 3.8). The increase in RSR under a very low N supply (+100%, *k* = 7) was more than 11fold stronger than under a medium N supply (+9%, *k* = 8); however the response of RSR at a medium N rate was still significantly different from zero (Fig. 3.9).

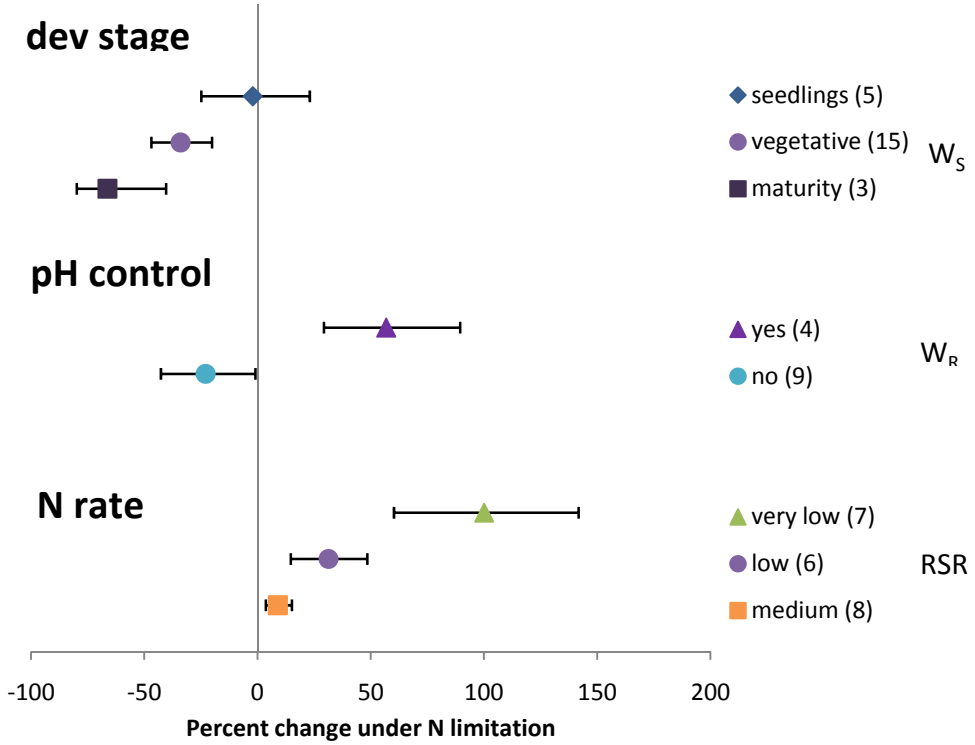


Figure 3.9: The response of shoot biomass (W_S) to N limitation at different development stages, the response of root biomass (W_R) as influenced by the categorical variable "pH control" and of the root-shoot-ratio (RSR) as influenced by the rate of N supply. Symbols show means surrounded by 95% confidence intervals. Sample sizes (*k*) for each point are in parenthesis.

Despite an increase in leaf carbohydrate contents (sugars, + 3% (n.s.), k = 14; non-structural carbohydrates, +71%, k = 14; starch, + 96%, k = 8) the specific leaf area (SLA) did not decline significantly under N shortage (Fig. 3.1). The response of SLA to N limitation was strongly affected by both crop species and crop type (Tab. 3.9), and these two categorical variables showed exactly the same Q_B, as in the SLA dataset every crop type was represented by a single crop species (see Appendix C, Tab. 7). The SLA of barley (*Hordeum vulgare*) increased by 25% (k = 2) while the SLA of cotton decreased by 17% (k = 6) (Fig. 3.10). Yet the significant study effect (Tab. 3.8) as well as the inconsistent responses of plants grown in pots of different sizes and at different N application frequencies (Fig. 3.10), suggest that there might be another underlying source of variation between studies and experiments not explained by the categorical analysis. Under this consideration also the differences in the response of different species and of crops in different development stages to N limitation (Fig. 3.10) have to be taken with reservation.

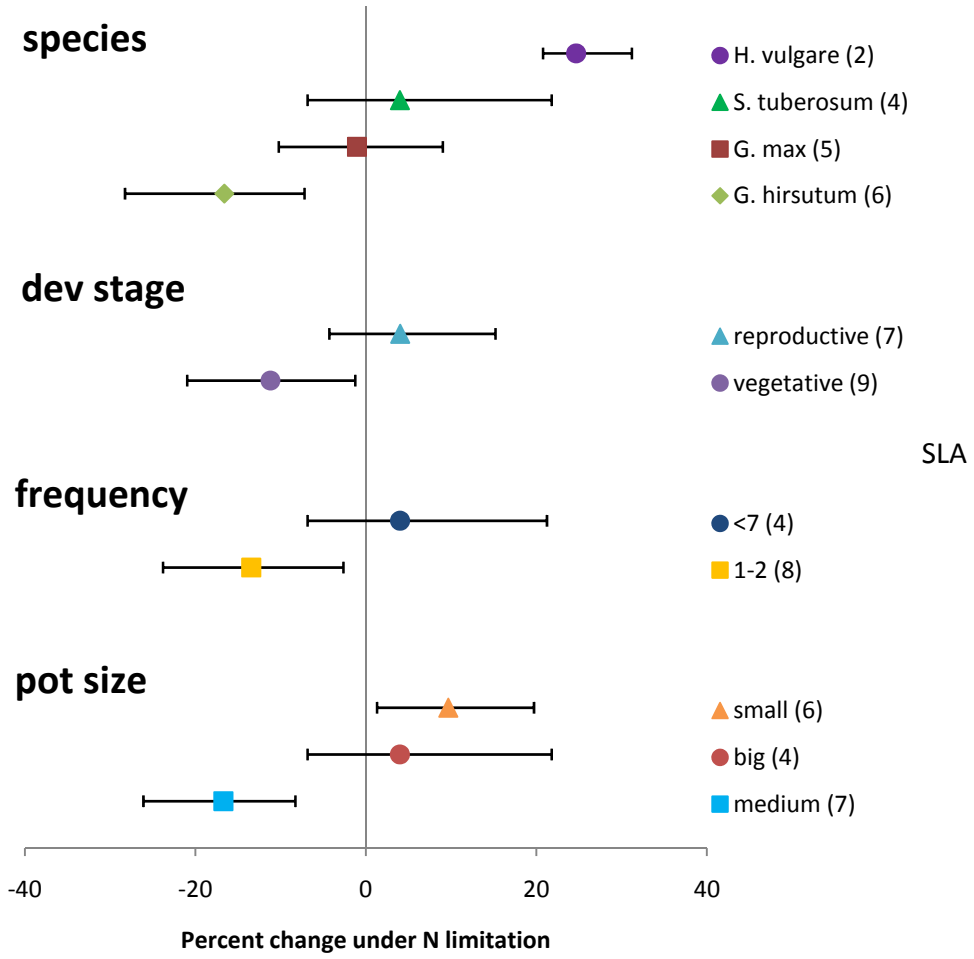


Figure 3.10: The response of specific leaf area (SLA) to N limitation as influenced by the categorical variables "crop species", "development stage", "frequency of N application" and "pot size". Symbols show means surrounded by 95% confidence intervals. Sample sizes (k) for each point are in parenthesis.

While an analysis across different biomes and plant life forms revealed a decrease in SLA with decreasing leaf N content (Reich *et al.* 1997), single studies often show no systematic response of SLA to N limitation (e.g. Chapin *et al.* 1988b; Vos & van der Putten 1998). It would be interesting to examine whether a broader analysis of SLA in crop species would confirm the non-response of SLA in crops to N limitation observed in the present meta-analysis. In addition for the modelling of N processes in crops it would be useful to know whether there really are such significant differences in the response of SLA between different crop species as suggested by the categorical analysis.

Leaf starch contents ($Stch_L$) increased much more than leaf sugar contents (Sug_L) under N limitation, and the increase in the contents of total non-structural carbohydrates (NSC_L) ranged between these two (Fig. 3.1). Response ratios for Sug_L varied considerably and covered a range from -50% to +320% (see Appendix C, Tab. 8). This variation could not be explained by the amount of N received or by any biological characteristic of the crop (Tab. 3.8 and 3.0). Instead Sug_L was affected significantly by N source, frequency of N application and pot size (Fig. 3.11). Yet the results for the categories "N source" and "pot size" are confounded, as all experiments with nitrate as N source used small pots and all experiments with nitrate and ammonium as N source used medium sized pots (see Appendix C, Tab. 8). Therefore the Q_B and the variance for both categorical variables are exactly the same (Tab. 3.8, Fig. 3.11). It is difficult to say whether the patterns in Fig. 3.11 really depict causes for the different responses of Sug_L to N limitation or whether the true causes are other underlying differences that plants experienced during their growth in the different experiments. More values from different studies needed to be included in a categorical analysis in order to be able to formulate hypothesis about the cause of variation in Sug_L.

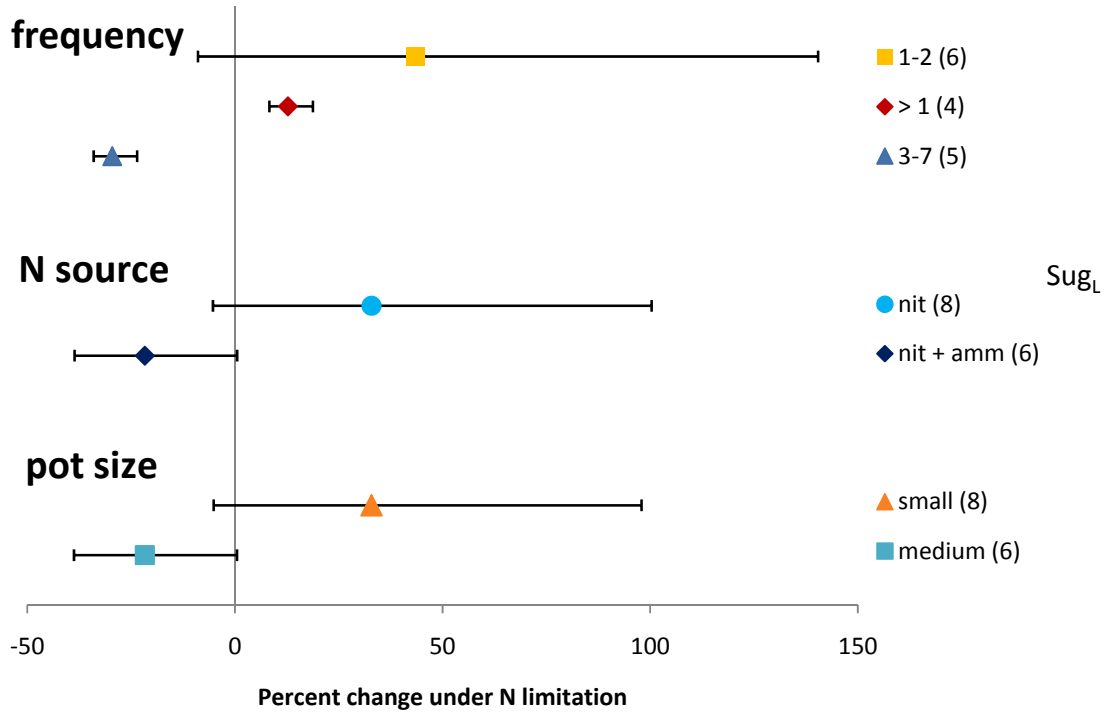


Figure 3.11: The response of leaf sugar contents (Sug_L) to N limitation, as influenced by the categorical variables "frequency of N application", "N source" and "pot size". Symbols show means surrounded by 95% confidence intervals. Sample sizes (*k*) for each point are in parenthesis.

There was no evidence for any significant categorical effect on $Stch_L$ under N limitation (Tab. 3.8 and 3.9), suggesting that there were no significant differences in the N limitation response of leaf starch contents between the categories examined. The response of NSC_L instead was significantly affected by the growth CO_2 concentration. The increase in NSC_L under ambient CO_2 concentrations (+ 119%, k = 9) was more than eightfold stronger than under elevated CO_2 concentrations (+ 14%, k = 5) (Fig. 3.12). This result could be due to the considerable accumulation of carbohydrates observed in plants grown at elevated CO_2 even under N-sufficient conditions (Stitt 1991; Bowes 1993). Thus, the increase in carbohydrates associated with N limitation might not be as marked under elevated CO_2. Still the analysis of Sug_L and $Stch_L$ showed no effect of growth CO_2 concentration on the N limitation response (Tab. 3.8). However, only two effect size values in these analyses were associated with an elevated CO_2 concentration (see Appendix C, Tab. 8 and 9) and the inclusion of more data from experiments with elevated CO_2 probably might show a difference in the N limitation response.

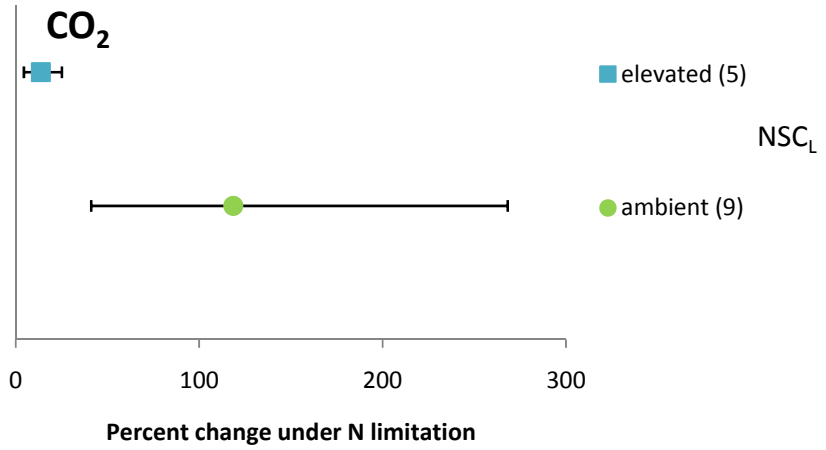


Figure 3.12: The response of leaf non-structural carbohydrate contents (NSC_L) to N limitation, as influenced by the growth CO_2 concentration. Symbols show means surrounded by 95% confidence intervals. Sample sizes (k) for each point are in parenthesis.

3.2.2 N allocation

All parameters describing N allocation declined significantly under N limitation (Fig. 3.13). Total N content (N_T) declined by -36% (k = 25), while the N content of leaves (N_L) declined by -38% (k = 24). As the unit, i.e. whether values were reported on a leaf area or a mass basis, had a significant effect on N_L (Tab. 3.8) the analysis of N_L was conducted a second time, constricted to effect sizes which were reported on an area basis. The analysis of effect sizes on a mass basis could not be carried out separately because there were not enough studies reporting N_L in this unit. N_L on an area basis decreased slightly less (- 33%, k = 18) than all N_L values together (Fig. 3.13). The magnitude of the N supply had a significant effect on the response of N_L to N limitation, whether reported on both area and weight basis or only on an area basis (Tab. 3.8). The categories “very low”, “low” and “medium” N supply differed significantly from each other

in their response to N limitation (Fig. 3.14). While N_L declined by -51% if the crop was exposed to a very low supply (-49% if only area based values were considered), the decline was only –17% under a medium N supply (-15% for area based values respectively).

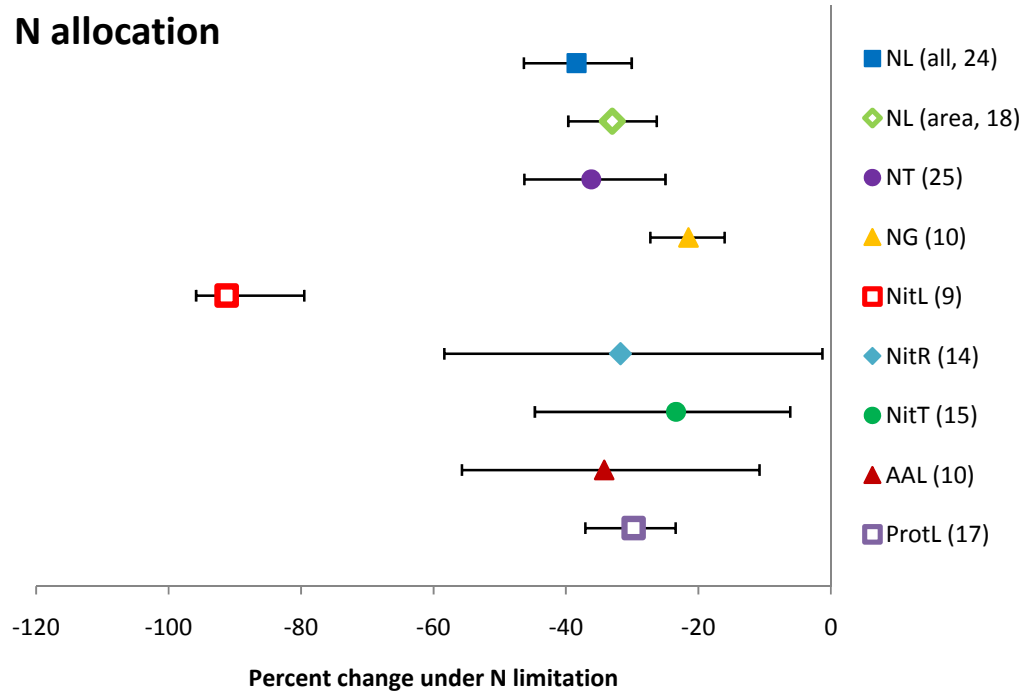


Figure 3.13: The response of parameters describing N allocation to a limiting N supply. Leaf N content (N_L) is shown for all effect sizes together as well as restricted to the effect sizes on an area basis. Symbols show means surrounded by 95% confidence intervals. Sample sizes (*k*) for each point are in parenthesis. Abbreviations: N_T: N content of whole plant; N_G: grain N content; Nit_L: leaf nitrate content; Nit_R: root nitrate content; Nit_T: whole plant nitrate content; AA_L: free amino acids in leaves; $Prot_L$: leaf soluble protein content.

The strong and significant decline of N_L yet was not affected by the crop species that was analyzed or by any other experimental or biological category (Tab. 3.8 and 3.9). The effect of the category "leguminous" on all N_L values pooled together (Tab. 3.9) was due to an underlying unit-effect, as four of the six weight based N_L effect sizes described a leguminous species (*Glycine max*; see Appendix C, Tab. 12). If just the pairs of means on a weight basis were analyzed, the category "leguminous" did not have any effect on N_L (data not shown). Here it would be interesting to examine whether there are any categorical effects within the different N rate classes in order to avoid the possible disguising effect of the N rate on the N limitation response. However as discussed before such a division of the dataset would need a larger sample size to yield useful results.

The strong decline of N_L under N limitation contradicts, at least for the crop species that were included in the analysis, the theory that plants do not change their leaf N content but instead adapt their canopy size to maintain a relatively constant, optimal leaf N content under N limitation (see discussion in 2.2.1). Crops probably follow a miscellaneous strategy and under a

limited N supply both decrease the canopy size (see 3.2.1) and the N content of leaves. Grindlay (1997) hypothesized that under N limitation plants might give priority to the maintenance of a constant N_L, compared to the maintenance of LA, except under severe N limitation, where they might reduce N_L more strongly. While the first part of this hypothesis was supported by the present analysis of crops, as LA declined more strongly (by -23% under medium N supply, Fig. 3.2) than N_L (by -15% at medium N, Fig. 3.14), showing a 1.5fold stronger decrease under medium N limitation, this priority did not change under stronger N limitation (under very low N supply LA decreased by 70% and N_L by 49%, which again marked an approximately 1.5fold stronger decrease in LA).

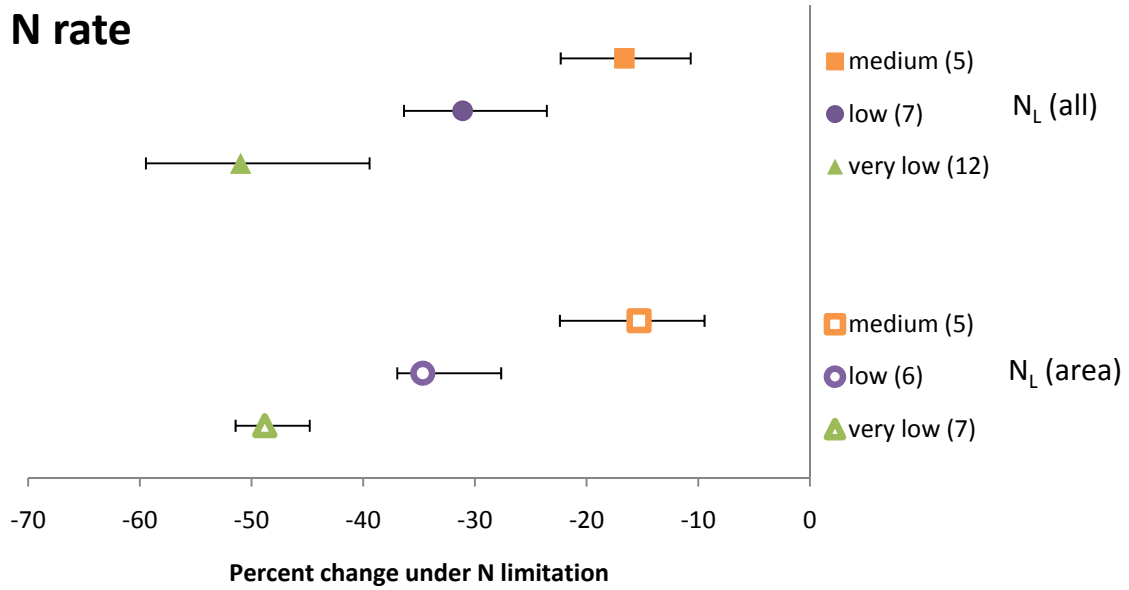


Figure 3.14: The effect of the magnitude of the N supply received by crops on the response to N limitation of leaf N contents (N_L) both on an area and a weight basis (i.e. all, above) or only on an area basis (below). Symbols show means surrounded by 95% confidence intervals. Sample sizes (*k*) for each point are in parenthesis.

The analysis of the N content of grains (N_G) was restricted to cereals, namely barley and wheat (see Appendix C, Tab. 13), as no data on seed or fruit N contents for other crops could be found. The duration of N limitation was the only categorical variable that had a significant effect on the response of N_G to N limitation (Tab. 3.8). The rate of the N supply probably did not have any effect on N_G because all N supplies given in experiments that measured N_G were either low or very low (see Appendix C, Tab. 13). If plants were grown for their entire growth with a limiting N supply the N_G declined by -28% (k = 4). If instead they were grown only for half of their growth under limited N, N_G declined only by -17% (k = 6; Fig. 3.15). Yet these differences between the categories describing the duration of N limitation were not significant, as their confidence intervals did overlap (Fig. 3.15). In general the categorical analysis of N_G is of only limited validity because of the small sample size (k = 10) and as several categories could not be analyzed at all. Overall N_G declined to a smaller degree (- 22%) than total or leaf N content. This held true even if the analysis of N_L was restricted to cereals (-39%, k = 11; data not shown). The values for single effect sizes of N_G ranged between -8% and -38% (see Appendix C, Tab. 13) but

were far from reaching the maximal responses observed for N_L for cereals (-61%; see Appendix C, Tab. 12). These results suggest that in cereals under N limitation seeds have the highest N allocation priority. It would be interesting to test whether this pattern can also be observed in other crop species.

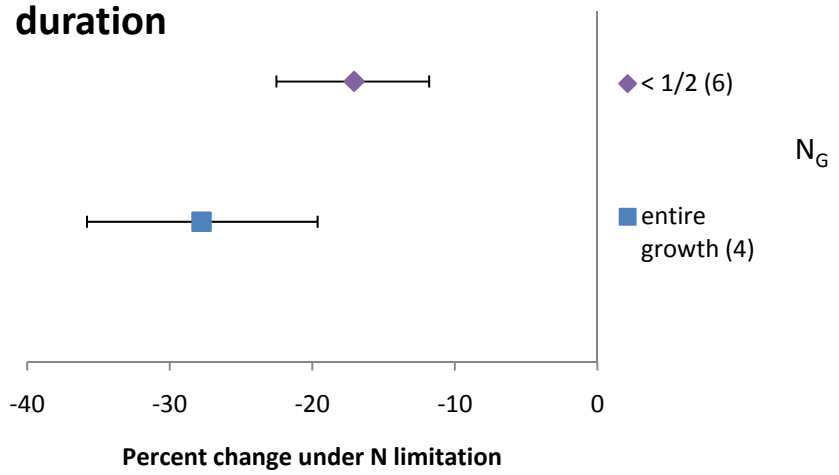


Figure 3.15: The effect of the duration of N limitation on the response of grain N contents (N_G) to N limitation. Symbols show means surrounded by 95% confidence intervals. Sample sizes (*k*) for each point are in parenthesis.

The results for N allocation from a single study (Devienne *et al.* 1994) were clearly distinct from those of other studies. The effect sizes for nitrate contents in roots (Nit_R), total nitrate contents in plant (Nit_T) and N_T from this study all showed considerable positive effects or small negative effects of N limitation (see Appendix C, Tab. 14, 16 and 17). The outlier role of this particular study merits further examination, wherefore an analysis was conducted excluding the values from Devienne *et al*. (1994). If the effect sizes from this study were excluded (Tab. 3.11), the category "study" no longer showed a significant effect on N_T and Nit_T. Thus the study effect revealed in the analysis of heterogeneity for these two parameters if all studies were included was due to the variation brought in by the study Devienne *et al.* (1994). Also the effects of the experimental and biological categorical variables differed considerably depending on whether the results from Devienne *et al.* (1994) were included or not. For N_T and Nit_T only the category "N rate" showed a significant effect if Devienne *et al.* (1994) was excluded (Tab. 3.11). For Nit_R instead there still was a study effect and several categories that varied in parallel with the different studies showed a significant effect. This suggests that the remaining studies still had some significant underlying variation in the response of root nitrate contents to N limitation that could either be explained by the significant categorical variables or - probably more likely - that was due to differences in the experimental setup, not described by the categorical variables.

The results concerning the effect of different N supplies on biomass (W_T) confirmed that the experimental N supplies (apart from the medium and high N rate; see Appendix C, Tab. 3) in the study of Devienne *et al.* (1994) were limiting for plant growth. Thus the unusual response of N allocation parameters in this study were not due to a wrong categorization of the relevant N rates

as limiting or non-limiting. What other reason there might be for the non-decrease of N and nitrate contents under N limitation in the study concerned remains uncertain. The authors themselves attribute the observed nitrate accumulation under N limitation to the water culture method. In the present analysis the category "medium" did show a significant effect on all three N allocation parameters in question (Tab. 3.8). Yet if the study Devienne *et al.* (1994) was excluded, the medium on which plants were grown no longer had any effect on the response variables (Tab. 3.11).

Table 3.11: Between-group heterogeneity (Q_B) for the categorical analysis of the response variables total plant N content (N_T), total plant nitrate content (Nit_T) and root nitrate content (Nit_R) among *k* pairs of means, excluding the study Devienne *et al.* (1994).

Variable	*k*	Study	N rate	Duration	Freq.	pH	Medium	Pot	$[CO_2]$	Species	Crop	C3/C4	Dev.
N_T	18	1.42	**15.98*****	-	-	-	1.28	1.28	1.87	2.08	0.96	0.18	1.42
Nit_T	8	0.44	**56.51*****	0.44	0.44	0.44	-	-	-	0.44	-	0.44	0.44
Nit_R	7	**4.74***	0.43	-	-	1.28	1.28	**7.87****	-	1.28	-	1.28	**7.87****

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

In addition also several other categories that varied between the studies showed a significant effect across all studies. The effect of the categories pot size, pH control, frequency of N application, duration of N limitation, species and C3/C4 photosynthetic pathway probably can be ruled out as explanations for the unusual response of Nit_T, Nit_R and N_T observed in Devienne *et al.* (1994), as the corresponding classes differed in a manner that would not be expected from theoretical considerations (e.g. the plants experiencing more frequent N application show a stronger decline in Nit_T; data not shown) and thus their significant effect probably was only due to a parallelism with the true cause for the observed variation. The category development stage in Nit_T instead differed in a manner that possibly could depict a true underlying cause (Fig. 3.16), i.e. the nitrate content of seedlings decreased more strongly than that of vegetative plants. Yet this category did not show any effect in Nit_R and N_T if the study Devienne *et al.* (1994) was considered (Tab. 3.8); if this study was excluded the response of nitrate in roots in different development stages even was opposite to that observed for total nitrate content (Fig. 3.16). Thus the significant effect of the category "development stage" probably also was an artefact of the variation between studies, which true cause remains unclear. If one wanted to dissect the true underlying reason for the observed accumulation of N under N limitation in the study of Devienne *et al.* (1994) and for the observed variation in the response of Nit_R between studies (Tab. 3.11), one would need to include more results from several more studies with varying experimental conditions in the analysis.

If the study Devienne *et al.* (1994b) was excluded from the categorical analysis of N_T and Nit_T, as mentioned above, no study effect was observed any more (Tab. 3.11). The results from this categorical analysis thus seem not to be confounded by an underlying study effect and can be interpreted more easily. The response to N limitation of both parameters was significantly affected by the magnitude of the N supply under which plants were grown (Fig. 3.17). No further categorical variable affected the response of Nit_T and N_T to N limitation (Tab. 3.11).

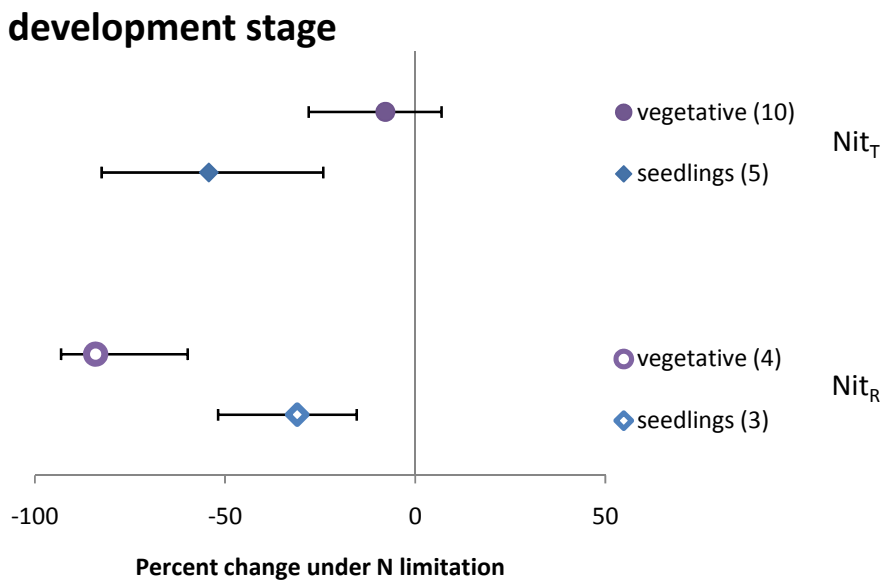


Figure 3.16: The effect of the category development stage on the response to N limitation of total nitrate contents in the plant (Nit_T) across all studies and of nitrate contents in root (Nit_R), when excluding the study of Devienne *et al.* (1994). Symbols show means surrounded by 95% confidence intervals. Sample sizes (*k*) for each point are in parenthesis.

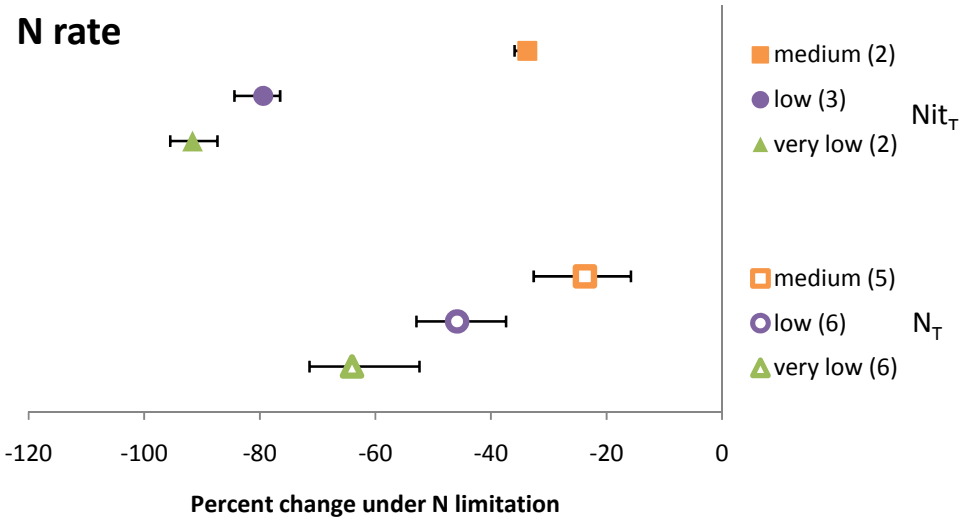


Figure 3.17: The effect of the magnitude of the N rate received by crops on the response to N limitation of total nitrate (Nit_T) and total N contents (N_T) in the plant. Symbols show means surrounded by 95% confidence intervals (omitted when smaller than the size of the symbol). Sample sizes (*k*) for each point are in parenthesis.

While the nitrate content in the leaf (Nit_L) declined strongly across all experiments (with single effect sizes ranging between -98% and -35%; see Appendix C, Tab. 15), the response of the nitrate contents in the root (Nit_R), as discussed above, varied much more considerably between studies and experiments (ranging between -94% and + 40%; see Appendix C, Tab. 16). Still N limitation showed a significant negative effect on both Nit_L (-91%, k = 9) and Nit_R (-32%, k = 14). If the study Devienne *et al.* (1994) was excluded from the analysis the decline in Nit_R was considerably stronger (-69%, k = 7) but still smaller than the decline in Nit_L (Fig. 3.18).

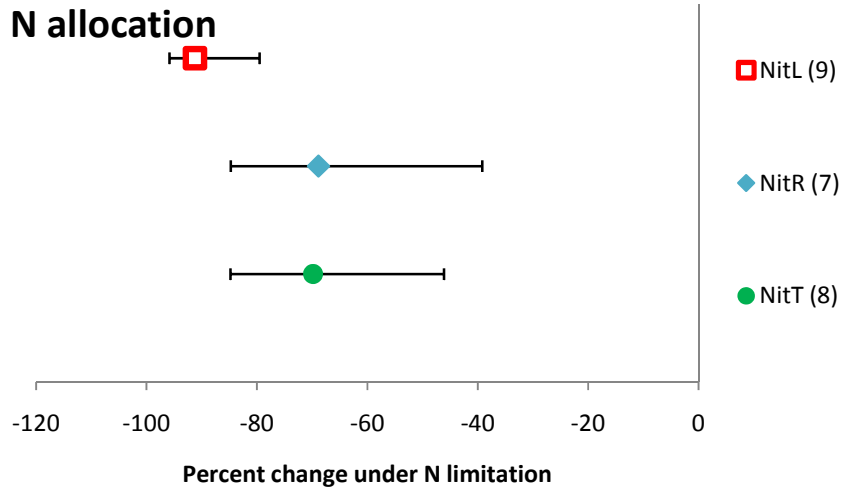


Figure 3.18: The response of nitrate contents in leaves (Nit_L), in roots (Nit_R) and in the whole plant (Nit_T) to N limitation if the study Devienne *et al.* (1994) was excluded from the analysis. Symbols show means surrounded by 95% confidence intervals. Sample sizes (k) for each point are in parenthesis.

It is generally believed that under a low external nitrate supply a high proportion of nitrate is reduced in the roots and transported to shoots in reduced form. With increasing supply of nitrate instead, the capacity for nitrate reduction in the roots becomes a limiting factor and an increasing proportion of total N is translocated to the shoot in the form of nitrate (Marschner 1995). This belief is corroborated by the results of the meta-analysis, as they show that under N limitation the nitrate contents in the leaves decline more than those in the roots. The strong decrease in leaf nitrate contents can also be interpreted as depletion of vacuolar nitrate storage pools (as discussed in 2.1.6.3).

The categorical analysis of Nit_L again showed a significant study effect on the response of Nit_L to N limitation (Tab. 3.8). Thus the response of leaf nitrate contents to N limitation differed between the studies incorporated in the analysis due either to differences in the experimental setup or due to biological differences of the crop species involved. The significant Q_B of the categorical variables “pH control” and “pot size” suggests that these experimental characteristics could influence the response of Nit_L to N limitation. In experiments, in which the acidity of the growth medium was controlled and held approximately constant, Nit_L declined only by -66% (k = 3); if the acidity instead was not controlled, Nit_L declined by -95% (k = 6) (Fig. 3.19). Yet as only one study included in the Nit_L analysis controlled the pH (being the same study that was

responsible for the significant pH effect on W_R and W_T; see 3.2.1) it is not clear whether this effect was really due to this experimental characteristic or whether the effect just depicts variation that is due to some other difference in this study. Similarly the effect of the category "pot size" seems not to depict a real effect, as the classes of the category responded in a manner that does not qualify them as explanatory effects (i.e. crops grown in medium pots had a larger decrease in Nit_L than both crops grown in small or big pots; Fig. 3.19). The classes of the category "pot size" differed between different studies (see Appendix C, Tab. 15) and their significant Q_B probably just depicts the significant variation between studies, without being the cause for this variation. The N rate classes instead also changed within studies (see Appendix C, Tab. 15) and the differences in the response of Nit_L between the N rate classes (Fig. 3.19) depict a pattern that is sensible, i.e. crops grown with very low N supply decreased Nit_L by -96% (k = 5), while crops grown with a medium N supply decreased Nit_L only by -44% (k = 3). Thus the response of Nit_L probably was truly affected by the rate of N supply. Biological categories like crop species or photosynthetic pathway interestingly had no effect on Nit_L (Tab. 3.9) or Nit_R (Tab. 3.11), suggesting that the species present in the analysis, namely maize and potato for Nit_L and maize and barley for Nit_R respectively, did not differ in the way or degree in which they depleted nitrate pools under N limitation. Yet the meta-analysis only examines the degree of change under N limitation and does not tell anything about the absolute amount of nitrate present in the plants under an optimal or limiting N supply.

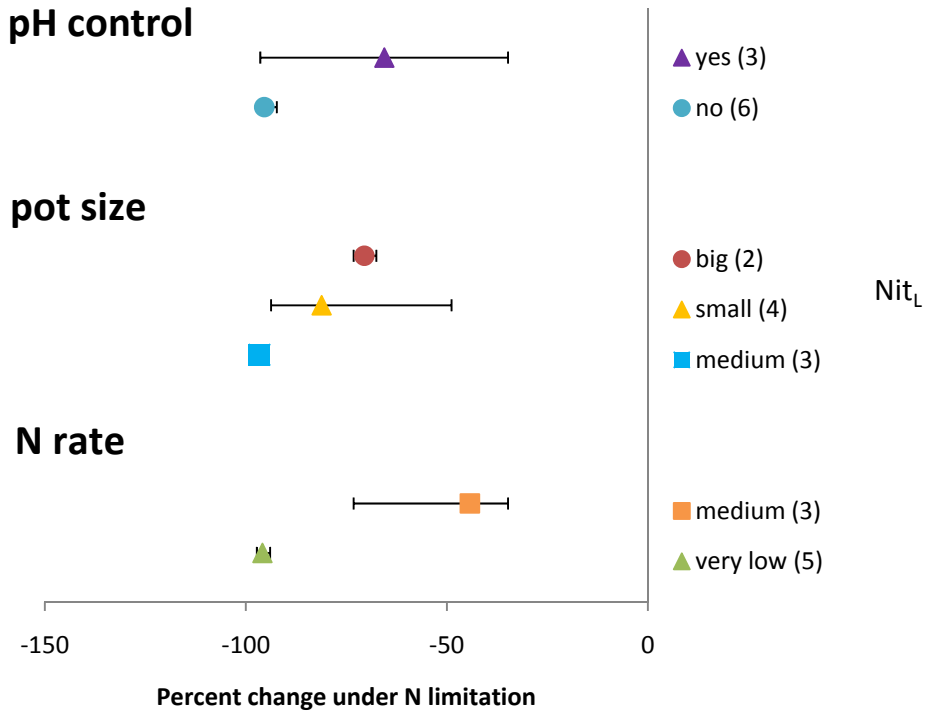


Figure 3.19: The response of nitrate contents in leaves (Nit_L) to N limitation, as influenced by the categorical variables "pH control" and "pot size" and "N rate". Symbols show means surrounded by 95% confidence intervals (omitted when smaller than the size of the symbol). Sample sizes (k) for each point are in parenthesis.

Nitrogen limitation caused a 34% decline in leaf free amino acid contents (AA_L, k = 10) and a 30% decline in leaf soluble protein contents ($Prot_L$, k = 17) (Fig. 3.13). Free amino acids in leaves, like nitrate, provide a storage pool of N when N is available to the plant at an abundant supply (see discussion in 2.1.6.3). Under N limitation the free amino acids are then used for the synthesis of proteins. Soluble proteins instead represent both a storage pool under abundant N (see 2.1.6.3) and a pool of active metabolic enzymes, which in leaves are involved mainly in photosynthesis (see 2.2.1.3). It is thus not surprising that free amino acid contents declined more than soluble proteins under N limitation, as N reserves - be they nitrate, amino acids or proteins - are used up under N shortage, while active metabolic proteins are still needed to maintain leaf processes. The amino acid contents measured in different experiments and studies included in the meta-analysis varied to a stronger degree than the protein contents (Fig. 3.13) but the overall ranges that values of single effect sizes spanned were similar (between +11% and -82% for AA_L and -2% to -86% for $Prot_L$; Appendix C Tab. 18 and 19). The unit in which AA_L or $Prot_L$ were expressed did not have an effect on their N limitation response (Tab. 3.8). There were instead significant differences in the response of AA_L and $Prot_L$ to N limitation between different N rates (Tab. 3.8). AA_L declined under very low supply almost 60 times as much as under medium N supply and it showed a fourfold stronger decrease under very low than under low N supply (Fig. 3.20). In $Prot_L$ the differences between the N rate classes were not as large, but there still was an almost threefold decrease under very low N supply compared to a medium N supply.

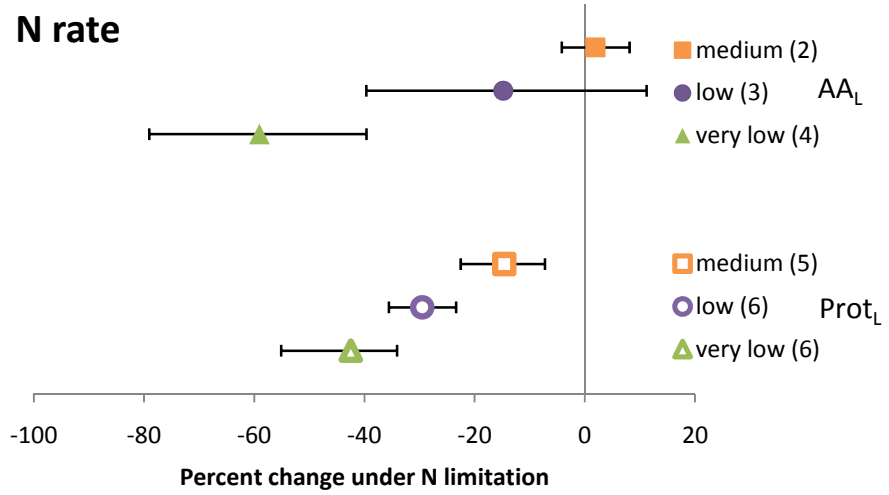


Figure 3.20: The effect of the magnitude of the N rate received by crops on the response to N limitation of leaf amino acid contents (AA_L) and leaf protein contents ($Prot_L$). Symbols show means surrounded by 95% confidence intervals. Sample sizes (k) for each point are in parenthesis.

The photosynthetic pathway of the crop species examined did have a significant effect on both AA_L and $Prot_L$ (Tab. 3.9). The decrease in the amino acid content of leaves of C3 species (-43%, k = 7) was 7 times stronger than that in C4 species (-6%, k = 3). The differences in the response of protein content were not as marked (-36%, k = 8 in C3 and – 24%, k = 9 in C4 species). Yet the results for AA_L are confounded by the fact that all experiments involving C4 species did

control the pH, while all experiments concerning C3 species did not control the pH of the growth medium (see Appendix C, Tab. 18) and the category "pH control" thus had exactly the same significant Q_B as the category "C3/C4" (Tab. 3.8 and 3.9). Thus both the effects of the categories "pH control" and "C3/C4" on AA_L have to be taken with caution, as it is not clear which of the two is the one that causes this pattern. The way in which the N_L of C3 and C4 species responds to N limitation could provide an indication whether there is a difference in N allocation under N limitation between species with different photosynthetic pathways, but unfortunately the effect of the category "C3/C4" on N_L could not be examined, as the data that was included in this analysis concerned only C3 species (see Appendix C, Tab. 12).

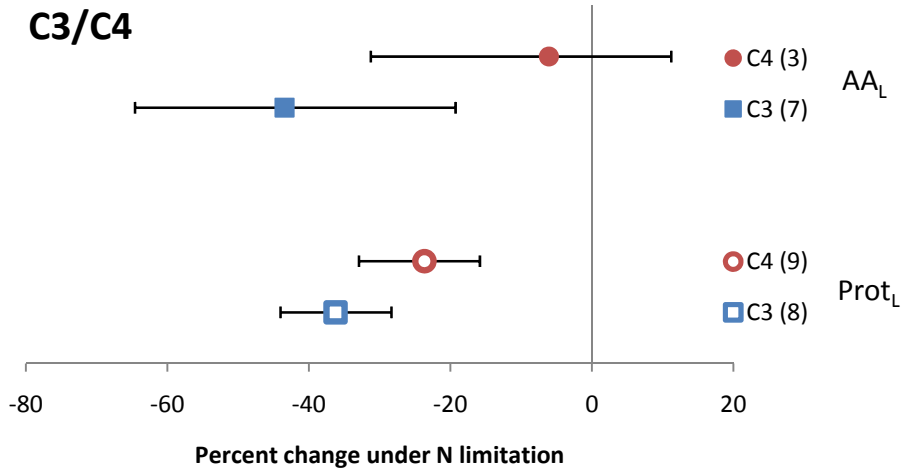


Figure 3.21: The differences in the response of leaf amino acid contents (AA_L) and leaf protein contents ($Prot_L$) to N limitation between C3 and C4 species. Symbols show means surrounded by 95% confidence intervals. Sample sizes (*k*) for each point are in parenthesis.

3.2.3 Photosynthesis and N uptake

While net photosynthesis (A) and the contents and activities of photosynthetic components, i.e. leaf chlorophyll content (Chl) and Rubisco activity (Rub), showed a significant response to N limitation and a comparably small variation, stomatal conductance (g_s) and N uptake (N_{up}) did not differ significantly from zero and showed a large variance (Fig. 3.22), on account of a small sample size involving effect sizes varying across a broad range. Values included in the analysis of N_{up} for example spanned a range from + 330% to -75% (see Appendix C, Tab. 24) and the analysis of heterogeneity not surprisingly showed a strong study effect (Tab. 3.8). It is therefore not possible to identify the source of this variation. The frequency of N application, the duration of N limitation and the control of the pH of the growth medium might all contribute to the differences observed in the response of N_{up} to N limitation, but this heterogeneity might as well be due to some other experimental difference between the studies that were included in the analysis. It is unlikely that the variation is due to biological characteristics as all experiments, except one, involved barley as study species (see Appendix C, Tab. 24). Probably in N uptake

experiments several other experimental characteristic not considered in the present categorical analysis (e.g. whether measurements were restricted to the low- or high-affinity uptake system, i.e. whether N_{up} was measured from low or high measurement solutions) might be relevant. A meta-analysis of N_{up} might therefore need an adaptation of methods, concerning the requirements for studies to be included in the analysis (in order to achieve better comparability of N uptake experiments) and an adaptation of the categorical variables examined (in order to be able to identify those experimental characteristics responsible for differences).

Similar holds true for the analysis of g_s: a large variation was observed in effect sizes (ranging between +95% and -58% change under N limitation; see Appendix C, Tab. 23) that probably could be attributed to experimental or biological differences between measurements. However no significant study effect was revealed (Tab. 3.8). Stomatal conductance is dependent on factors like light intensity, CO_2 concentration and humidity (Farquhar & Sharkey 1982). Thus probably – as for N_{up} – a more detailed meta-analysis of g_s might need an adaptation of methods, e.g. by including categorical variables like "air water pressure" or "light intensity during measurements". Of course any useful meta-analysis of N_{up} and g_s first needed to cover a broader range of data in order to be able to identify reasons for the large variation in the response to N limitation.

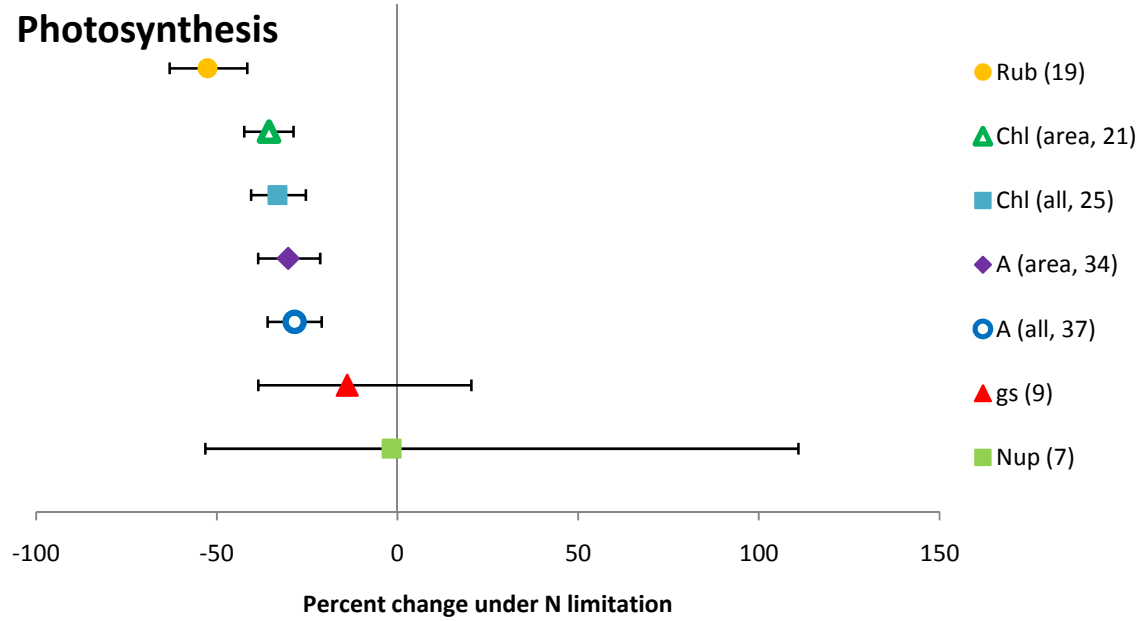


Figure 3.22: The response of parameters concerned with photosynthesis and N uptake to N limitation. Symbols show means surrounded by 95% confidence intervals. Sample sizes (*k*) for each point are in parenthesis. Abbreviations: Rub: Rubisco activity; Chl: chlorophyll content; A: photosynthesis; g_s: stomatal conductance; N_{up}: N uptake rate.

Photosynthesis on the contrary showed a significant response despite the variation in experimental methods and measurement characteristics. The values included in the analysis derived both from measurements of A under growth light intensities (k = 21) as well as under light saturation (k = 16). A categorical analysis of the light intensity during measurements

however did not show a significant effect on the response of A to N limitation (Q_B = 0.32, p = 0.57). The category "unit" did neither have a clearly significant effect on photosynthesis (Q_B = 3.09, p = 0.079) or Chl content (Q_B = 3.15, p = 0.076; see Tab. 3.8). But as the Q_B for "unit" was close to the significance level I decided to conduct a separate analysis restricted to area-based values (there were not enough values for a separate analysis of A or Chl on a weight-basis). This also eases the interpretation of results. The significance of Q_B for the different categories did not differ if the analysis of photosynthesis was restricted to area based values or if all values were included, except for the category "duration of N limitation" which only had a significant effect on area-based photosynthesis (Tab. 3.8). All results discussed in the following section will therefore just refer to A on an area basis. The significance levels of Q_B for Chl instead differed depending on whether all Chl effect sizes were taken or whether only those on an area-basis were considered (Tab. 3.8) For all Chl values pooled together there appeared significant effects of "frequency", "medium" and "pot size", but these significances could be put down to single classes all belonging to weight-based values from the study Khamis & Lamaze (1990). Thus – as for A – only the results for Chl on an area basis will be further discussed.

Wheat was the crop species with the smallest decline in A under N limitation (-5%, *k* = 6) and this decline was not significantly different from zero (Fig. 3.23); it was instead significantly different from the response of maize (-31%, *k* = 11), cotton (-34%, *k* = 6) and rice (*Oryza sativa*; -53%, *k* = 4). The crop type of the study species did not have any effect on the response of A to N limitation and neither did the photosynthetic pathway or the fact whether it was a leguminous species (Tab. 3.9). The missing significance of the category "crop type" probably could be attributed to the large difference in the response of wheat and rice which both are cereals (Fig. 3.23). Interestingly wheat showed only a very small and non-significant decrease in A (although the respective experiments involved mainly very low N rates; see Appendix C, Tab. 22), whereas its decrease in LA was very marked (-87% across all data; see 3.2.1). However, four out of the six effect sizes for A in wheat came from the same study that delivered the data for LA of wheat (Evans 1983; see Appendix C, Tab. 2 and 22) and it is therefore yet not possible to conclude that the pattern depicted shows a general strategy of wheat. In both maize and cotton the degree of decrease in A compared to LA was similar: while maize showed a -48% decline of LA (Fig. 3.3) and a -31% decline in A (Fig. 3.23), cotton decreased LA by -58% and A by -34%, which in both species marked a 1.5- to 1.7fold stronger decrease of LA compared to A. Thus while A (-30% across all species, Fig. 3.22) and N_L (-33% across all species, Fig. 3.13) on an area basis responded in a very similar manner to N limitation, LA of maize and cotton decreased approximately one and a half times stronger than both A and N_L under N limitation. Soybean instead differed from this pattern and showed a slightly stronger decrease of A (-20%, *k* = 6) than of LA (-16%), but with both responses being considerably smaller than the cumulative means of all species. This smaller decrease of LA and A probably can be attributed to the N_2-fixing activity of soybean nodules.

Photosynthesis declined stronger under elevated (-44%, *k* = 10) than under ambient (-23%, *k* = 24) growth CO_2 concentrations (Fig. 3.23). A possible explanation for this difference could be based on the fact that at an optimal N supply an increase in the CO_2 concentration leads to an increase in the photosynthetic rate (Bowes 1993; Stitt & Krapp 1999). Under N limitation instead elevated CO_2 concentrations might bring no further or at least not as large a benefit as under N sufficient conditions, leading to a larger decrease of A under N limitation in elevated CO_2. Bowes (1993) however states that plants under N-limited conditions still experience a stimulation of A by CO_2 enrichment, often to about the same degree as under optimal N supply.

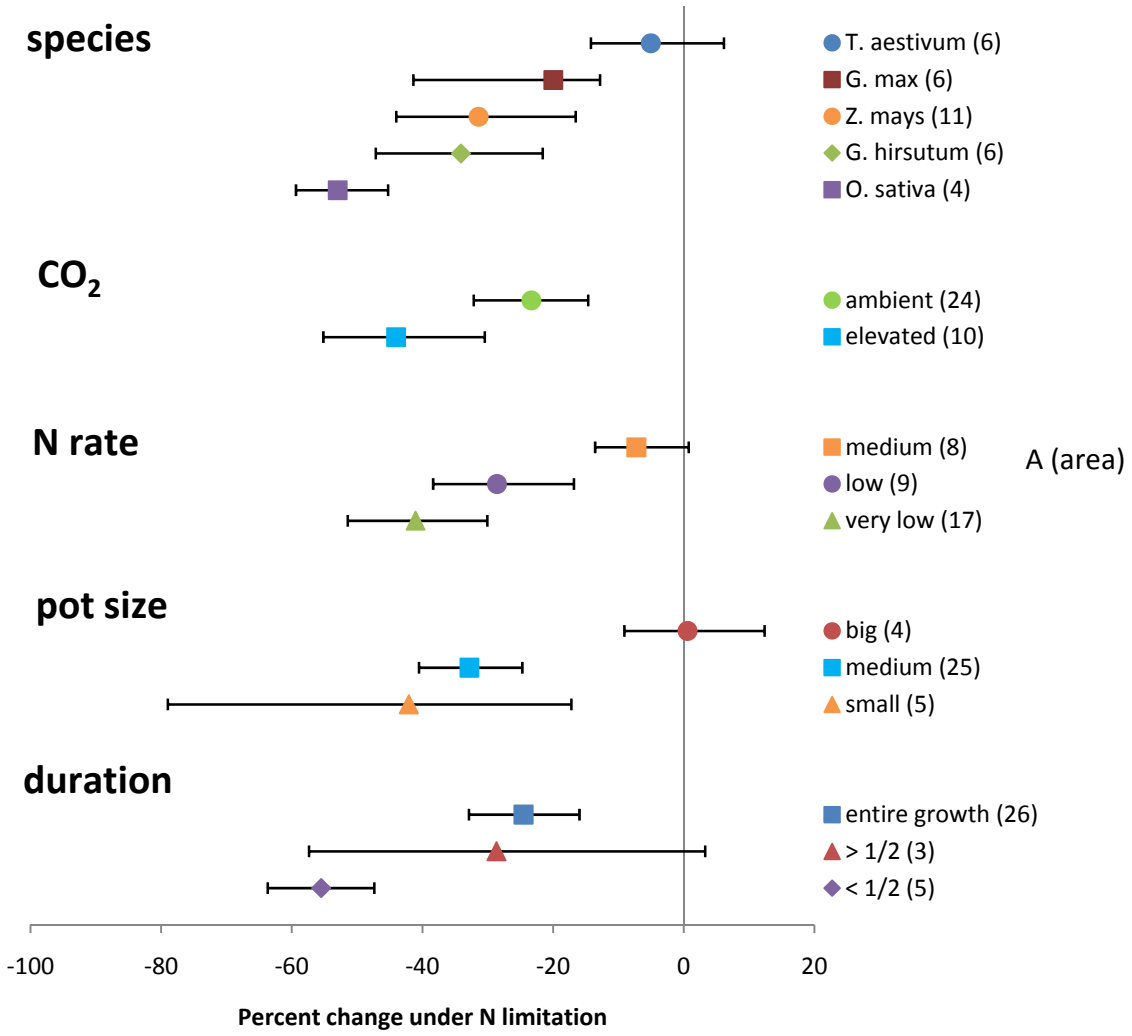


Figure 3.23: The response of photosynthesis (A) on an area basis to N limitation, as influenced by the categorical variables "species", "[CO_2]", "N rate", "pot size" and "duration of N limitation". Symbols show means surrounded by 95% confidence intervals. Sample sizes (k) for each point are in parenthesis.

The only other parameters that were influenced by the CO_2 concentration in the present meta-analysis were NSC_L and RGR (Tab. 3.8) (N_T showed no effect of CO_2 concentration if the study effect was excluded, Tab. 3.11). However the response of RGR under elevated CO_2 rather suggests that the decline in growth under N limitation is less pronounced at elevated CO_2 (see 3.2.1), which would fit more into the view expressed by Bowes (1993). It would therefore be interesting to test whether the observed difference in the response of A and RGR in crops to N limitation under different growth CO_2 concentrations can still be observed if a larger dataset is analysed by means of a meta-analysis.

Crops grown for less than half of their growth period with limited N (-56%, k = 5) showed more than twice the reduction in A under N limitation than crops grown for their entire growth period under limited N supply (-25%, k = 26; Fig. 3.23). This pattern could be interpreted as an

indication for photosynthetic acclimation to N limitation. Photosynthetic acclimation involves changes in the composition of the photosynthetic apparatus (see 2.2.1.3). From the review of the literature in part 2 no consistent pattern emerged as to the response of the photosynthetic apparatus to N limitation. Thus a meta-analytic examination of how different photosynthetic components change in crops under N limitation would help to assess whether the response at different durations of N limitation depicted in Figure 3.23 can be reduced to an acclimation of the photosynthetic components. Unfortunately the present meta-analysis could not examine the response of Rubisco contents to N limitation, as not enough data on Rubisco content could be gathered. However the response of Chl and Rubisco activity (Rub) could give indications about a possible photosynthetic acclimation. Chl on an area basis declined by -36% (k = 21) across all experiments (Fig. 3.22). The N rate was the only categorical variable that affected the response of Chl (Tab. 3.8). All N rates differed significantly from each other, with Chl declining by -46% (k = 9), -31% (k = 8) and -15% (k = 4) in the very low, low and medium class respectively (Fig. 3.24). If one compares this response with the response of total N_L, a remarkable similarity appears: N_L on an area basis declined by -49% (k = 7), -35% (k = 6) and -15% (k = 5) in the very low, low and medium class respectively (Fig. 3.14). This parallelism suggests that the proportion of Chl to N_L does not change strongly under N limitation. Rub instead declined much more (-53%, k = 19) and the response was not affected by the rate of the N supply (Tab. 3.8). There was a significant study effect on the response of Rub to N limitation and the significance of the category "frequency" – which showed exactly the same Q_B as the study effect (Tab. 3.8) - also was due to differences between the studies which involved different N application frequencies (see Appendix C, Tab. 21). While the crop species did not explain heterogeneity within the Rub dataset, the crop type had a slight effect on the response of Rub to N limitation (Tab. 3.9); but as their confidence intervals did overlap, the classes "cereals" (represented by wheat and barley) and "fibre crops" (represented by cotton) were not significantly different from each other (Fig. 3.24).

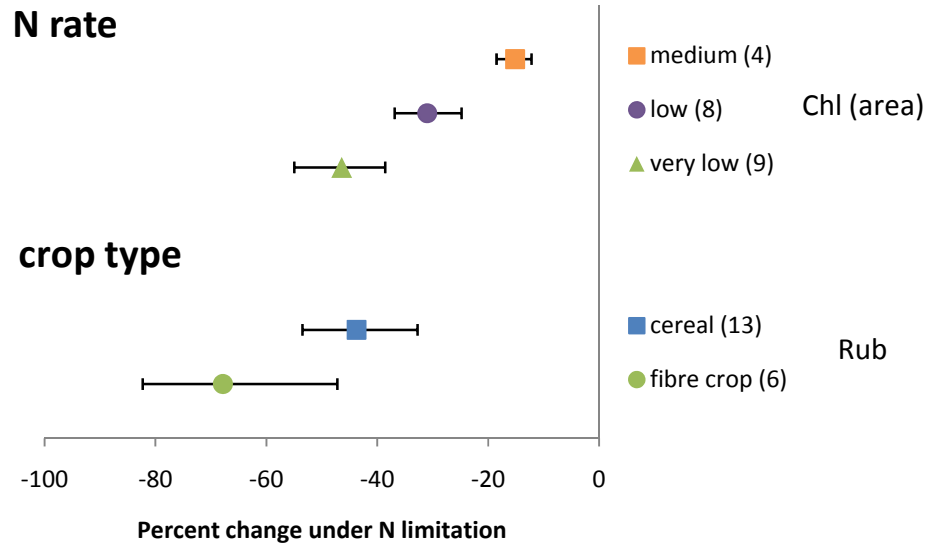


Figure 3.24: The response of chlorophyll content on an area basis (Chl) as influenced by the rate of N supply and of Rubisco activity (Rub) as influenced by the crop type. Symbols show means surrounded by 95% confidence intervals. Sample sizes (k) for each point are in parenthesis.

Thus, while the chlorophyll content changed in line with leaf N content and to about the same degree as leaf photosynthesis, Rubisco activity declined far stronger under N limitation. This pattern suggests that there does take place an acclimation of photosynthetic components to N limitation in crops, involving a stronger decrease of Rubisco than of thylakoid N associated with light capture and electron transport (for which the Chl content is an approximation, see 2.2.1.3). The stronger decrease in Rub compared to Chl, N_L and A could be due to catalytically active Rubisco acting as N store at high N supply (see 2.1.6.3) or due to a decrease in the internal CO_2 transfer resistance at low N supply, resulting higher CO_2 concentrations at the carboxylation site and an associated increase in the efficiency of Rubisco under N limitation (see 2.2.1.3). However the total soluble protein content of leaves did not decline as strongly as the Rubisco activity but instead to more or less the same degree as other photosynthetic parameters (-30% across all studies, k = 17; Fig. 3.13) and within the different N rate classes it again showed a remarkable parallelism with Chl and A (-42%, -30%, -15% in the very low, low and medium N rate classes; Fig. 3.20). As the Rubisco content is generally believed to be proportional to $Prot_L$ (Evans 1989) this result could suggest that the activation state of Rubisco changes under N limitation, with more Rubisco being present in an inactive state. This however would not make much sense if N constitutes a limiting nutrient. Another explanation could be that the analysis of Rub and $Prot_L$ involved different species with different strategies, but this was not the case as the main species present in both analysis were maize, wheat and cotton (see Appendix C, Tab. 19 and 21). Yet an alternative explanation is that the proportion of Rubisco to $Prot_L$ declines under N limitation and is thus not constant as generally believed. It would therefore be interesting to examine the change of Rubisco contents under N limitation and to compare if these change concomitantly with Rubisco activity and thus to a stronger degree than leaf soluble protein contents. In addition it would be useful to put the observations of this meta-analysis and the associated implications about the response of the photosynthetic apparatus of crops to N limitation on a sturdier basis by including more data from different studies.

3.3 Evaluation of methods used in the meta-analysis of the N limitation effect

Although meta-analysis tries to provide a *more* objective method for a literature review than the classical "narrative review" - as it was conducted in part 2 of this thesis – it is not truly objective, as one has to make several decisions, e.g. to define boundaries and to code the variables (DeCoster 2004).

The attribution of the N rate categories probably was the most critical point of the present meta-analysis. Because of the large variation in experimental methods (e.g. N rates, N frequencies and N forms) different N experiments are difficult to compare. For this reason I decided to restrict the analysis to controlled conditions, as here experiments are a little more comparable than under field conditions (see description of methods in 3.1.2). However the problem of the classification of an N rate as non-limiting for plant growth still remains. For an analysis of the effect of N limitation it seems essential to have a control group consisting of a non-limiting N supply. The fact that most effect sizes for the parameter W_T showed a negative response (see Appendix C, Tab. 3) cannot be taken as indication that the classification of the control N rates was appropriate

and that they really constituted non-limiting rates, as even if a limiting N supply is compared with a smaller limiting N supply, one would observe a negative response ratio. It remains to be discussed whether there is a significant difference in the response of a plant if one compares a high limiting with a low limiting N rate or if one compares a non-limiting, i.e. optimal N rate with a suboptimal one. Considering the tentativeness in the classification of an N rate as non-limiting for plant growth, probably it would be more appropriate to talk about a meta-analysis of the "effect of decreased N availability" than of an "N limitation effect", as in most experiments and studies it could not be accurately determined whether the maximum N rate really was non-limiting for plant growth. This would imply that also studies comparing two low N rates (e.g. Lawlor *et al.* 1987 a, b, c) could be included in the meta-analysis. It seems however likely that there are differences when one compares the response ratio calculated from a control plant that is supplied with a high N rate of 12 mM with an experimental plant supplied with 1 mM N (thus constituting a very low N rate, following the definition of N rate classes; see Tab. 3.5) with the response ratio based on a control N supply of 1.2 mM and an experimental N supply of 0.1 mM (which would also fall into the very low N rate class). Although the classification of non-limiting N rates thus is tentative, I still suggest applying some minimum requirements for the maximum N rate supplied in an experiment to be used as control N rate. And the standard concentrations of complete nutrient solutions used to define boundaries (see description of methods in 3.1.2) do provide such a basis for the classification and comparison of maximum N rates. The method used for the classification of different experimental N rate classes relative to the control N rate (see Tab. 2.5) appears to be useful, as the so attributed N rate classes were the categorical variable that most frequently could explain variation within the dataset (see Tab. 3.8) and the different N rate classes always responded in a sensible pattern (e.g. Fig. 3.9, 3.14 and 3.20).

The categorical analysis however also led to several strange effects caused by differences between effect sizes from different studies that were attributed different categorical variables. If for example there were significant differences in the response of a parameter from several experiments from two studies and these studies grew plants in pots of different sizes, then the categorical variable "pot size" yielded a significant Q_B, without probably being the cause for the differences between the studies. Thus the problem was based on the fact that several experiments from a single study, e.g. experiments involving different N rates, were included separately in the same analysis although they might not be totally independent. The problem may be avoided if a larger sample size was used. Hedges *et al.* (1999) define a large sample size for a meta-analysis using the response ratio as an effect size as $k \geq 50$, an intermediate sample size as $20 \leq k \leq 50$ and a small sample size as $k \leq 20$. Thus most sample sizes for the response variables in the present meta-analysis – which ranged between $k = 7$ and $k = 46$ – would be classified as small sample sizes, with the remaining being intermediate ones. However even several response variables with an intermediate sample size (e.g. A, $k = 37$) showed a significant study effect, i.e. significant heterogeneity between different studies (Tab. 3.8). Thus it appears that if several values from different experiments from the same study, sharing the same attributes of categorical variables are included in the same meta-analysis, an even larger sample size is needed in order to be able to conduct a valuable categorical analysis. Probably it would be useful to apply the sample size categorizations described by Hedges *et al.* (1999) for the number of effect sizes to the number of different studies included in a meta-analysis, i.e. if for example more than 50 single effect sizes were included in an analysis but these belonged only to less than 20 different studies, then the dataset should be classified as having a small sample size.

An alternative strategy to avoid such experimental bias probably would be to apply more strict

requirements for studies to be included in the analysis. In most studies included in the present meta-analysis a nutrient solution was supplied to the crops with a defined N concentration and at a defined frequency (e.g. 12 mM, once a week). Yet as plants continuously take up N from the solution or the medium, this method does not imply truly controlled (i.e. steady-state) conditions. Some authors (e.g. Ingestad & Agren 1992) criticise that with such a methodology – which is very customary in N experiments - the plant does not experience stable nutritional conditions, but instead it is exposed to a highly varying N availability in the medium at different points in time, leading to strongly varying results that are difficult to interpret. If a plant for example is grown under low light conditions, an N supply of 5 mM in a nutrient solution, given once a week, might mean sufficient N for maximal plant growth. Under high light conditions on the contrary the plant might show a larger growth rate, take up more N, the medium might therefore impoverish faster and the plant might experience severe N deficiency a few days after the application of the nutrient solution. Much of the variation observed between studies might therefore be put down to such non-stable nutritional conditions. Yet if one would limit a meta-analysis to studies that are better controlled and that supply a stable and defined N concentration, not many studies would be left to be included in the analysis.

A method that has been proposed to better define and describe N limitation than the "external concentration approach" (i.e. the monitoring of plant N status through the monitoring of the concentration of the nutrient solution) is the so called relative addition rate (RAR). This implies a continuously monitored but non-constant N concentration in the nutrient solution that allows the maintenance of a constant limiting or non-limiting target N concentration in the plant (Ingestad 1982). Only a single study that was included in the present meta-analysis used RAR (Fricke *et al.* 1997). Macduff *et al*. (1993) compare the two nutritional methods (i.e. controlled concentrations in the nutrient solution and RARs) and come to the conclusion that plant responses to N supply are intrinsically independent of the method employed. Thus it seems justified to also include N experiments that monitor the external concentration instead of the internal N concentration through RARs in a meta-analysis of the N limitation effect. However the "external concentration method" applied by Macduff *et al.* (1993) involved continuously monitored constant N concentration in the growth medium, i.e. the hydroponic solution. Most studies reporting N experiments still do not even maintain a constant N concentration in the growth medium but supply N at seemingly arbitrary concentrations and frequencies, without stating the reason why N is applied in the respective manner. And most studies do also not monitor and maintain a constant acidity in the growth medium (see Tab. 3.7) or report the initial N content of the medium if a solid growth medium is used. Considering these strongly varying and mostly not well defined methods applied in N experiments, it is not surprising that the analysis of heterogeneity often revealed a significant study effect (see Tab. 3.8) and that several parameters showed a considerable variance (see Fig. 3.1, 3.13 and 3.22). However if one wanted to define quality criteria for studies to be included in the meta-analysis but still to conduct a weighted meta-analysis and thus require primary data to be reported with sample sizes and variances, even more studies would have to be excluded. It therefore appears that as long as the majority of N experiments use such undefined and little controlled nutritional methods, a meta-analysis should also include these common "semi-controlled" N experiments and rather try to include a large number of different studies, thus putting quantity above quality.

3.4 Conclusion

The present meta-analysis leads to a number of conclusions that can be stated with relative certainty as they agree with the picture that emerged from the review of the literature in part 2. The meta-analysis confirmed that crops under limiting N supply produce less biomass, decrease the canopy size, increase the root-shoot-ratio, accumulate less N, have lower concentrations of nitrate, amino acids, proteins and chlorophyll, have a decreased Rubisco activity and photosynthetic rate, and show increased levels of leaf carbohydrates. Considering the questions emerging from the review of the literature concerning the N processes in plants (see 2.3) it also could be concluded that:

I. Leaf N content does change significantly in crops under N limitation and is thus not just dependent on the light environment of the leaf.

II. The photosynthetic rate on a leaf area basis does also change under N limitation but generally to a smaller degree than leaf area. Photosynthesis does however not seem to be primarily limiting for growth under N limitation as carbohydrates accumulate in leaves.

III. Rubisco activity seems to decline more strongly under N limitation than the Chl content, but total soluble protein content declines to a similar extent as Chl, suggesting a change in the proportion of soluble protein present as Rubisco.

IV. Crop species within a crop type, e .g. cereals, often do not respond in a similar manner to N limitation and crop types are in most cases inferior predictors for the response to N limitation than crop species.

Still many questions remain to be answered. For example meta-analyses conducted here could not satisfactorily answer the following questions:

I. Different crop species differ in the extent in which they reduce photosynthesis and leaf area under N limitation. Probably wheat follows a strategy that is distinct from that of soybean and mainly reduces its leaf area while keeping the photosynthetic rate more constant, while soybean reduces photosynthesis to a stronger degree than leaf area.

II. Are there really differences in the proportion of Rubisco in total soluble protein or are the different patterns observed for Rubisco activity and soluble protein contents due to some other cause?

III. Is the decline in different photosynthetic components under N limitation affected by interactions like light or atmospheric CO_2?

IV. How does the CO_2 concentration affect the N limitation response of photosynthesis and growth and is there really a stronger decline of photosynthetic rate under N limitation at elevated CO_2, as suggested by the present results?

V. Can the remarkable parallelism observed in the response of photosynthesis, Chl content and leaf soluble protein content be formulated as a general relation and thus be used to predict the response of crop photosynthesis to N limitation?

VI. Do C3 and C4 species really differ in the degree of decrease of leaf soluble protein contents under N limitation and do leguminous species really differ in the response of leaf area to N limitation?

VII. How do different N sources influence plant growth and is the less pronounced decrease in leaf area and plant biomass under N limitation in plants supplied with ammonium as sole N source observed in the present analysis consistent in a larger dataset?

Several responses to N limitation showed no clear direction in the present analysis (e.g. stomatal conductance, N uptake, RGR) and/or a great variation (e.g. leaf starch content, root nitrate content). The missing response to N limitation could either be due to the fact that the relevant parameter simply does not change significantly under N limitation or it could be due to large variations in the results from different experiments and different studies. In the latter case it would be interesting to examine the reasons for this variation and to dissect the experimental methods and/or plant characteristics that are responsible for the different responses to N limitation. Unfortunately the categorical analysis in the present meta-analysis is of only limited validity due to small sample sizes for most response variables. Yet the meta-analytic method used and applied here for the first time to the analysis of the N limitation effect proved to be useful and emerged as a promising tool for a further examination of the response of crops to N limitation.

Chapter 4
Discussion

In the last two chapters I have tried to draw a complete as possible picture of the N processes in crops and of the effect of N limitation on crop physiology and growth. Here I will summarize the findings about relevant processes and interactions that were elaborated through the means of literature review and meta-analysis and discuss the implications derived for the representation of crop C-N interactions in a global model. I will shortly present a conceptual framework of C-N fluxes and pools and their interdependencies. I will then look at how the processes that were concluded to be important are represented in present N-inclusive global models of natural vegetation and also consider shortly the representation of crop C-N interactions in crop models. Lastly I will evaluate the achievements of this study and I will put them into a context, considering the matters that were addressed by this thesis and the matters that remain to be analyzed and that need future research.

4.1 Summary of crop N processes and interactions most relevant for the implementation in a global model

An extensive summary of conclusions from the literature review has already been given in section 2.3. The focus there however was on primary dependencies. Here I want to summarize which more general interactions can be deduced from these primary regulations and controls. Table 4.1 depicts the interactions as extracted in section 2. I distinguish between regulations (i.e. signals influencing the transcription of genes, the post-translational modification of proteins or protein activity) and controls (i.e. which processes impose feedbacks without regulating directly).

Table 4.1: C-N processes in the plant, how they are regulated and by what they are controlled. Summary of findings from chapter 2.

Process	Regulated by	Controlled by
N uptake	external N conc root N conc carbohydrates amino acids	energy and reducing equivalents (respiration)
N fixation	external nitrate conc root nitrate conc carbohydrates amino acids temperature water status	energy and reducing equivalents (respiration)
N assimilation	internal nitrate conc carbohydrates amino acids	C skeletons (photosynthesis, respiration) energy and reducing equivalents (photosynthesis, respiration)
N allocation	phytohormones (?)	metabolic activity/age development stage plant N status
Photosynthesis	phytohormones carbohydrates amino acids	N status of the root
Respiration	root nitrate conc	metabolic activity (i.e. protein quantity) carbohydrates (as substrates)
C allocation	phytohormones root: carbohydrates external nitrate conc	N status of the root (for shoot growth) N status of the shoot (for root growth) N available for growth

N assimilatory enzymes for example are regulated directly by nitrate, amino acids and carbohydrates (see 2.1.5.2), while C skeletons (i.e. organic acids) impose a control on N assimilation, as they are needed for the synthesis of amino acids (see 2.2.3.2) and ATP and

reducing equivalents are needed in several enzymatic reactions of N assimilation (see 2.1.3). Photosynthetic proteins on the other hand are regulated by phytohormones, amino acids and carbohydrates (see 2.2.3), but the N status of the root also controls photosynthesis, although not regulating it directly, mediated by phytohormones.

Figure 4.1 shows a model of C and N pools and fluxes in plants (adapted from Kattge 2002), where N_{inorg} and C_{inorg} are inorganic, mobile N and C pools (i.e. nitrate and CO_2), N_{org} and C_{org} are organic, mobile N and C pools (i.e. amino acids and carbohydrates) which are either allocated to structural (N_{struc} and C_{struc} in fine roots (FR), roots (R), stem (S) and leaves (L)) or storage pools (N_{res} and C_{res}, mainly representing storage proteins and starch). This model allows the differentiation between mobile C and N pools and structural C and N contents of tissues. Thus N_L (i.e. the N content of leaves) here represents the amount of N incorporated into leaves, i.e. proteins (including Rubisco and thylakoid proteins), Chl, nucleic acids, secondary metabolites and others, minus the N present as amino acids, minus the N in nitrate and minus N stores (be they nitrate, proteins or amino acids). N_L thus is not the whole leaf N content, which may appear an uncommon approach. This division of N_{anorg}, N_{org}, N_{res} and N_{struc} has however several advantages which will be discussed in the following.

It is obvious from the summary in Table 4.1 that carbohydrates, i.e. the C_{org} pool from Figure 4.1, and amino acids, i.e. the N_{org} pool from Figure 4.1, represent key pools in plant C-N interactions. As C_{org} and N_{org} often are signals that mediate the plant C:N status, the shoot C:N status or the root C:N status to relevant processes like N assimilation or photosynthesis (see 2), it appears sensible to define the N status of the plant as based on these key pools. If C_{org}/N_{org} is greater than a certain critical value, then this expression would define the N demand of the plant. If however C_{org}/N_{org} is smaller than a certain critical value, it would describe the C demand of the plant. This expression could thus be used to achieve a simulated balance between N and C assimilation that is very near to the actual coordination observed in plants.

Table 4.2 transfers the regulations and controls from Table 4.1 to the different C and N pools from Figure 4.2 and thus describes the dependencies of processes on C and N pools as well as other factors. It includes several further assumptions:

- The dependence of N uptake, N fixation and N assimilation on energy and reducing equivalents and of N uptake on C skeletons from photosynthesis and/or respiration (see Tab. 4.1) is already implicitly considered in the dependence of these processes on C_{org}.
- The mediatory role of phytohormones is not considered, instead the regulation of photosynthesis and of C allocation to the shoot through phytohormones that mediate the root nitrate status is interpreted as dependence on N_{inorg}.
- The dependence of growth on growth proteins and thus on the amount of N available for growth is interpreted as a dependence of C allocation on N_{org}, as amino acids form the basis for the synthesis of growth proteins.

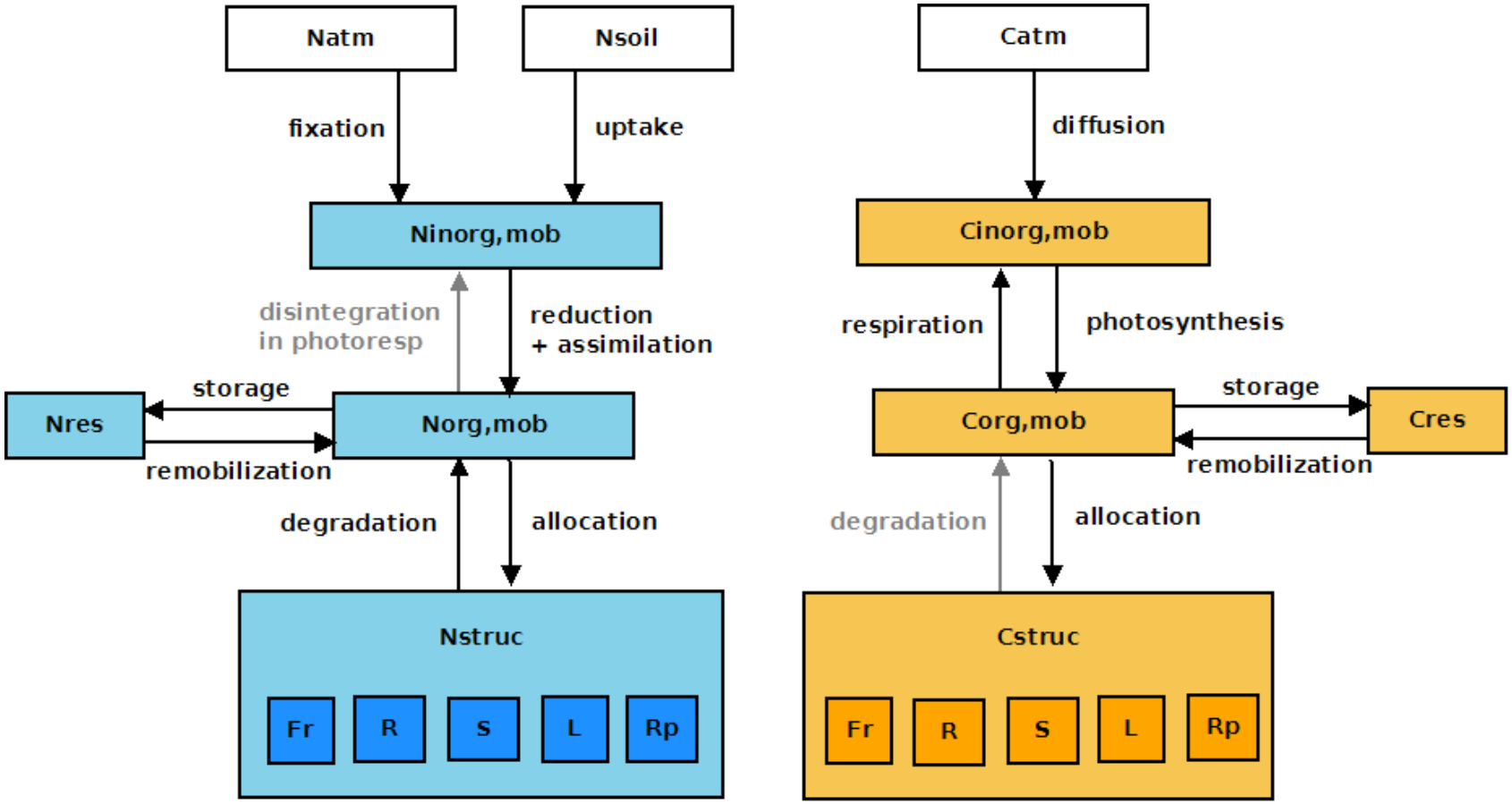


Figure 4.1: Conceptual model of C (orange) and N (blue) pools and fluxes. Processes in grey are not considered further as they either do hardly occur (e.g. C degradation and recycling from senescing tissue) or their magnitude is not relevant in the whole plant budget (e.g. the release of inorganic N in photorespiration). Abbreviations: N_{atm}: atmospheric N_2; N_{soil}: soil N content; N_{inorg}: inorganic N; N_{org}: organic mobile N; N_{res}: storage N; N_{struc}: structural N; C_{atm}: atmospheric CO_2; C_{inorg}: inorganic C, equivalent to C_i (i.e. internal CO_2 concentration); C_{org}: organic mobile C; C_{res}: storage C; C_{struc}: structural C; Fr: fine root compartment; R: root compartment; S: stem compartment; L: leaf compartment; Rp: reproductive compartment.

Table 4.2: C-N processes in the plant and the pools and variables on which they depend. Abbreviations: C_{FR}: C in fine roots; T: temperature; microorg: inoculation with Rhizobium; dev: development stage; LA: leaf area; SLA: specific leaf area (for further abbreviations see Fig. 4.1).

Process	Dependent on
N uptake	N_{soil}, N_{inorg}, C_{org}/N_{org}, C_{FR}
N fixation	N_{soil}, N_{inorg}, C_{org}/N_{org}, T, H_2O, microorg
N assimilation	N_{inorg}, C_{org}/N_{org}
N allocation/ degradation	C allocation, age, light, N_{org}, dev
N storage/ remobilization	C_{org}/N_{org}, dev
Photosynthesis	C_{org}/N_{org}, N_{inorg}, LA (i.e. C_L and SLA), C_{inorg}, T, light
Respiration	N_{struc}, N_{res}, C_{org}, T
C allocation	C_{org}/N_{org}, N_{inorg}, (N_{soil}), dev
C storage/ remobilization	C_{org}/N_{org}, dev

As discussed in section 2.1, environmental factors like temperature and water do not regulate N uptake and N assimilation directly, but impose a control by influencing the growth of the plant and thus the demand for N as well as the availability of N in the soil. N fixation instead is regulated directly by temperature and water status, independently from the influence of water and temperature and plant growth and soil N.

Respiration is dependent on the metabolic activity and thus on the protein content of tissues (see 2.2.2). Proteins however are not considered explicitly in the pools in Figure 4.1. And as the amount of N in tissues scales with the protein content (see 2.2) and as N storage also involves respiratory costs (see 2.1.6), as an approximation respiration is considered to be dependent on both N_{struc} and N_{res} (Tab. 4.2). As N_{struc} represents the amount of N incorporated into new growth and can thus be taken as a measure for growth, this dependence of respiration on the N_{res} and N_{struc} pools possibly makes the rather non-mechanistic division of respiration into maintenance and growth respiration unnecessary. This however constitutes just a provisional hypothesis that needs to be evaluated and discussed in more detail.

New growth can only occur if there are both enough growth proteins available and enough photosynthates present. Thus the incorporation of C_{org} and N_{org} into C_{struc} and N_{struc} depends on a balance between the two organic pools and is limited by the smaller one of the two. If N_{org} is insufficient and thus C_{org}/N_{org} is larger than a critical value, then C_{org} accumulates and N uptake, N fixation, N assimilation and C allocation to the root are stimulated while photosynthesis decreases. If instead there is enough N_{org} but C_{org} is limited and thus C_{org}/N_{org} is smaller than the critical value, then N_{org} accumulates and photosynthesis is stimulated. Photosynthesis and C allocation to the canopy are in addition stimulated at high N availability and thus high N_{inorg} content, independent of the C_{org}/N_{org} ratio, thus even if C_{org} is not limiting for new growth.

The stimulation of lateral root growth into nitrate-rich patches by local high nitrate concentrations (see 2.2.4.2) and thus the dependence of C allocation to the root on soil N availability (N_{soil}; Tab. 4.2) is only relevant if the N availability in the soil is simulated with a patchy distribution and if the distribution of fine roots in the soil is modelled.

The description of the balanced need for both N_{org} and C_{org} for new growth does however not imply that the C_{struc}/N_{struc} content of tissues is constant. Instead the critical values, where growth ceases because of either a limitation through N_{org} or through C_{org}, should be interpreted as the minimum and maximum C_{struc}/N_{struc} ratios required in new tissue, thus allowing for a dynamic N content in plant compartments. The allocation of N to different organs does largely scale with the allocation of C (see 2.1.6); however there are several exceptions, including the change in the N content with (i) the age of a tissue (i.e. highest concentrations in young, metabolically active tissue and lowest concentrations in senescing organs with considerable N degradation and recycling), (ii) the depth in the canopy and thus with light availability, (iii) N availability (represented by the size of the N_{org} pool), (iv) development stage (in the reproductive stage reproductive organs have an especially high N allocation priority).

The control of the photosynthetic rate by leaf N content discussed in chapter 2 is an extremely critical connection between C and N cycles (Field & Mooney 1986) and it could be argued that it thus should be an essential part of a model of crop C-N processes. As the Rubisco carboxylation capacity and the capacity of the electron transport (for which the Chl content is an approximation, see 2.2.1) are key parameters in the Farquhar *et al.* (1980a) photosynthesis model, in addition it is desirable to describe these as separate pools. In the framework described above however photosynthesis is dependent only on carbohydrate (C_{org}) and amino acid (N_{org}) concentrations and neither on N_L, Rubisco or Chl content. This shortcoming of the concept could be confronted by avoiding the summarization of different photosynthetic processes but instead considering the individual components of photosynthesis separately (Tab. 4.3). This shows that the degree of detail of the mechanistic Farquhar *et al.* (1980a) model allows the representation of individual photosynthetic components, while for the other processes (e.g. N uptake, N assimilation) different components (e.g. transporters and enzymes) have to be summarized, as yet no such universally valid, process-based and easily parameterized model is available.

Table 4.3: Photosynthetic components and variables on which they depend. Rub (Rubisco protein) and Thyl (thylakoid protein) can be estimated either based on actual regulation (second column) or on a correlation (third column). Abbreviations as in Fig. 4.1 and Tab. 4.2.

Process	Dependent on (reg)	Dependent on (correl)
Allocation to Rubisco	C_{org}/N_{org}, N_{inorg}, light	N_L
Allocation to Thylakoid	C_{org}/N_{org}, N_{inorg}, light	N_L
Photosynthesis	Rub, Thyl, LA (i.e. C_L and SLA), C_{inorg}, T, light	

As discussed in 2.2.1.2, the correlation of photosynthesis and N_L is an essentially empirical relationship and it does not imply that photosynthesis is regulated according to N_L. Instead it has been shown that the expression of both Rubisco and several components of thylakoid proteins are directly regulated by carbohydrates, amino acids, cytokinins (mediating the root nitrate concentration) and light (2.2.3.1). Thus in a mechanistic approach it would be desirable to describe both the Rubisco and the thylakoid protein fraction as dependent on the C_{org}, N_{org} and N_{inorg} pools as well as on light. This also would allow – through a specific description of the different form and degree of regulation by different signals - for the representation of photosynthetic acclimation, i.e. the change in the relative proportion of different photosynthetic components according to environmental conditions. In a more empirical approach instead it would also be possible to derive the amount of N allocated to Rubisco and thylakoid proteins as a constant fraction of N_{struc} in leaves (i.e. N_L). Here the fact that the N_L pool does not include N storage compounds is advantageous, as the curvilinear relation between A_{max} and N_L observed in several studies most probably is due to N storage at high N_L levels, while if N storage forms are accounted for, the relationship is usually linear across the entire range of N_L (Sage & Pearcy 1987a).

To summarize, the differentiation of the C and N pools as described in Figure 4.1 allows to

I. represent a mechanistic regulation of several processes (e.g. N uptake, N assimilation, root C allocation) by C_{org} and N_{org},

II. represent a balanced regulation of new growth and the incorporation of the organic pools into C_{struc} and N_{struc} by the amount of C and N available for new growth (i.e. the C_{org} and N_{org} pools),

III. obtain either a mechanistic representation of different photosynthetic components, regulated by C_{org}, N_{org} and N_{inorg}, or a representation based on the linear relationship between photosynthesis and N_L.

4.2.Representation of relevant processes and interactions in present N-inclusive models

In several aspects of their physiology (e.g. N uptake, photosynthesis) crops do not differ substantially from natural vegetation. Therefore for the simulation of crop processes it is possible to derive several formulations which were initially developed for natural vegetation. Patterns that differ in the representation of crops compared to natural plant types include (i) allocation (e.g. time course of allocation and allocation to harvestable storage organs), (ii) phenology (e.g. as controlled by sawing and harvest), (iii) management (e.g. irrigation, grazing, harvest, sowing) and (iv) distribution (which is not driven solely by climatic factors but dependent also on management decisions) (Bondeau *et al.* 2007). However for many more general processes it is useful to look at their representation in models which only consider natural vegetation. Table 4.3 summarizes the representation of plant N processes and the representation of the N control on C processes in selected models of the natural vegetation.

Table 4.4: Representation of plant N processes and of N controls on plant C processes in an ecosystem model (FOREST-BGC) and in selected global terrestrial biosphere models (CLM-CN, TEM, Hybrid 3.0). Abbreviations used: decomp: decomposition; min: mineralization; nitr: nitrification; denitr: denitirification; leach: N leaching; BNF: biological N_2 fixation; dep: N deposition; N_L: leaf N content; V_{Cmax}: maximal carboxylation activity of Rubisco; T: temperature; LA: leaf area; LAI: leaf area index; SLA: specific leaf are; R_M: maintenance respiration; R_G: growth respiration; GPP: gross primary production; NPP: net primary production; PFT: plant functional type

Model	Crops	N Cycle	Photosynthesis	Respiration	C allocation	N allocation	N demand/ N uptake	N limitation
FOREST-BGC[1]	no	yes (decomp, min, nitr, leach)	calculation based on CO_2 diffusion gradient, canopy water conductance + mesophyll CO_2 conductance (dependent on T, N_L and light)	R_M dependent on T; R_G as fixed fraction of C available for growth	root, leaf and stem compartments; C available for growth dependent on water, C and N limitation; dynamic root-shoot ratio; no C storage or reproductive compartment	N in canopy dependent on N availability, N demand and C allocation to root and shoot; N_L ranges between min and max boundaries; root N content static in relation to N_L; retranslocation of N_L at senescence	N demand relative to hypothetical optimal leaf N pool (calculated as maximal LA x maximal N_L); N uptake not considered explicitly (N in canopy defines N in plant)	imposes constraint on C use for growth; influences root-shoot-ratio; determines N_L
CLM-CN[2]	no	yes (dep, BNF (as fraction of annual NPP), denitr, leach)	differential calculation of sun and shade-leaves; dynamic V_{Cmax} dependent on N_L and PFT-specific fraction of N_L in Rubisco[5]; leaf photosyn based on F & C[6] models; canopy GPP downregulated if produced C cannot be used due to N limitation	R_M dependent on T and N content of tissues; R_G as constant fraction of total C in new growth	short-term C storage pool is depleted if R_M > GPP, has first allocation priority when GPP > R_M; constant proportion of allocation in leaves, fine roots and wood; PFT-specific constant long-term C storage pool built up for use for growth in subsequent year; no predefined maximal boundary for growth, growth is reduced when high LAI leads to higher proportion of shaded canopy	N_L as function of SLA and (fixed) C:N ratio in leaves → variation of N_L in canopy through variation in SLA; C:N ratios of tissues are constant for PFT; short-term N storage pool built up from retranslocation of N from senescing tissue; PFT-specific constant long-term N storage pool built up for use in growth in subsequent year	N demand dependent on PFT-specific C:N ratios of tissues; N demand met first from short-term storage pool	downregulates GPP by producing excess C that cannot be used for growth

TEM[3]	no	yes (min, decomp, N loss, N input)	GPP dependent on N available for growth, light, CO_2, H_2O, T and LA; GPP reduced in parabolic form with increasing N limitation	R_M as function of plant biomass and T; R_G dependent on GPP and R_M	no representation of different plant compartments	amount N in growth determined by N uptake and recycling of N in vegetation	N demand based on optimal C:N ratio of new growth; N uptake determined by soil N availability, soil moisture, T, maximal N uptake capacity and C available for N uptake	occurs when C:N ratio of new growth > than optimal C:N ratio; reduces GPP
Hybrid 3.0[4]	no	yes (decomp, min, dep, BNF (fixed), leach)	canopy photosyn calculated from leaf photosyn, that declines according to light intensity within canopy; leaf photosyn based on F & C models; form of dependence of V_{Cmax} on N_L calculated daily for each PFT, actual V_{Cmax} of individuals then scaled against individual N_L	R_M in roots and leaves based on T and N contents of tissues, in stem based on total biomass and T; R_G based on C allocation in different compartments	constant allocation to leaves, support structures, fine roots and storage	N allocation in canopy according to light intensity assuming optimization; constant relative C:N ratios between compartments are maintained (i.e. C:N ratio within tissue can vary but not relative relation between tissues); allocation of N_L to Rubisco, Chl and other N (fractions constant)[7]	N demand based on C:N ratio of whole plant; N uptake calculated from N demand, soil N availability and biomass in fine roots	influences plant C:N ratios and though this N_L and photosynthesis

[1]Running & Coughlan (1988) and Running & Gower (1991)
[2]Thornton *et al.* (2007) and Thornton & Zimmermann (2007)
[3]Raich *et al.* (1991) and McGuire *et al.* (1992)
[4]Friend et al. (1997)
[5]based on Niinemets & Tenhunen (1997)
[6]F standing for Farquhar *et al.* (1980a), C for Collatz *et al.* (1991, 1992)
[7]based on parameters derived from Evans (1989)

It can be observed that several relationships that were assessed as being important for the simulation of crop N processes (see 4.1.1) are represented in the models that are described in Table 4.4. I will not go into a detailed analysis of the different approaches and calculations used in the different models, but I want to highlight some particular interesting aspects and some shortcomings in the representation of plant N processes in these models.

Some sort of dependence of photosynthesis on N is represented in all models. However the degree to which relevant mechanisms are described varies strongly. While TEM involves an empirically derived function for the N dependence of GPP and simply scales GPP down under N limitation according to a parabolic curve (McGuire *et al.* 1992), both CLM-CN and Hybrid 3.0 describe the variation in leaf N content (N_L) and photosynthesis with canopy depth and include a dynamic calculation of the carboxylation capacity of Rubisco (V_{Cmax}) as a function of N_L, which then is used in the calculation of photosynthesis based on the Farquhar *et al.* (1980) model (Friend *et al.* 1997; Thornton *et al.* 2007). V_{Cmax} is one of the most sensitive parameters in photosynthesis (Collatz *et al.* 1991) and resulting terrestrial biosphere models (White *et al.* 2000; Kattge *et al.* 2009). The dynamic calculation of V_{Cmax} as a function of N_L appears more appropriate than the often observed use of a prescribed value for V_{Cmax} for each PFT (e.g. Collatz *et al.* 1991). It also appears useful when one wants to integrate plant N processes into a model like LPJmL that calculates photosynthesis based on Farquhar *et al.* (1980) but that so far derives V_{Cmax} from an optimization algorithm that predicts the V_{Cmax} that gives the maximum rate of net photosynthesis (i.e. at an optimal balance between gross photosynthesis and leaf respiration rate) under optimal (i.e. non-limited) conditions, without explicitly considering N_L (Haxeltine & Prentice 1996).

The calculation of V_{Cmax} from N_L in CLM-CN is based on the formulation from Niinemets & Tenhunen (1997):

$$V_{C\max} = N_m * F_{LNR} * 6.25 * SLW * a_R \tag{4.1}$$

where N_m is leaf N on a mass basis, F_{LNR} is the proportion of leaf N in Rubisco protein (a PFT-specific parameter), SLW is specific leaf weight (leaf dry mass per unit area), a_R is the specific carboxylation activity of Rubisco (which is a function of temperature) and 6.25 is the g N in the Rubisco protein (converts N content to protein content). This is a rather mechanistic representation, as V_{Cmax} is dependent on the carboxylation turnover number and the number of catalytic sites (Farquhar *et al.* 1980), which again depends on leaf Rubisco content, which in turn can be estimated from N_L (Evans 1989). Friend (1995) derives a very similar formulation for the calculation of V_{Cmax} from N_L.

This formulation can easily be adapted to values on a leaf area basis (Thornton & Zimmermann 2007):

$$V_{C\max} = N_a * F_{LNR} * \frac{1}{F_{NR}} * a_R \tag{4.2}$$

(where N_a is leaf N content on an area basis and F_{NR} is the mass fraction of N in the Rubisco molecule (constant across PFTs)) and it can be adapted to derive V_{Cmax} from leaf C:N ratios (CN_L) and SLA (Thornton *et al.* 2002):

$$V_{C\max} = \frac{F_{LNR} * a_R}{F_{NR} * SLA * CN_L} \tag{4.3}$$

Thus there already exist robust formulations to derive V_{Cmax} from some sort of information about the allocation of N to leaves. Niinemets & Tenhunen (1997) and Friend (1995) in addition propose to also predict J_{max} (the maximal potential electron transport rate), which is another important parameter in the Farquhar *et al.* (1980) photosynthesis model, from a parameter describing the fraction of leaf N bound in light harvesting and/or electron transport and N_L. These approaches

however emphasize the need of an adequate prediction of N_L as driven and influenced by environmental conditions.

Several of the models described involve a dynamic calculation of N_L. In CLM-CN N_L is dependent on SLA and a PFT-specific fixed leaf C:N ratio (Thornton & Zimmermann 2007). N_L thus varies in accordance with SLA with canopy depth and thus with light intensity; it is however not influenced by N availability. This leads thereto that photosynthesis – which calculation is dependent on N_L (as discussed above) - is influenced by light availability and by the architecture of the canopy but not directly by N availability. N limitation instead is implemented into CLM-CN instead as a correction of C assimilation down to a level where it matches N supply, i.e. primary production is reduced when the C produced can no longer be used for growth due to a limited availability of N for new growth. This approach is similar to that in FOREST-BGC, where the use of C for new growth is based on GPP but is in addition limited and reduced by either water or N limitation (Running & Gower 1991).

Such representation of N limitation is in line with the conclusion drawn from the literature review that the primary effect of limited N is on the use of C for growth and not on photosynthesis (see 2.2). However – as the meta-analysis showed – N_L and thus photosynthesis (whether on mass or area basis) do also decline under limited N availability (see 3.2), probably as a secondary effect resulting from the downregulation of photosynthetic protein synthesis due to photosynthate accumulation. Thus the constant C:N ratios assumed in CLM-CN do not describe the real physiological processes appropriately. FOREST-BGC on the contrary involves a prediction of N_L based on PFT-specific minimum and maximum concentration limits and dependent on the amount of N taken up by the plant.

In Hybrid 3.0 N is allocated in leaves according to light availability, assuming optimization, and leaf C:N ratio is influenced by the amount of N taken up by the plant (Friend *et al.* 1997). However as discussed in chapter 2 (2.1.6 and 2.2.1), actual N allocation in plants often deviates from the calculated optimum. In the Hybrid 3.0 model therefore a maximal LAI boundary had to be defined, as a simulation with equations derived from optimization theory lead to unrealistically high LAI values (Friend *et al.* 1997). Hybrid 3.0 does not involve any explicit consideration of the effect of N limitation, but simply reduced N_L and thus photosynthesis and thus growth under low N availability. Probably a mixture of a reduction in N_L (and through it a reduction in photosynthesis) and a limitation of C use for new growth due to N limitation would mimic physiological interactions in plants more appropriately.

Hybrid 3.0 includes an allocation of N_L to three different leaf N pools: Rubisco, thylakoid N (represented by Chl) and the other N fraction. From the Rubisco and the Chl contents then V_{Cmax} and J_{max} are estimated (see discussion above). This approach is especially useful if one wants to represent the acclimation of the photosynthetic apparatus to different environmental factors (see 2.2.1.3). As the fraction of N_L in the different photosynthetic components is in fact not constant, but changes with light, CO_2 concentration and probably N availability, it is not realistic to define a single value for F_{LNR} (see equation 4.1) across all PFTs (as done in Hybrid 3.0) or as a single value for each PFT (as done in CLM-CN). Instead it would be more appropriate to predict the partitioning of N between Rubisco and Chl, for example based on optimization theory (e.g. Hikosaka & Terashima 1995; Hikosaka & Hirose 1998).

Several of the models presented in Table 4.4 calculate R_M as dependent on temperature and on N contents of tissues, while R_G is mostly calculated based on C in new growth. This approach is essentially empirical and does not describe and represent any underlying mechanisms. However respiration is a very complex process consisting of several sub-processes and it cannot - like photosynthesis - be put down to a limited number of key compounds or enzymes. Therefore so far no process-based model, comparable to the Farquhar *et al.* (1980a) model for photosynthesis, exists that would allow a more mechanistic description of respiration. This is also the reason why respiration has just been dealt with superficially in the context of this thesis (see 2.2.2).

The fraction of C allocation in different plant compartments is held constant in CLM-CN and Hybrid 3.0 (Tab. 4.4). This approach however does not account for an increased allocation of C into roots under low N and an associated increase in the N uptake capacity. The change of the root-shoot ratio is a fundamental strategy of plants to respond to limited N availability and it therefore should be a central part in the representation of plant N processes in a model. FOREST-BGC includes such a dynamic calculation of the root-shoot ratio, based on N and water availability and derived from experimental values on dry matter partitioning in trees (Running & Gower 1991).

As with the allocation of N (see above) also the allocation of C between root and shoot can be predicted based on optimization theory (e.g. Johnson & Thornley 1987; Hilbert 1990). But this concept – similarly to the optimization theory for allocation of N within a canopy (see above) - shows major limitations. Coordination theory, which has been used to model N allocation within the canopy (Chen *et al.* 1993), has also been applied to predict whole plant allocation (Reynolds & Chen 1996). In general all these approaches predict C allocation between root and shoot in a satisfactory manner, yet without incorporating a realistic physiological allocation model (Van der Werf & Nagel 1996).

BNF in plants is not considered explicitly in any of the models from Table 4.4 However, BNF is a key component of the N cycle; before invention of the Haber-Bosch process, BNF was the only way to assimilate N_{atm} into the biological system and to compensate for losses from the system occurring inevitably. Today, BNF derived from agricultural cultivation of legumes contributes substantially to the human induced alteration of the N cycle (Vitousek *et al.* 1997; see 2.1.4) and it also is important in the N-budgets of single fields (e.g. Adu-Gyamfi *et al.* 2007). In a global model of the coupled C-N cycle that considers crop growth and management it therefore appears essential to include an explicit representation of N_2 fixation in leguminous crops. So far there are however very few simulation models of BNF (Vitousek *et al.* 2002). The simulation of leguminous N_2 fixation therefore probably is a major challenge in the implementation of crop C-N interactions in a global model.

Jeuffroy *et al.* (2002) provide a review of the representation of N processes in agricultural models. These models often are built for a quite different purpose than global terrestrial biosphere models. They mainly aim at providing strategies for farmers to (i) minimize N loss through N leaching, (ii) enhance crop productivity with the smallest possible amount of N fertilizer and (iii) improve quality (which is often determined by N content) of harvest products (Willigen 1991; Jeuffroy *et al.* 2002). All the same crop models include some interesting concepts that could be useful for the implementation of crop N processes in a global model of the managed land surface.

All crop models are based on a similar conceptual framework; they compare N availability and the crop N demand and from this they predict crop growth and development (see Fig. 4.2, Jeoffroy *et al.* 2002). They often derive crop N-demand from the concept of the critical N concentration ($\%N_{crit}$; defined as the minimum N concentration permitting maximal crop growth; e.g. Greenwood *et al.* 1990; Justes *et al.* 1994). This approach is quite similar to the representation of the N demand in relation to an optimal C:N ratio of plant tissues observed in several terrestrial biosphere models (Tab. 4.4). However a constant optimal C:N ratio for each PFT, as used for example in CLM-CN, does not account for the observed change in plant N content with time and increasing biomass (see 2.1.6.2). The concept of a critical N dilution curve describes $\%N_{crit}$ as a function of dry mass (DM):

$$\%N_{crit} = a(DM)^{-b} \qquad (4.4)$$

where *a* is a parameter describing the concentration of N in plant biomass and *b* is a parameter describing the shape of decline in N content with increasing biomass. Several such N dilution curves have been established based on experimental data, e.g. for wheat (Justes *et al.* 1994), rape (Colnenne *et al.* 1998) as well as general equations for C3 and C4 crops (Greenwood *et al.* 1990) (see Lemaire 1997 for potatoes, grain legumes, maize, sorghum, wheat, barley, durum wheat and grassland). Although this is a strongly empirical concept, it allows the definition of a dynamic

threshold value at which the plant switches from N deficiency to N storage.

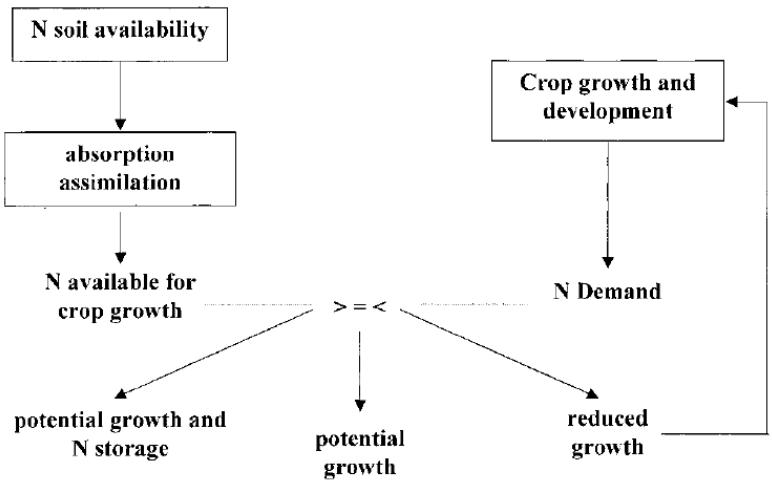


Figure 4.2: General framework used in agronomical models. From Jeuffroy *et al.* (2002).

The representation of N uptake in crop models also provides interesting components that could be adapted for use in a global model of crop processes (see description in Willigen 1991 and Jeuffroy *et al.* 2002).

This short discussion of several concepts included in selected crop and global natural system models points out some possibilities about how to represent relevant crop C-N processes in a global model. However most of the models described only represent a number of the important processes and interactions that emerged in this thesis from a look at crop physiology (see 4.1.1).

4.3 Conclusions and outlook

When constructing a model of biological processes one always has to assess the adequacy of mechanistic vs. empirical approaches. Thornley (1976) describes the steps in the construction of a mechanistic model as follows:

I. Look at the structure of the system.

II. Divide the system into components.

III. Try to understand the behaviour of the whole system in terms of the behaviour of the individual system components and their interactions with one another.

IV. Make some assumptions:

   a. Which are important components of the system?

   b. How do they behave?

   c. Which can be ignored?

V. Mathematical description and formulation of assumptions as equations.

VI. Solution of the equations.

VII. Comparison of predicted values with experimental data.

In an empirical model instead one tries to understand the responses of a system, without going through the step of understanding system structure and without making assumptions and trying to work out mathematical consequences of assumptions. Thornley (1976) describes the stages of the construction of an empirical model as:

I. Look at experimental data.

II. Possibly do some analysis of the data.

III. Make an intelligent guess at the (usually simple) form of equation or set of equations that can be used, fitted to the data.

Both approaches have their advantages and disadvantages – which will not be further discussed here-, and often a combination of mechanistic and empirical methods is applied (Thornley 1976).

Some global models centre on empirical functions (e.g. TEM, Raich *et al.* 1991), while others try a more mechanistic representation of processes (e.g. CLM-CN, Thornton *et al.* 2007 and LPJ, Sitch *et al.* 2003), but most involve both mechanistic and empirical derivations. The dependence of photosynthesis on N_L for example is an essentially empirical observation. And as discussed in chapter 2 in all probability it does not depict a primary control. However the relation can be used as a basis to predict V_{Cmax}, which then can be used in the comparably mechanistic photosynthesis model of Farquhar *et al.* (1980a) (see 4.1.2). The Farquhar *et al.* (1980a) model is in fact a very nice example for a mechanistic representation of physiological processes that reduces and simplifies the relative profound knowledge on the multiple steps and complex interactions in photosynthesis to such a degree that it can be easily and widely applied in ecological models up to the global scale. Such a physiological model that reduces complex processes to a few essential controls and that does not need extensive parameterization is the best case for modeling. However it requires good knowledge on physiological interactions and controls.

In the present thesis I have first looked at the mechanisms governing the N metabolism in crops. I have tried to dissect the controls imposed by N on several components of crop physiology and I have looked at how the plant achieves a coordination of metabolic processes by joint N and C signals. This evaluation resulted in an integrated and simplified picture of crop C-N interactions. In the second part of the thesis I have instead adopted a more empirical approach and tried to dissect quantitative relationships of crop responses to N availability by making a statistical analysis of experimental data. In chapter 2 I have thus undertaken steps I to III of the construction of a mechanistic model, with some considerations on step IV, and in chapter 3 I have conducted steps I and II of the empirical approach described by Thornley (1976).

For the prediction of system responses, models should be as mechanistic as possible, as this allows for an operational simulation that enables to predict changes in processes and interactions under changing environmental conditions. For the incorporation of processes into models it is therefore important to first develop a sufficiently robust understanding of mechanisms. This requires the integration of advances from plant physiology and molecular biology, as done in section 2 of this study. Several N processes and C-N interactions discussed in that chapter have been implemented in physiological plant models (e.g. Bijlsma & Lambers 2000; Bijlsma *et al.* 2000). But the step from such mechanistic and parameter-intensive simulations of processes in single plants to a simulation of plant processes adequate for a global model is quite large. For an implementation of crop N processes into a global model therefore the understanding of the mechanism underlying plant growth and plant responses needs to be used to reduce and to generalize where possible. Meta-analysis has proved to be a promising tool for priority setting with regard to such general dependencies and for the definition of the shape of responses. It can also be applied to assess differences between species in the response to N limitation and potentially for the derivation of parameters for modelling.

The system components divided and described here, their behaviour and their interactions then need

to be translated into mathematical formulations (steps V and III respectively, as described by Thornley 1976). This necessitates both the discussion of the utility of existing concepts derived from different types of models (as discussed on some examples in 4.1.2) and possibly their incorporation into the model in question. But it also necessitates the mathematical formulation of interactions that so far have not been considered (e.g. N_2 fixation in leguminous crops or the regulation of the dynamic N allocation into different photosynthetic components) or which formulation needs to be improved (e.g. the dynamic and N-dependent allocation of C to root and shoot).

As mentioned in the introduction, I have in this thesis only looked at the crop plant from an autecological perspective, thus just considering what the plant does with certain environmental conditions and external driving variables. The isolated crop plant however represents just a small section of the environmental system. In reality the plant cannot be assessed detached from its biotic and abiotic surrounding, as it is both influenced by and shapes its environment.

In a model of terrestrial C-N interactions the representation of the pedosphere and of the interplay between the biotic and abiotic environment, both above- and belowground, are at least as important as the representation of primary production in plants. Willigen (1991) concluded from a comparison of the description of N turnover in the soil-crop system in crop models that the representation of belowground processes and especially of biological soil processes was more problematic and less accurate than the representation of aboveground processes. In fact many processes in the pedosphere and their response to environmental change are still poorly understood (e.g. Powlson 1993; Rustad 2001; Neff *et al.* 2002).

As could be concluded from the review of the literature (see 2.3) as well as from the results of the meta-analysis (see 3.4) even within the plant numerous effects and mechanisms - e.g. the response of the A_{max}-N_L relationship and the degree of acclimation of photosynthesis to changing environmental conditions, the regulation of photosynthetic proteins or the mechanisms underlying the control of O_2 diffusion into root nodules - remain largely uncertain. The plant however is a by far better studied system than the soil or even than the whole ecosystem. The example of the Farquhar *et al.* (1980a) photosynthesis model emphasizes that a solid understanding of processes is essential for their representation in models. Therefore, in addition to the gathering and interpretation of information on processes (as done in this thesis), and to the mathematical description of these processes for a representation in models, an increased understanding of the underlying mechanisms through primary biochemical, physiological and ecological research appears to be a prerequisite for the improvement of the forecasting capacity of global terrestrial biosphere models.

Acknowledgements

First of all I want to thank Christoph Müller for being my tutor. He always was there – although geographically quite far away - with help, advice and encouragement and he showed great patience with regard to my sometimes strange ideas and longwinded E-mails. The contact and exchange with him helped me to bring more order and systematic into my doings. I am also grateful to Werner Kaiser and Wolfgang Cramer for agreeing to supervise my diploma thesis. Thanks to my brother Luca who helped with editorial matters. Special thanks go to my parents: my mother Susanne for being a very reliable, enduring and entirely overqualified assistant, helping with data extraction and the literature list, and my father Günther for many fruitful discussions, inspiring ideas and inputs and also for helping me to keep the necessary critical distance to my subject.

References

Adalsteinsson S. & Jensen P. (1988). Root development in winter wheat grown at different N/P supply: Root length patterns and N-P interactions in phosphate uptake. *Physiologia Plantarum* 72: 271-278.

Adam N. R., Wall G. W., Kimball B. A., Pinter P. J., LaMorte R. L., Hunsaker D. J., Adamsen F. J., Thompson T., Matthias A. D., Leavitt S. W. & Webber A. N. (2000). Acclimation response of spring wheat in a free-air CO_2 enrichment (FACE) atmosphere with variable soil nitrogen regimes. 1. Leaf position and phenology determine acclimation response. *Photosynthesis Research* 66: 65-77.

Adams D. C., Gurevitch J. & Rosenberg M. S. (1997). Resampling tests for meta-analysis of ecological data. *Ecology* 78 (5): 1277-1283.

Adu-Gyamfi J. J., Myaka F. A., Sakala W. D., Odgaard R., Veseterager J. M. & Hogh-Jensen H. (2007). Biological nitrogen fixation and nitrogen and phosphorus budgets in farmer-managed intercrops of maize-pigonpea in semi-arid southern and eastern Africa. *Plant and Soil* 295: 127-136.

Agren G. I. (1985). Limits to plant production. *Journal of Theoretical Biology* 113: 89-92.

Ainsworth E. A., Davey P. W., Bernacchi C. J., Dermody O. C., Heaton E. A., Moore D. J., Morgan P. B., Naidu S. L., Ra H.-S. Y., Zhu X.-G., Curtis P. S. & Long S. P. (2002). A meta-analysis of elevated (CO_2) effects on soybean (Glycine max) physiology, growth and yield. *Global Change Biology* 8: 695-709.

Ali A., Rab A. & Hussain S. A. (2003). Yield and nutrients profile of potato tubers at various stages of development. *Asian Journal of Plant Sciences* 2 (2): 247-250.

Améziane R., Richard-Molard C., Deléens E., Morot-Gaudry J.-F. & Limami A. M. (1997). Nitrate ($^{15}NO_3$) limitation affects nitrogen partitioning between metabolic and storage sinks and nitrogen reserve accumulation in chicory (*Cichorium intybus* L.). *Planta* 202: 303-312.

Amthor J. S. (1984). The role of maintenance respiration in plant growth. *Plant, Cell and Environment* 7: 561-569.

Amthor J. S. (2000). The McCree-de Wit-Penning de Vries-Thornley respiration paradigms: 30 years later. *Annals of Botany* 86: 1-20.

Anderson P. M., Oelke E. A. & Simmons S. R. (1985a). *Growth and Development Guide for Spring Wheat*. University of Minnesota Agricultural Extension Folder AG-FO-2547.

Anderson P. M., Oelke E. A. & Simmons S. R. (1985b). *Growth and Development Guide for Spring Barley*. University of Minnesota Agricultural Extension Folder AG-FO-2548.

Anten N. P. R., Schieving F. & Werger M. J. A. (1995). Patterns of light and nitrogen distribution in relation to whole canopy carbon gain in C_3 and C_4 mono- and dicotyledonous species. *Oecologia* 101: 504-513.

Arft A. M., Walker M. D., Gurevitch J., Alatalo J. M., Bret-Harte M. S., Dale M., Diemer M., Gugerli F., Henry G. H. R., Jones M. H., Hollister R. D., Jónsdóttir I. S., Laine K., Lévesque E., Marion G.M., Molau U., Molgaard P., Nordenhall U., Raszhivin V., Robinson C. H., Starr G., Stenström A., Stenström M., Totland O., Turner P. L., Walker I. J., Webber P. J., Welker J. M. & Wookey P. A. (1999). Responses of tundra plants to experimental warming: Meta-analysis of the international tundra experiment. *Ecological Monographs* 69: 491-511.

Arp W. J. (1991). Effects of source-sink relations on photosynthetic acclimation to elevated CO_2. *Plant, Cell and Environment* 14: 869-875.

Atkin O. K. (1996). Reassessing the nitrogen relations of Arctic plants: a mini-review. *Plant, Cell and Environment* 19: 695-704.

Atkinson L. J., Hellicar M. A., Fitter A. H. & Atkin O. E. (2007). Impact of temperature on the relationship between respiration and nitrogen concentration in roots: an analysis of scaling relationships, Q_{10} values and thermal acclimation ratios. *New Phytologist* 173: 110-120.

Barneix A. J., James D. M., Watson E. F. & Hewitt E. J. (1984). Some effects of nitrate abundance and starvation on metabolism and accumulation of nitrogen in barley (*Hordeum vulgare* L. cv Sonja). *Planta* 162: 469-476.

Barneix A. J., Arnozis P. A. & Guitman M. R. (1992). The regulation of nitrogen accumulation in the grain of wheat plants (*Triticum aestivum*). *Physiologia Plantarium* 86: 609-615.

Beck E. H. (1996). Regulation of shoot/root ratio by cytokinins from roots in *Urtica dioica*: Opinion. *Plant and Soil*: 1-12.

Berges J. A., Charlebois D. O., Mauzerall D. C. & Falkowski P. G. (1996): Differential effects of nitrogen limitation on photosynthetic efficiency of photosystems I and II in microalgae. *Plant Physiology* 110: 689-696.

Bhat K. K. S., Nye P. H. & Brereton A. J. (1979). The possibility of predicting solute uptake and plant growth response from independently measured soil and plant characteristics. VI. The growth and uptake of rape in solutions of constant nitrate concentration. *Plant and Soil* 53: 137-167.

Biemond, H. & Vos J. (1992). Effects of nitrogen on the development and growth of the potato plant. 2. The partitioning of dry matter, nitrogen and nitrate. *Annals of Botany* 70: 37-45.

Bijlsma R. J. & Lambers H. (2000). A dynamic whole-plant model of integrated metabolism of nitrogen and carbon. 2. Balanced growth driven by C fluxes and regulated by signals from C and N substrate. *Plant and Soil* 220: 71-87.

Bijlsma R. J., Lambers H. & Kooijman S. A. L. M. (2000). A dynamic whole-plant model of integrated metabolism of nitrogen and carbon. 1. Comparative ecological implications of ammonium-nitrate interactions. *Plant and Soil* 220: 49-69.

Billes G., Rouhier H. & Bottner P. (1993). Modifications of the carbon and nitrogen allocations in the plant (*Triticum aestivum* L.) soil system in response to increased atmospheric CO_2 concentration. *Plant and Soil* 157: 215-225.

Bingham I. J., Blackwood J. M. & Stevenson E. A. (1998). Relationship between tissue sugar content, phloem import and lateral root initiation in wheat. *Physiologia Plantarum* 103: 107-113.

Bloom A. J. & Chapin F. S. (1981). Differences in steady-state net ammonium and nitrate influx by cold- and warm-adapted barley varieties. *Plant Physiology* 68: 1064-1067.

Bloom A. J., Chapin F. S. & Mooney H. A. (1985). Resource limitation in plants. An economic analogy. *Annual Review of Ecology and Systematics* 16: 363.392.

Bloom A. J., Jackson L. E. & Smart D. R. (1993). Root growth as a function of ammonium and nitrate in the root zone. *Plant, Cell and Environment* 16: 199-206.

Bloom A. J., Sukrapanna S. S. & Warner R. L. (1992). Root respiration associated with ammonium and nitrate absorption and assimilation by Barley. *Plant Physiology* 99: 1294-1301.

Boddey R. M., de Oliveira O. C., Urquiaga S., Reis V. M., de Olivares F. L., Baldani V. L. D. & Döbereiner J. (1995). Biological nitrogen fixation associated with sugar cane and rice: contributions and prospects for improvement. *Plant and Soil* 174: 195-209.

Boddey R. M., Urquiaga S., Alves B. J. R. & Reis V. (2003). Endophytic nitrogen fixation in

sugarcane: present knowledge and future applications. *Plant and Soil* 252: 139-149.

Bondeau A., Smith P. C., Zaehle S., Schaphoff S., Lucht W., Cramer W., Gerten D., Lotze-Campen H., Müller C., Reichstein M. & Smith B. (2007). Modelling the role of agriculture for the 20th century global terrestrial carbon balance. *Global Change Biology* 13: 679-706.

Bouma D. (1970). Effects of nitrogen nutrition on leaf expansion and photosynthesis of *Trifolium subterraneum L.* 1. Comparison between different levels of nitrogen supply. *Annals of Applied Botany* 34: 1131-1142.

Bowes. G. (1993). Facing the inevitable: Plants and increasing atmospheric CO_2. *Annual Review of Plant Physiology and Plant Molecular Biology* 44: 309-332.

Brewitz E., Larsson C.-M. & Larsson M. (1995). Influence of nitrate supply on concentrations and translocation of absisic acid in barley (*Hordeum vulgare*). *Physiologia Plantarum* 95: 499-506.

Britto D. T. & Kronzucker H. J. (2002). NH_4^+ toxicity in higher plants: a critical review. *Journal of Plant Physiology* 159: 567-584.

Brovkin V., Sitch S., Von Bloh W., Claussen M., Bauer E. & Cramer W. (2004). Role of land cover changes for atmospheric CO_2 increase and climate change during the last 150 years. *Global Change Biology* 10: 1253-1266.

Caloin M. & Yu O. (1984). Analysis of the time course of change in nitrogen content in *Dactylis glomerata* L. using a model of plant growth. *Annals of Botany* 54: 69-76.

Campbell W. H. (1999). Nitrate reductase structure, function and regulation: bridging the gap between biochemistry and physiology. *Annual Review of Plant Physiology and Plant Molecular Biology* 50: 277-303.

Caputo C. & Barneix A. J. (1997). Export of amino acids to the phloem in relation to N supply in wheat. *Physiologia Plantarum* 101: 853-860.

Carvajal M., Cooke D. T. & Clarkson D. T. (1996). Responses of wheat plants to nutrient deprivation may involve the regulation of water-channel function. *Planta* 199: 372-381.

Chapin F. S. III (1980). The mineral nutrition of wild plants. *Annual Review of Ecology and Systematics* 11: 233-260.

Chapin F. S. III, Bloom A. J., Field C. B. & Waring R. H. (1987). Plant responses to multiple environmental factors. *BioScience* 37 (1): 49-57.

Chapin F. S., Clarkson D. T., Lenton J. R. & Walter C. H. S. (1988a). Effect of nitrogen stress and abscisic acid on nitrate absorption and transport in barley and tomato. *Planta* 173: 340-351.

Chapin F. S. III, Walter C. H. S. & Clarson D. T. (1988b). Growth response of barley and tomato to nitrogen stress and its control by abscisic acid, water relations and photosynthesis. *Planta* 173: 353-366.

Chapin F. S. III, Schulze E.-D. & Mooney H. A. (1990). The ecology and economics of storage in plants. *Annual Review of Ecology and Systematics* 21: 423-447.

Chapin F. S. III, Matson P. A. & Mooney H. A. (2002). *Principles of Terrestrial Ecosystem Ecology*. Springer, New York, USA. 472 pp.

Chen B. & Coops N. C. (2009). Understanding of coupled terrestrial carbon, nitrogen and water dynamics – an overview. *Sensors* 9: 8624-8657.

Chen J.-L., Reynolds J. F., Harley P. C. & Tenhunen J. D. (1993). Coordination theory of leaf nitrogen distribution in a canopy. *Oecologia* 93: 63-69.

Clark H. E. (1936). Effect of ammonium and of nitrate nitrogen on the composition of the tomato

plant. *Plant Physiology*: 5-24.

Claussen M. (1994). On coupling global biome models with climate models. *Climate Research* 4: 203-221.

Cliquet J. B., Murray P. J. & Boucaud J. (1997). Effect of arbuscular mycorrhizal fungus *Glomus fasciculatum* on the uptake of amino nitrogen by *Lolium perenne*. *New Phytologist* 137: 345-349.

Cooper H. D. & Clarkson D. T. (1989). Cycling of amino-nitrogen and other nutrients between shoots and roots in cereals – a possible mechanism integrating shoot and root in the regulation of nutrient uptake. *Journal of Experimental Botany* 40: 753-762.

Collatz G. J., Ball J. T., Grivet C. & Berry J. A. (1991). Physiological and environmental regulation of stomatal conductance, photosynthesis and transpiration: a model that includes a laminar boundary layer. *Agricultural and Forest Meteorology* 54: 107-136.

Collatz G. J., Ribas-Carbo M. & Berry J. A. (1992). Coupled photosynthesis-stomatal conductance model for leaves of C_4 plants. *Australian Journal of Plant Physiology* 19: 519-538.

Colnenne C., Meynard J. M., Reau R., Justes E. & Merrien A. (1998). Determination of a critical dilution curve for winter oilseed rape. *Annals of Botany* 81: 311-317.

Cotrufo M. F., Ineson P. & Scott A. (1998). Elevated CO_2 reduces the nitrogen concentration of plant tissues. *Global Change Biology* 4: 43-54.

Cox W. J. & Reisenauer H. M. (1973). Growth and ion uptake by wheat supplied nitrogen as nitrate, or ammonium, or both. *Plant and Soil* 38: 363-380.

Cramer M. C. & Lewis O. A. M. (1993). The influence of nitrate and ammonium nutrition on the growth of wheat (*Triticum aestivum*) and Maize (*Zea mays*) plants. *Annals of Botany* 72: 359-365.

Cramer W., Kicklighter D. W., Bondeau A., Moore B., Churkina G., Nemry B., Ruimy A., Schlosse A. L. & Participants of the Potsdam NPP Model Intercomparison (1999). Comparing global models of terrestrial net primary productivity (NPP): overview and key results. *Global Change Biology* 5 (Suppl. 1): 1-15.

Cramer W., Bondeau A., Woodward F. I., Prentice I. C., Betts R. A., Brovkin V., Cox P. M., Fisher V., Foley J. A., Friend A. D., Kucharik C., Lomas M. R., Ramankutty N., Sitch S., Smith B., White A. & Young-Molling C. (2001). Global response of terrestrial ecosystem structure and function to CO_2 and climate change: results from six dynamic global vegetation models. *Global Change Biology* 7: 357-373.

Crawford N. M. & Glass A. D. M. (1998). Molecular and physiological aspects of nitrate uptake in plants. *Trends in Plant Science* 3(10): 389-395.

Crawford N. M. (1995). Nitrate: Nutrient and signal for plant growth. *The Plant Cell* 7: 859-868.

Cren M. & Hirel B. (1999). Glutamine synthetase in higher plants: regulation of gene and protein expression from the organ to the cell. *Plant Cell Physiology* 40(12): 1187-1193.

Crookshanks M., Taylor G.. & Dolan L. (1998). A model system to study the effects of elevated CO2 on the developmental physiology of roots: the use of *Arabidopsis thaliana*. *Journal of Experimental Botany* 49(320): 593-597.

Curtis P. S. & Wang X. (1998). A meta-analysis of elevated CO_2 effects on woody plant mass, form, and physiology. *Oecologia* 113: 299-313.

Curtis P. S. (1996). A meta-analysis of leaf gas exchange and nitrogen in trees grown under elevated carbon dioxide. *Plant, Cell and Environment* 19: 127-137.

D´Agostino I. B., Deruère & Kieber J. J. (2000). Characterization of the response of the Arabidopsis response regulator gene family fo cytokinin. *Plant Physiology* 124: 1706-1717.

DeCoster J. (2004). *Meta-Analysis Notes*. Department of Psychology, University of Alabama <http://www.stat-help.com/notes.html> (last access on 23.09.2009).

Delgado E., Mitchell R. A. C., parry M. A. J., Driscoll S. P., Mitchell V. J. & Lawlor D. W. (1994). Interacting effects of CO_2 concentration, temperature and nitrogen supply on the photosynthesis and composition of winter wheat leaves. *Plant, Cell and Environment* 17: 1205-1213.

Devienne F., Mary B. & Lamaze. T. (1994). Nitrate transport in intact wheat roots. II. Long-term effects of NO_3^- concentration in the nutrient solution on NO_3^- unidirectional fluxes and distribution within the tissues. *Journal of Experimental Botany* 45(274): 677-684.

Dijkwel P.P., Kock P. A. M., Bezemer R., Weisbeek P. J. & Smeekens S. C. M. (1996). Sucrose represses the developmentally controlled transient activation of the plastocyanin gene in *Arabidopsis thaliana* seedlings. *Plant Physiology* 110: 455-463.

Dixon R. & Kahn D. (2004). Genetic regulation of biological nitrogen fixation. *Nature Reviews Microbiology* 2: 621-631.

Drew M. C. (1975). Comparison of the effects of a localized supply of phosphate, nitrate, ammonium and potassium on the growth of the seminal root system, and the shoot, in barley. *New Phyotologist* 75: 479-490.

Egli D. B. (1997). Cultivar maturity and response of soybean to shade stress during seed filling. *Field Crops Research* 52: 1-8.

Ellsworth D. S., Reich P. B., Naumburg E. S., Koch G. W., Kubiske M. E. & Smith Stan D. (2004). Photosynthesis, carboxylation and leaf nitrogen responses of 16 species to elevated pCO_2 across four free-air CO_2 enrichment experiments in forest, grassland and desert. *Global Change Biology* 10: 2121-2138.

Ericsson T. (1995). Growth and shoot: root ratio of seedlings in relation to nutrient availability. *Plant and Soil* 168-169: 205-214.

Evans L. T. (1975). Beyond photosynthesis – the role of respiration, translocation and growth potential in determining productivity. In: Cooper J. P. (ed.). *Photosynthesis and productivity in different environments*, pp. 501-507. International Biological Programme III. Cambridge University Press, Cambridge.

Evans J. R. (1983). Nitrogen and photosynthesis in the flag leaf of wheat (*Triticum aestivum* L.). *Plant Physiology* 72: 297-302.

Evans J. R. (1989). Photosynthesis and nitrogen relationships in leaves of C_3 plants. *Oecologia* 78: 9-19.

Evans J. R. (1996). Development constraints on photosynthesis: effects of light and nutrition. In: Baker N. R. (ed.): *Photosynthesis and the environment*, pp.281-304. Advances in Photosynthesis, Volume 5. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Evans J. R. & Terashima (1988). Photosynthetic characteristics of spinach leaves grown with different nitrogen treatments. *Plant and Cell Physiology* 29(1): 157-165.

Evans J. R. & Poorter, H. (2001). Photosynthetic acclimation of plants to growth irradiance: the relative importance of specific leaf area and nitrogen partitioning in maximizing carbon gain. *Plant, Cell and Environment* 24: 755-767.

Fair P., Tew J. & Cresswell C. F. (1974). Enzyme activities associated with carbon dioxide exchange in illuminated leaves of *Hordeum vulgare* L. III. Effects of concentration and form

of nitrogen supplied on carbon dioxide compensation point. *Annals of Botany* 38: 39-43.

Fangmeier A., Hadwiger-Fangmeier A., Van der Eerden L. & Jäger H.-J. (1994). Effects of atmospheric ammonia on vegetation – a review. *Environmental Pollution* 86: 43-82.

FAOSTAT (2007). *FAOSTAT data*. URL: <http://faostat.fao.org/site/291/default.aspx> (last access on 26.11.2009)

Farquhar G. D. & Sharkey T. D. (1982). Stomatal conductance and photosynthesis. *Annual Review of Plant Physiology and Plant Molecular Biology* 33: 317-345.

Farquhar G. D., von Caemmerer S. & Berry J. A. (1980a). A biochemical model of photosynthetic CO_2 assimilation in leaves of C_3 species. *Planta* 149: 78-90.

Farquhar G. D., Firth P. M., Wetselaar R. & Weir B. (1980b). On the gaseous exchange of ammonia between leaves and the environment: determination of the ammonia compensation point. *Plant Physiology* 66: 710-714.

Farrar J. (1996). Regulation of root weight ratio is mediated by sucrose: Opinion. *Plant and Soil* 185: 13-19.

Fenn M. E., Baron J. S., Allen E. B., Rueth H. M. Nydick K. R., Geiser L., Bowman W. D., Sickman J. O., Meixner T., Johnson D. W. & Neitlich P. (2003). Ecological effects of nitrogen deposition in the western united states. *BioScience* 53(4): 404-420.

Field C. & Mooney H. A. (1983). Leaf age and seasonal effects on light, water, and nitrogen use efficiency in a California shrub. *Oecologia* 56: 348-355.

Field C. & Mooney H. A. (1986). The photosynthesis-nitrogen relationship in wild plants. In: Givnish T. J. (ed.): *On the economy of plant form and function*, pp. 25-54. Cambridge University Press, Cambridge.

Field C. (1983). Allocating leaf nitrogen for the maximization of carbon gain: leaf age as a control on the allocation program. *Oecologia* 56: 341-347.

Finck A. (2007). *Pflanzenernährung und Düngung in Stichworten,* 6. edition. Borntraeger, Berlin. 253 pp.

Finnemann J. & Scjoerring J. K. (2000). Post-translational regulation of cytosolic glutamine synthetase by reversible phosphorylation and 14-3-3 protein interaction. *The Plant Journal* 24(2): 171-181.

Fischer H.-M. (1994). Genetic regulation of nitrogen fixation in Rhizobia. *Microbiological Reviews* 58(3): 352-386.

Flores S. & Tobin E. M. (1988). Cytokinin modulation of LHCP mRNA levels: the involvement of post-transcriptional regulation. *Plant Molecular Biology* 11: 409-415.

Foehse D. & Jungk A. (1983). Influence of phosphate and nitrate supply on root hair formation of rape, spinach and tomato plants. *Plant and Soil* 74: 359-368.

Foley J. A., Prentice I. C., Ramankutty N., Levis S., Pollard D., Sitch S. & Haxeltine A. (1996). An integrated biosphere model of land surface processes, terrestrial carbon balance, and vegetation dynamics. *Global Biogeochemical Cycles* 10(4): 603-628.

Foley J. A., Levis S., Prentic C., Pollard D. & Thompson S. L. (1998). Coupling dynamic models of climate and vegetation. *Global Change Biology* 4: 561-579.

Foley J. A., DeFries R., Asner G. P., Barford C., Bonan G., Carpenter S. R., Stuart Chapin F., Coe M. T., Daily G. C., Gibbs H. K., Helkowski J. H., Holloway T., Howard E. A., Kucharik C. J., Monfreda C., Patz J. A., Prentice C., Ramankutty N. & Snyder P. K. (2005). Global consequences of land use. *Science* 309: 570-574.

Forde B. G. (2000). Nitrate transporters in plants: structure, function and regulation. *Biochimica et Biophysica Acta* 1465: 219-235.

Forde B. G. (2002). Local and long-range signalling pathways regulating plant responses to nitrate. *Annual Revies of Plant Biology* 53: 203-224.

Foyer C. H., Lescure J.-C., Lefebvre C., Morot-Gaudry J.-F. Vincentz M. & Vaucheret H. (1994). Adaptations of photosynthetic electron transport, carbon assimilation, and carbon partitioning in transgenic *Nicotiana plumbaginifolia* plants to changes in nitrate reductase activity. *Plant Physiology* 104: 171-178.

Freeden A. L., Gamon J. A. & Field C. B. (1991). Responses of photosynthesis and carbohydrate-partitioning to limitations in nitrogen and water availability in field-grown sunflower. *Plant, Cell and Environment* 14; 963-970.

Fricke W., McDonald A.J.S. & Mattson-Djos L. (1997). Why do leaves and leaf cells of N-limited barley elongate at reduced rates? *Planta* 202: 522-530.

Friedlingstein P., Cox P., Betts R., Bopp L., Von Bloh W., Brovkin V., Cadule Pl, Doney S., Eby M., Fung I., Bala G., John J., Jones C., Joos F., Kato T., Kawamiya M., Knorr W., Lindsay K., Metthews H. D., Raddatz T., Rayner P., Reick C., Roeckner E., Schnitzler K.-G., Schnur R., Strassmann K., Weaver A. J., Yoshikawa C., & Zeng N., (2006). Climate-carbon cycle feedback analysis: Results from the C^4MIP model intercomparison. *Journal of Climate* 19: 3337-3353.

Friend A. D. (1995). PGEN: an integrated model of leaf photosynthesis, transpiration, and conductance. *Ecological Modelling* 77: 233-255.

Friend A. D., Stevens A. K., Knox R. G.. & Cannell M. G. R. (1997). A process-based, terrestrial biosphere model of ecosystem dynamics (Hybrid v3.0). *Ecological Modelling* 95: 249-287.

Fuentes S. I., Allen D. J., Ortiz-Lopez A. & Hernández G. (2001). Over-expression of cytosolic glutamine synthetase increases photosynthesis and growth at low nitrogen concentrations. *Journal of Experimental Botany* 52(358): 1071-1081.

Galloway J. N., Townsend A. R., Erisman J. W., Bekunda M., Cai Z., Freney J. R., Martinelli L. A., Seitzinger S. P. & Sutton M. A. (2008). Tranformation of the nitrogen cycle: recent trends, questions and potential solutions. *Science* 320: 889-892.

Gansel X., Muños S., Tillard P. & Gojon A. (2001). Differential regulation of the NO_3^- and NH_4^+ transporter genes *AtNrt2.1* and *AtAmt1.1* in *Arabidopsis*: relation with long-distance and local controls by N status of the plant. *The Plant Journal* 26(2): 143-156.

Garcia R., Kanemasu E. T., Blad B. L., Bauer A., Hatfield J. L., Major D. J., Reginato R. J. & Hubbard K. G. (1988). Interception and use efficiency of light in winter wheat under different nitrogen regimes. *Agricultural and Forest Meteorology* 44: 175-186.

Gastal F. & Belanger G. (1993). The effects of nitrogen fertilization and the growing season on photosynthesis of field-grown tall fescue (*Festuca arundinacea* Schreb.) canopies. *Annals of Botany* 72: 401-408.

Gastal F. & Lemaire G. (2002). N uptake and distribution in crops: an agroeconomical and ecophysiological perspective. *Journal of Experimental Botany* 53: 789-799.

Gastal F., Belanger, G. & Lemaire G. (1992). A model of the leaf extension rate of tall fescue in response to nitrogen and temperature. *Annals of Botany* 70: 437-442.

Gervois S., Noblet-Ducoudré N. de, Viovy N. & Ciais P. (2004). Including croplands in a global biosphere model: Methodology and evaluation at specific sites. *Earth Interactions* 8(16): 1-25.

Geßler A., Rienks M. & Rennenberg H. (2002). Stomatal uptake and cuticular adsorption contribute to dry deposition of NH_3 and NO_2 to needles of adult spruce (*Picea abies*) trees. *New Phytologist* 156: 179-194.

Gilbert G. A., Gadush M. V., Wilson C. & madore M. A. (1998). Amino acid accumulation in sink and source tissues of *Coleus blumei* Benth. during salinity stress. *Journal of Experimental Botany* 49: 107-114.

Glass A. D. M., Brito D. T., Kaiser B. N., Kronzucker H. J., Kumar A., Okamoto M., Rawat S. R., Siddiqi M. Y., Silim S. M., Vidmar J. J. & Zhuo D. (2001). Nitrogen transport in plants, with an emphasis on the regulation of fluxes to match plant demand. *Journal of Plant Nutrition and Soil Science* 164: 199-207.

Glass A. D. M, Britto D. T., Kaiser B. N., Kinghom J. R., Kronzucker H. J., Kumar A., Okamoto M., Rawat S., Siddiqi M. Y., Unkles S. E. & Vidmar J. J. (2002). The regulation of nitrate and ammonium transport systems in plants. *Journal of Experimental Botany* 53: 855-864.

Goldberg D. E., Rajaniemi T., Gurevitch J. & Stewart-Oaten A. (1999). Empirical approaches to quantifying interaction intensity: Competition and facilitation along productivity gradients. *Ecology* 80(4): 118-1131.

Gómez-Aparicio L. (2009). The role of plant interactions in the restoration of degraded ecosystems: a meta-analysis across life-forms and ecosystems. *Journal of Ecology* 97: 1202-1214.

Graham P. H. & Vance C. P. (2000). Nitrogen fixation in perspective: an overview of research and extension needs. *Field Crops Research* 65: 93-106.

Green C. F. (1987). Nitrogen nutrition and wheat growth in relation to absorbed solar radiation. *Agricultural and Forest Meteorology* 41: 207-248.

Greenwood D. J., Lemaire G., Gosse G., Cruz P., Draycott A. & Neeteson J. J. (1990). Decline in percentage N of C3 and C4 crops with increasing plant mass. *Annals of Botany* 66: 425-436.

Greenwood D. J., Gastal F., Lemaire G., Draycott A., Millard P. & Neeteson J. J. (1991). Growth Rate and % N of Field Grown Crops: Theory and Experiments. *Annals of Botany* 67: 181-190.

Grindlay D. J. C. (1997). Towards an explanation of crop nitrogen demand based on the optimization of leaf nitrogen per unit leaf area. *Journal of Agricultural Science* 128: 377-396.

Guitman M. R., Arnozis P. A. & Barneix A. J. (1991). Effect of source-sink relations and nitrogen nutrition on senescence and N remobilization in the flag leaf of wheat. *Physiologia Plantarium* 82: 278-284.

Gulmon S. L. & Chu C. C. (1981). The effects of light and nitrogen on photosynthesis, leaf characteristics and dry matter allocation in the chaparall schrub, *Diplacus aurantiacus*. *Oecologia* 49: 207-212.

Gurevitch K. & Hedges L. V. (1999). Statistical issues in ecological meta-analyses. *Ecology* 80(4): 1142-1149.

Gurevitch K., Morrison J. A. & Hedges L. V. (2000). The interaction between competition and predation: A meta-analysis of field experiments. *The American Naturalist* 155(4):435-453.

Harbur M. M. & Owen M. D. K. (2004). Light and growth rate effects on crop and weed responses to nitrogen. *Weed Science* 52: 578-583.

Harley P. C. & Sharkey D. (1991). An improved model of C_3 photosynthesis at high CO_2: Reversed O_2 sensitivity explained by lack of glycerate reentry into the chloroplast. *Photosynthesis Research* 27: 169-178.

Hawkes C. V. & Sullivan J. J. (2001). The impact of herbivory on plants in different resource conditions: A meta-analysis. *Ecology* 82(7): 169-178.

Haxeltine A. & Prentice I. C. (1996). A general model for the light-use efficiency of primary production. *Functional Ecology* 10(5): 551-561.

Hedges L. V., Gurevitch J. & Curtis P. W. (1999): The meta-analysis of response ratios in experimental ecology. *Ecology* 80 (4): 1150-1156.

Heilmeier H., Schulze E.-D. & Whale D. M. (1986). Carbon and nitrogen partitioning in the biennial monocarp *Arctium tomentosum* Mill. *Oecologia* 70: 466-474.

Herridge D. F., Peoples M. B. & Boddey R. M. (2008). Global inputs of biological nitrogen fixation in agricultural systems. *Plant Soil* 311: 1-18.

Hewitt E. J. & Smith T. A. (1975). *Plant mineral nutrition*. The English University Press, London. 298 pp.

Hikosaka K. & Hirose T. (1998). Leaf and canopy photosynthesis of C_3 plants at elevated CO_2 in relation to optimal partitioning of nitrogen among photosynthetic components: theoretical prediction. *Ecological Modelling* 106: 247-259.

Hikosaka K. & Terashima I. (1995). A model of the acclimation of photosynthesis in the leaves of C_3 plants to sun and shade with respect to nitrogen use. *Plant, Cell and Environment* 18: 605-618.

Hikosaka K. & Terashima I. (1996). Nitrogen parititioning among photosynthetic components and its consequence in sun and shade plants. *Functional Ecology* 10: 335-343.

Hilbert D. W. (1990). Optimization of plant root:shoot ratios and internal nitrogen concentration. *Annals of Botany* 66: 91-99.

Hill J. E. & Williams J. F. (1997). Rice development stages. In: *UC IPM Pest management guidelines: Rice*. University of California Agriculture and Natural Resources, Publication 3465.

Hirose T. (1984). Nitrogen use efficiency in growth of *Polygonum cuspidatum* Sieb. et Zucc. *Annals of Botany* 54: 695-704.

Hirose T. & Werger M. J. A. (1987a). Nitrogen use efficiency in instantaneous and daily photosynthesis of leaves in a canopy of a *Solidago altissima* stand. *Physiologia Plantarum* 70: 215-222.

Hirose T. & Werger M. J. A. (1987b). Maximizing daily canopy photosynthesis with respect to the leaf nitrogen allocation pattern in the canopy. *Oecologia* 72: 520-526.

Hoagland D. R. & Arnon D. I. (1950). *The water culture method for growing plants without soil*. California Agricultural Experiment Station Circular 347. 39 pp.

Hodge A., Robinson D. & Fitters A. (2000a). Are microorganisms more effective than plants at competing for nitrogen? *Trends in Plant Science* (7): 304-308.

Hodge A., Robinson D. & Fitter A. H. (2000b). An arbuscular mycorrhizal inoculum enhances root proliferation in, but not nitrogen capture from, nutrient-rich patches in soil. *New Phytologist* 145: 575-584.

Hoff T., Truong H.-N. & Caboche M. (1994). The use of mutants and transgenic plants to study nitrate assimilation. *Plant, Cell and Environment* 17: 489-506.

Hogan K. P., Smith A. P. (1991). Potential effects of elevated CO_2 and changes in temperature of tropical plants. *Plant, Cell and Environment* 14: 763-778.

Howitt S. M. & Udvardi M. K. (2000). Structure, function and regulation of ammonium transporters in plants. *Biochimica et Biophysica Acta* 1465: 152-170.

Huber S. C., MacKintosh C. & Kaiser W. M. (2002). Metabolic enzymes as targets for 14-3-3

proteins. *Plant Molecular Biology* 50: 1053-1063.

Hungate B. A., Dukes J. S., Shaw M. R., Luo Y. & Field C. B. (2003). Nitrogen and climate change. *Science* 302: 1512-1513.

Hunt S. & Layzell D. B. (1993). Gas exchange of legume nodules and the regulation of nitrogenase activity. *Annual Review of Plant Physiology and Plant Molecular Biology* 44: 483-511.

Hutmacher (2002). Heat unit averages and time to mature bolls. *California Cotton Review* 63: 4-6.

Ingestad T. (1979). Nitrogen stress in birch seedlings. II. N, K, P, Ca, and Mg nutrition. *Physiologia Plantarum* 45: 149-157.

Ingestad T. (1982). Relative addition rate and external concentration; Driving variables used in plant nutrition research. *Plant Cell and Environment*: 443-453.

Ingestad T. & Agren G. I. (1992). Theories and methods on plant nutrition and growth. *Physiologia Plantarum* 84: 177-184.

Irving A. A. & Hankinson R. (1908). The presence of a nitrate reducing enzyme in green plants. *Bio-Chemical Journal*: 87-96.

Irving D. E. & Silsbury J. H. (1987). A comparison of the rate of maintenance respiration in some crop legumes and tobacco determined by three methods. *Annals of Botany* 59: 257-264.

Jablonski L. M., Wang X. & Curtis P. S. (2002). Plant reproduction under elevated CO_2 conditions: a meta-analysis of reports on 79 crop and wild species. *New Phytologist* 156: 9-26..

Jackson W. A., Kwik K. D., Volk R. J. & Butz R. G. (1976). Nitrate influx and efflux by intact wheat seedlings: effects of prior nitrate nutrition. *Planta* 132: 149-156.

Jain A., Yang X., Kheshgi H., McGuire A. D., Post W. & Kicklighter D. (in press). Nitrogen attenuation of terrestrial carbon cycle response to global environmental factors. *Global Biogeochemical Cycles*.

James E. K. (2000). Nitrogen fixation in endophytic and associative symbiosis. *Field Crops Research* 65: 197-209.

Jang J.-C. & Sheen J. (1994). Sugar sensing in higher plants. *The Plant Cell* 6: 1665-1679.

Jeuffroy M. H., Ney B. & Ourry A. (2002). Integrated physiological and agronomic modelling of N capture and use within the plant. *Journal of Experimental Botany* 53: 809-823.

Johnson I. R. & Thornley J. H. M. (1987). A model of shoot:root partitioning with optimal growth. *Annals of Botany* 60: 133-142.

Jones D. L., Owen A. G. & Farrar J. F. (2002). Simple method to enable the high resolution determination of total free amino acids in soil solutions and soil extracts. *Soil Biology and Biochemistry* 34: 1893-1902.

Jung M., Vetter M., Herold M., Churkina G., Reichstein M., Zaehle S., Ciais P., Viovy N., Bondeau A. Chen Y., Trusilova K., Feser F. & Heimann M. (2007). Uncertainties of modeling gross primary productivity over Europe: A systematic study on the effects of using different drivers and terrestrial biosphere models. *Global Biogeochemical Cycles* 21: GB 4021.

Justes E., Mary B., Meynard J.-M., Machet J.-M. & Thelier-Huche L. (1994). Determination of a critical nitrogen dilution curve for winter wheat crops. *Annals of Botany* 74: 397-407.

Kaiser W. M. & Huber S. C. (2001). Post-translational regulation of nitrate reductase: mechanisms, physiological relevance and environmental triggers. *Journal of Experimental Botany* 52(363): 1981-1989.

Kaiser W. M., Weiner H. & Huber S. C. (1999). Nitrate reductase in higher plants: A case study for

transduction of environmental stimuli into control of catalytic activity. *Physiologia Plantarum* 105: 385-390.

Kaiser W. M., Stoimenova M. & Man H. (2002). What limits nitrate reduction in leaves? In: Foyer C. H. & Noctor G. (eds.). *Photosynthetic nitrogen assimilation and associated carbon and respiratory metabolism*, pp. 63-70. Advances in Photosynthesis and Respiration, Vol. 12. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Kattge J. (2002). *Zur Bedeutung von Stickstoff für den CO_2-Düngungseffekt*. Dissertation, Justus-Liebig Universität Gießen, 285 pp.

Kattge J., Knorr W., Raddatz T. & Wirth C. (2009). Quantifying photosynthetic capacity and its relationship to leaf nitrogen for global-scale terrestrial biosphere models. *Global Change Biology* 15: 976-991.

Khamis S. & Lamaze T. (1990). Maximal biomass production can occur in corn (*Zea mays*) in the absence of NO_3^- accumulation in either leaves or roots. *Physiologia Plantarum* 78: 388-394.

Khamis S., Lamaze T., Lemoine Y. & Foyer C. (1990). Adaptation of the photosynthetic apparatus in maize leaves as a result of nitrogen limitation. *Plant Physiology* 94: 1436-1443.

Khamis S., Lamaze T. & Farineau J. (1992). Effect of nitrate limitation on the photosynthetically active pools of aspartate and malate in maize, a NADP malic enzyme C_4 plant. *Physiologia Plantarum* 85: 223-229.

King B. J., Siddiqi M. Y., Ruth T. J., Warner R. L. & Glass A. D. M. (1993). Feedback regulation of nitrate influx in barley roots by nitrate, nitrite, and ammonium. *Plant Physiology* 102: 1279-1286.

Koch K. E. (1996). Carbohydrate-modulated gene expression in plants. *Annual Review of Plant Physiology and Plant Molecular Biology* 47: 509-540.

Kolber Z., Zehr J. & Falkowski P. (1988). Effects of growth irradiance and nitrogen limitation on photosynthetic energy conversion in photosystem II. *Plant Physiology* 88: 923-929.

Krapp A., Hofmann B., Schäfer C. & Stitt M. (1993). Regulation of the expression of rbcS and other photosynthetic genes by carbohydrates: a mechanism for the ´sink regulation` of photosynthesis? *The Plant Journal* 3(6): 817-828.

Krapp A., Ferrario-Mery S. & Touraine B. (2002). Nitrogen and signaling. In: Foyer C. H. & Noctor G. (eds.). *Photosynthetic nitrogen assimilation and associated carbon and respiratory metabolism*, pp. 205-225. Advances in Photosynthesis ad Respiration, Vol. 12. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Kucharik C. J. & Brye K. R. (2003). Integrated biosphere simulator (IBIS) yield and nitrate loss predicitons for Wisconsin maize receiving varied amounts of nitrogen fertilizer. *Journal of Environmental Quality* 32: 247-268.

Kuiper D., Kuiper P. J. C., Lambers H., Schuit J., Staal M. (1988). Cytokinin concentration in relation to mineral nutrition and benzyladenine treatment in *Plantago major ssp. pleiosperma*. *Physiologia Plantarum* 75(4): 511-517.

Kumar P. A., Parry M. A. J., Mitchell R. A. C., Ahmad A. & Abrol Y. P. (2002). Photosynthesis and nitrogen-use efficiency. In: Foyer C. H. & Noctor G. (eds.): *Photosynthetic nitrogen assimilation and associated carbon and respiratory metabolism*, pp.23-34. Advances in Photosynthesis and Respiration, Volume 12. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Laine P., Ourry A. & Boucaud J. (1995). Shoot control of nitrate uptake rates by roots of *Brassica napus* L.: effects of localized nitrate supply. *Planta* 196: 77-83.

Lambers H. & Poorter H. (1992). Inherent variation in growth rate between higher plants: a search for physiological causes and ecological consequences. *Advances in Ecological Research* 34: 187-261.

Lambers H., Chapin F. S. III & Pons T. L. (2008): *Plant Physiological Ecology*, 2. edition. Springer, New York. 604 pp.

Lancien M., Gadal P. & Hodges M. (2000). Enzyme redundancy and the importance of 2-oxoglutarate in higher plant ammonium assimilation. *Plant Physiology* 123: 817-824.

Larcher W. (1976). *Ökologie der Pflanzen*. 2. edition. Ulmer, Stuttgart. 320 pp.

Lauerer M., Saftic D., Quick W. P., Labate C., Fichtner K., Schulze E.-D., Rodermel, S. R., Bogorad L. & Stitt M. (1993). Decreased ribulose-1,5-bisphosphate carboxylase-oxygenase in transgenic tobacco transformed with ´antisense´ *rbc*S. VI. Effect on photosynthesis in plants grown at different irradiance. *Planta* 190: 332-345.

Lawlor D. W. (2002). Carbon and nitrogen assimilation in relation to yield: mechanisms are the key to understanding production systems. *Journal of Experimental Botany* 53(370): 773-787.

Lawlor D. W., Boyle F. A., Kendall A. C. & Keys A. J. (1987a). Nitrate nutrition and temperature effects on wheat: enzyme composition, nitrate and total amino acid content of leaves. *Journal of Experimental Botany* 38(188): 378-392.

Lawlor D. W., Boyle F. A., Young A. T., Keys A. J. & Kendall A. C. (1987b). Nitrate nutrition and temperature effects on wheat: photosynthesis and photorespiration of leaves. *Journal of Experimental Botany* 38(188): 393-408.

Lawlor D. W., Boyle F. A., Young A. T., Kendall A. C. & Keys A. J. (1987c). Nitrate nutrition and temperature effects on wheat: soluble components of leaves and carbon fluxes to amino acids and sucrose. *Journal of Experimental Botany* 38(192): 1091-1103.

Lawlor D. W., Kontturi M. & Young A. T. (1989). Photosynthesis by flag leaves of wheat in relation to protein, ribulose bisphoshate carboxylase activity and nitrogen supply. *Journal of Experimental Botany* 40(210): 43-52.

Leakey A. D. B., Bernacchi C. J., Dohleman F. G., Ort D. R. & Long S. P. (2004). Will photosynthesis of maize (*Zea mays*) in the US corn belt increase in future (CO_2) rich atmospheres? An analysis of diurnal courses of CO_2 uptake under free-air concentration enrichment (FACE). *Global Change Biology* 10: 951-962.

Lee R. B. (1993). Control of net uptake of nutrients by regulation of influx in barley plants recovering from nutrient deficiency. *Annals of Botany* 72: 223-230.

Lee R. B. & Rudge K. A. (1986). Effects of nitrogen deficiency on the absorption of nitrate and ammonium by barley plants. *Annals of Botany* 57: 471-486.

Leggett J. E. & Frere M. J. (1971). Growth and nutrient uptake by soybean plants in nutrient solutions of graded concentrations. *Plant Physiology* 48: 457-460.

Lemaire G. (ed., 1997). *Diagnosis of the nitrogen status in crops*. Springer-Verlag Berlin, Heidelberg, New York. 239 pp.

Lemaire G. & Gastal F. (1997). N uptake and distribution in plant canopies. In: Lemaire G. (ed.). *Diagnosis of the nitrogen status in crops*, pp. 3-43. Springer, Berlin, Heidelberg, New York.

Lemaire G., Onillon B., Gosse G., Chartier M. & Allirand J. M. (1991). Nitrogen distribution within a lucerne canopy during regrowth: relation with light distribution. *Annals of Botany* 68: 483-488.

Lerbs S., Lerbs W., Klyachko N. L., Romanko E. G., Kulaeva O. N., Wollgiehn R. & Parthier B. (1984). Gene expression in cytokinin- and light-mediated plastogenesis of Cucurbita

cotyledons: ribulose-1,5-bisphosphate carboxylase/oxygenase. *Planta* 162: 289-298.

Lewis O. A. M., James D. M. & Hewitt E. J. (1982). Nitrogen assimilation in barley (*Hordeum vulgare* L. cv. Mazurka) in response to nitrate and ammonium nutrition. *Annals of Botany* 49: 39-49.

Li M. & Jones M. B. (1992). Effect of nitrogen status on the maintenance respiration of C_3 and C_4 *Cyperus* species. In: Lambers H. & van der Plas L.H.W. (eds.): *Molecular, biochemical and physiological aspects of plant respiration*, pp. 509-514. SPB Academic Publishing, The Hague.

Lima L., Seabra A., Melo P. & Cullimore J. (2006a). Phosphorylation and subsequent interaction with 14-3-3 proteins regulate plastid glutamine synthetase in *Medicago trunculata*. *Planta* 223: 558-567.

Lima L., Seabra A., Melo P., Cullimore J. & Carvalho H. (2006b). Post-translational regulation of cytosolic glutamine synthetase of *Medicago truncatula*. *Journal of Experimental Botany* 57(11): 2751-2761.

Lipson D. & Näsholm T. (2001). The unexpected versatility of plants: organic nitrogen use and availability in terrestrial ecosystems. *Oecologia* 128: 305-316.

Lockyer D. R. & Whitehead D. C. (1986). The uptake of gaseous ammonia by the leaves of italian ryegrass. *Journal of Experimental Botany* 37(180): 919-927.

Logan B. A., Demmig-Adams B., Rosenstiel T. N. & Adams W. W. (1999). Effect of nitrogen limitation on foliar antioxidants in relationship to other metabolic characteristics. *Planta* 209: 213-220.

Long S. R. (1996). *Rhizobium* symbiosis: Nod factors in perspective. *The Plant Cell* 8: 1885:1898.

Longnecker N. & Robson A. (1994). Leaf emergence of spring wheat receiving varying nitrogen supply at different stages of development. *Annals of Botany* 74: 1-7.

Longstreth D. J. & Nobel P. S. (1980). Nutrient influences on leaf photosynthesis. Effects of nitrogen, phosphorus, and potassium for *Gossypium hirsutum* L. *Plant Physiology* 65: 541-543.

Macduff J. H., Hopper M. J. & Wild A. (1987). The effect of root temperature on growth and uptake of ammonium and nitrate by *Brassica napus* L. cv. Bien venu in flowing solution culture, II. Uptake from solutions containing NH_4NO_3. *Journal of Experimental Botany* 38(186): 53-66.

Macduff J. H., Jarvis S. C., Larsson C.-M. & Oscarson P. (1993). Plant growth in relation to the supply and uptake of NO_3^-: A comparison between relative addition rate and external concentration as driving variables. *Journal of Experimental Botany* 44(266): 1475-1484.

Machado A. T., Sodek L. & Fernandes M. S. (2001). N-partitioning, nitrate reductase and glutamine synthetase activities in two contrasting varieties of maize. *Pesquisa Agropecuária Brasileira* 36(2): 249-256.

Makino A. & Osmond B. (1991). Effects of nitrogen nutrition on nitrogen partitioning between chloroplasts and mitochondria in pea and wheat. *Plant Physiology* 96: 355-362.

Makino A., Mae T. & Ohira K. (1984a). Relation between nitrogen and ribulose-1,5-bisphosphate carboxylase in rice leaves from emergence through senescence. *Plant & Cell Physiology* 25(3): 429-437.

Makino A., Mae T. & Ohira K. (1984b). Effect of nitrogen, phosphorus or potassium on the photosynthetic rate and ribulose-1,5-bisphosphate carboxylase content in rice leaves during expansion. *Soil Science and Plant Nutrition* 30(1): 63-70.

Makino A., Mae T. & Ohira K. (1988). Differences between wheat and rice in the enzymic

properties of ribulose-1,5-bisphosphate carboxylase/oxygenase and the relationship to photosynthetic gas exchange. *Planta* 174: 30-38.

Makino A., Sakashita H., Hidema J., Mae T., Ojima K. & Osmond B. (1992). Distinctive responses of ribulose-1,5-bisphosphate carboxylase and carbonic anhydrase in wheat leaves to nitrogen nutrition and their possible relationships to CO_2-transfer resistance. *Plant Physiology* 100: 1737-1743.

Makino A., Nakano H. & Mae T. (1994). Response of ribulose-1,5-bisphosphate carboxylase, cytochrome f, and sucrose synthesis enzyme in rice leaves to leaf nitrogen and their relationship to photosynthesis. *Plant Physiology* 105: 173-179.

Makino A., Sato T., Nakano H. & Mae T. (1997). Leaf photosynthesis, plant growth and nitrogen allocation in rice under different irradiances. *Planta* 203: 390-398.

Malagoli P., Meuriot F., Laine P., Le Deunff E. & Ourry A. (2008). Modeling nitrogen uptake and nitrogen dynamics in winter oilseed rape (*Brassica napus* L.). In: Ma L., Ahuja L. R. & Bruulsema T. W. (eds.). *Quantifying and understanding plant nitrogen uptake for systems modeling*, pp. 47-69. CRC press, Boca Raton, USA.

Manh C. T., Bismuth E., Boutin J.-P., Provot M. & Champigny M.-L. (1994). Metabolite effectors for short-term nitrogen-dependent enhancement of phophoenolpyruvate carboxylase activity and decrease of net sucrose synthesis in wheat leaves. *Physiologia Plantarum* 89: 460-466.

Man H.-M. & Kaiser W. M. (2001). Increased glutamine synthetase activity and changes in amino acid pools in leaves treated with 5-aminoimidazole-4-carboxiamide ribonucleoside (AICAR). *Physiologia Plantarum* 111: 291-296.

Marschner H. (1995). *Mineral nutrition of higher plants*, 2. edition. Academic Press, London. 889 pp.

Masuda T., Fujita K. & Kogure K. (1989a). Effect of CO_2 enrichment and nitrate application on vegetative growth and dinitrogen fixation of wild and cultivated soybean varieties. *Soil Science and Plant Nutrition* 35(3): 357-366.

Masuda T., Fujita K. & Ogata S. (1989b). Effect of CO_2 enrichment and nitrate application on vegetative growth and dinitrogen fixation of wild and cultivated soybean plants during pod-filling stage. *Soil Science and Plant Nutrition* 35(3): 405-416.

Mattson W. J. (1980). Herbivory in relation to plant nitrogen content. *Annual Review of Ecology and Systematics* 11: 119-161.

Mattsson M., Johansson E., Lundborg T., Larsson M. & Larsson C.-M. (1991). Nitrogen utilization in N-limited barley during vegetative and generative growth. I. Growth and nitrate uptake kinetics in vegetative cultures grown at different relative addition rates of nitrate-N. *Journal of Experimental Botany* 42(235): 197-205.

Mattsson M., Lundborg T., Larsson M. & Larsson C.-M. (1992a). Nitrogen utilization in N-limited barley during vegetative and generative growth. II. Method for monitoring generative growth and development in solution culture. *Journal of Experimental Botany* 43(246): 15-23.

Mattsson M., Lundborg T., Larsson M. & Larsson C.-M. (1992a). Nitrogen utilization in N-limited barley during vegetative and generative growth. III. Post-anthesis kinetics of net nitrate uptake and the role of the relative root size in determining the capacity for nitrate acquisition. *Journal of Experimental Botany* 43(246): 25-30.

McDonald A. J. S. (1989). Nitrate availability and shoot area development in small willow (*Salix viminalis*). *Plant, Cell and Environment* 12: 417-424.

McDonald A. J. S., Lohammar T. & Ericsson A. (1986). Growth response to step-decrease in

nutrient availability in small birch (*Betula pendula* Roth). *Plant, Cell and Environment* 9: 427-432.

McGuire A. D., Melillo J. M., Joyce L. A., Kicklighter D. W., Grace A. L., Morre B. & Vorosmarty C. J. (1992). Interactions between carbon and nitrogen dynamics in estimating net primary productivity for potential vegetation in North America. *Global Biogeochemical Cycles* 6(2): 101-124.

McNeal F. H., Berg M. A. & Watson C. A. (1966). Nitrogen and dry matter in five spring wheat varieties at successive stages of development. *Agronomy Journal* 58: 605-608.

Medlyn B. E., Badeck F.-W., De Pury D. G. G.., Barton C. V. M., Broadmeadow M., Ceulemans R., De Angelis P., Forstreuter M., Jach M. E., Kellmokaki S., Laitat E., Marek M., Philippot S., Rey S., Strassemeyer J., Laitinen K., Liozon R., Portier B., Roberntz Pl, Wang K. & Jarvis P. G. (1999). Effects of elevated (CO_2) on photosynthesis in European forest species: a meta-analysis of model parameters. *Plant, Cell and Environment* 22: 1475-1495.

Mellilo J. M., McGuire A. D., Kicklighter D. W., Moore B., Vorosmyrty C. & Schloss A. L. (1993). Global climate change and terrestrial net primary production. *Nature* 363: 234-240.

Mellilo J. M., Steudler P. A., Aber J. D., Newkirk K., Lux H., Bowles F. P., Catricala C., Magill A., Ahrens, T. & Morisseau S. (2002). Soil warming and carbon-cycle feedbacks to the climate system. *Science* 298: 2173-2176.

Miflin B. J. & Lea P. J. (1977). Amino acid metabolism. *Annual Reviews of Plant Physiology* 28: 299-329.

Miflin B. J. & Habash D. Z. (2002). The role of glutamine synthetase and glutamate dehydrogenase in nitrogen assimilation and possibilities for improvement in the nitrogen utilization of crops. *Journal of Experimental Botany* 53(370): 979-987.

Millard P. (1988). The accumulation and storage of nitrogen by herbaceous plants. *Plant, Cell and Environment* 11: 1-8.

Millard P. & Catt J. W. (1988). The influence of nitrogen supply on the use of nitrate and ribulose 1,5-*bis*phosphate carboxylase/oxygenase as leaf nitrogen stores for growth of potato tubers (*Solanum tuberosum* L.). *Journal of Experimental Botany* 39(198): 1-11.

Miller A. J. & Smith S. J. (1996). Nitrate transport and compartimentation in cereal root cells. *Journal of Experimental Botany* 47(300): 843-854.

Miller A. J. & Cramer M. D. (2004). Root nitrogen acquisition and assimilation. *Plant and Soil* 274: 1-36.

Miller T. & Street J. E. (2009). *Growth stages of the rice plant*. Mississippi State University Extension Service, Publication 1624.

Mitchell R. A. C., Mitchell V. J., Driscoll S. P., Franklin J. & Lawlor D. W. (1993). Effects of increased CO_2 concentration and temperature on growth and yield of winter wheat at two levels of nitrogen application. *Plant, Cell and Environment* 16: 521-529.

Monreal C. M. & McGill W. B. (1985). Centrifugal extraction and determination of free amino acids in soil solutions by TLC using tritiated 1-Fluoro-2,4-Dinitrobenzene. *Soil Biology and Biochemistry* 17(4): 533-539.

Mooney H. A., Field C., Gulmon S. L. & Bazzaz F. A. (1981). Photosynthetic capacity in relation to leaf position in desert versus old-field annuals. *Oecologia* 50: 109-112.

Morgan J. A. (1984). Interaction of water supply and N in wheat. *Plant Physiology* 76: 112-117.

Muchow R. C. & Sinclair T. R. (1994). Nitrogen response of leaf photosynthesis and canopy radiation use efficiency in field-grown maize and sorghum. *Crop Science* 34: 721-727.

Muller B. & Touraine B. (1992). Inhibition of NO_3^- uptake by various phloem-translocated amino acids in soybean. *Journal of Experimental Botany* 43(250): 617-623.

Müntz K. (1994). Storage and reactivation of high molecular weight nitrogen compounds in plants. In: Mohr H. & Müntz K. (organizers): *The terrestrial nitrogen cycle as influenced by man*, pp. 183-200. Leopoldina Symposium, Halle (Saale), September 29, 1993 to October, 1, 1993. Deutsche Akademie der Naturforscher Leopoldina, Halle.

Murchie E. H., Ferrario-Méry S., Valadier M.-H. & Foyer C. H. (2000). Short-term nitrogen-induced modulation of phospho*enol*pyruvate carboxylase in tobacco and maize leaves. *Journal of Experimental Botany* 51(349): 1349-1356.

Nakamura T., Koike T., Lei T., Ohashi K., Shinano T. & Tadano T. (1999). The effect of CO_2 enrichment on the growth of nodulated and non-nodulated isogenic types of soybean raised under two nitrogen concentrations. *Photosynthetica* 37(1): 61-70.

Nakano H., Makino A. & Mae T. (1997). The effect of elevated partial pressures of CO_2 on the relationship between photosynthetic capacity and N content in rice leaves. *Plant Physiology* 115: 191-198.

Näsholm T., Ekblad A., Nordin A., Giesler R., Högberg M. & Högberg P. (1998). Boreal forest plants take up organic nitrogen. *Nature* 139: 914-916.

Natr L. (1975). Influence of mineral nutrition on photosynthesis and the use of assimilates. In: J. P. Cooper (ed.): *Photosynthesis and productivity in different environments*, pp. 537-556. Cambridge University Press.

Neff J. C., Townsend A. R., Gleixner G., Lehman S. J., Turnbull J. & Bowman W. D. (2002). Variable effects of nitrogen additions on the stability and turnover of soil carbon. *Nature* 419: 915-917.

Neff J. C., Chapin F. S. III & Vitousek P. M. (2003). Breaks in the cycle: dissolved organic nitrogen in terrestrial ecosystems. *Frontiers in Ecology and the Environment* 1(4): 205-211.

Newsham K. K. & Robinson S. A. (2009). Responses of plants in polar regions to UVB exposure: a meta-analysis. *Global Change Biology* 15: 2574-2589.

Nightingale G. T. (1937). The nitrogen nutrition of green plants. *The Botanical Review* 3: 85-174.

Niinemets Ü. & Tenhunen J. D. (1997). A model separating leaf structural and physiological effects on carbon gain along light gradients for the shade-tolerant species *Acer saccharum*. *Plant, Cell & Environment* 20: 845-866.

Niinemets Ü., Kull O. & Tenhunen J. D. (1998). An analysis of light effects on foliar morphology, physiology, and light interception in temperate deciduous woody species of contrasting shade tolerance. *Tree Physiology* 18: 681-696.

Novoa R. & Loomis R.S. (1981). Nitrogen and plant production. *Plant and Soil* 58: 177-204.

Öhlen E. & Larsson C.-M. (1992). Nitrate assimilatory properties of barley grown under long-term N limitation: effects of local nitrate supply in split-root cultures. *Physiologia Plantarum* 85: 9-16.

Oliveira I. C., Brenner E., Chiu J., Hsieh M.-H., Kouranov A., Lam H.-M., Shin M.-J. & Coruzzi G. (2001). Metabolite and light regulation of metabolism in plants: lessons from the study of a single biochemical pathway. *Brazilian Journal of Medical and Biological Research* 34: 567-575.

Ortiz-Lopez A., Chang H.-C. & Bush D. R. (2000). Amino acid transporters in plants. *Biochimica et Biophysica Acta* 1465: 275-280.

Osenberg C. W., Sarnelle O., Cooper S. D. & Holt R. D. (1999). Resolving ecological questions

through meta-analysis: Goals, metrics, and models. *Ecology* 80(4): 1105-1117.

Ostle N. J., Smith P., Fisher R., Woodward F. I., Fisher J. B., Smith J. U., Galbraith D., Levy P., Meir P., McNamara N. P. & Bardgett R. (2009). Integrating plant-soil interactions into global carbon cycle models. *Journal of Ecology* 97: 851-863.

Owen A. G. & Jones D. L. (2001). Competition for amino acids between wheat roots and rhizosphere microorganisms and the role of amino acids in plant N acquisition. *Soil Biology and Biochemistry* 33: 651-657.

Pain B. F., Phillips V. R., Clarkson C. R. & Klarenbeck J. V. (1989). Loss of nitrogen through ammonia volatilisation during and following the application of pig or cattle slurry to grassland. *Journal of the Science of Food and Agriculture* 47: 1-12.

Parsons R., Stanforth A., Raven J. A. & Sprent J. I. (1993). Nodule growth and activity may be regulated by a feedback mechanism involving phloem nitrogen. *Plant, Cell and Environment* 16: 125-136.

Paul M. J. & Stitt M. (1993). Effects of nitrogen and phosphorus deficiencies on levels of carbohydrates, respiratory enzymes and metabolites in seedlings of tobacco and their response to exogenous sucrose. *Plant, Cell and Environment* 16: 1047-1057.

Paul M. J. & Driscoll S. P. (1997). Sugar repression of photosynthesis: the role of carbohydrates in signalling nitrogen deficiency through source: sink imbalance. *Plant, Cell and Environment* 20: 110-116.

Paul M. J, & Foyer C. H. (2001). Sink regulation of photosynthesis. *Journal of Experimental Botany* 52(360): 1282-1400.

Pearman I., Thomas S. M. & Thorn G. N. (1977). Effects of nitrogen fertilizer on growth and yield of spring wheat. *Annals of Botany* 41: 93-108.

Pearman I., Thomas S. M. & Thorne G. N. (1978). Effects of nitrogen fertilizer on the distribution of photosynthate during grain growth of spring wheat. *Annals of Botany* 42: 91-98.

Pearman I., Thomas S. M., Thorne G. N. (1979). Effect of nitrogen fertilizer on photosynthesis of several varieties of winter wheat. *Annuals of Botany* 43: 613-621.

Pego J. V., Kortstee A. J., Huijser C. & Smeekens S. C. M. (2000). Photosynthesis, sugars and the regulation of gene expression. *Journal of Experimental Botany* 51: 407-416.

Peng S., Cassman K. G. & Kropff M. J. (1995). Relationship between leaf photosynthesis and nitrogen content of field-grown rice in tropics. *Crop Science* 35: 1627-1630.

Peoples M. B. & Craswell E. T. (1992). Biological nitrogen fixation: investments, expectations and actual contributions to agriculture. *Plant and Soil* 141: 13-39.

Peterson A. G., Ball T. J., Luo Y., Field C. B., Reich P. B., Curtis P. S., Griffin K. L., Gundreson C. A., Norby R. J., Tissue D. T., Forstreuter M., Rey A., Vogel C. S. & CMEAL participants (1999). The photosynthesis – leaf nitrogen relationship at ambient and elevated atmospheric carbon dioxide: a meta-analysis. *Global Change Biology* 5: 331-346.

Plhak F. (2003). Nitrogen supply through transpiration mass flow can limit nitrogen nutrition of plants. *Plant, Soil and Environment* 49(10): 473-479.

Plumley F. G. & Schmidt G. W. (1989). Nitrogen-dependent regulation of photosynthetic gene expression. *Proceedings of the National Academy of Sciences* USA 86: 2678-2682.

Plumley F. G., Douglas S. E., Branagan-Switzer A. & Schmidt G. W. (1989). Nitrogen-dependent biogenesis of chlorophyll-protein complexes. In: Briggs W. R. (ed.). *Photosynthesis*, pp. 311-329. Plant Biology series, Vol. 8. Alan R Liss, Inc, New York.

Pons T. L. & Pearcy R. W. (1994). Nitrogen reallocation and photosynthetic acclimation in response to partial shading in soybean plants. *Physiologia Plantarium* 92: 636-644.

Pons T. L., van Rijnberk H., Scheurwater I. & van der Werf A. (1993). Importance of the gradient in photosynthetically active radiation in a vegetation stand for leaf nitrogen allocation. *Oecologia* 95: 416-424.

Pons TL, van der Werf A, Lambers H (1994). Photosynthetic nitrogen use efficiency of inherently slow- and fast-growing species: possible explanations for observed differences. In: Roy J & Garnier E (eds). *A whole plant perspective on carbon-nitrogen interactions*, pp. 61-77. SPB Academic Publishing. The Hague.

Powlson D. S. (1993). Understanding the soil nitrogen cycle. *Soil Use and Management* 9(3): 86-94.

Pugnaire F. I. & Chapin F. S. (1992). Environmental and phyisological facors governing nutrient resorption efficiency in barley. *Oecologia* 90: 120-126.

Quebedeaux B. & Hardy R. W. F. (1975). Reproductive growth and dry matter production of *Glycine max* (L.) Merr. In response to oxyen concentration. *Plant Physiology* 55: 102-107.

Quick W. P., Schurr U., Fichtner K., Schulze E. D., Rodermel S. R., Bogorad L. & Stitt M. (1991). The impact of decreased Rubisco on photosynthesis, growth, allocation and storage in tobacco plants which have been transformed with antisense *rbcS*. *The Plant Journal*: 51-58.

Radin J. W. (1983). Control of plant growth by nitrogen: differences between cereals and broadleaf species. *Plant, Cell and Environment* 6: 65-68.

Radin J. W. (1990). Responses of transpiration and hydraulic conductance to root temperature in nitrogen- and phosphorus-deficient cotton seedlings. *Plant Physiology* 92: 855-857.

Radin J. W. & Ackerson R. C. (1981). Water relations of cotton plants under nitrogen deficiency. III. Stomatal conductance, photosynthesis, and absisic acid accumulation during drought. *Plant Physiology* 67: 115-119.

Radin J. W. & Boyer J. S. (1982). Control of leaf expansion by nitrogen nutrition in sunflower plants. Role of hydraulic conductivity and turgor. *Plant Physiology* 69: 771-775.

Radin J. W. & Elmor C. D. (1980). Concepts of translocation with special reference to the assimilation of nitrogen and its movement into fruits. In: Hesketh J. D. & Jones J. W. (eds.). *Predicting photosynthesis for ecosystem models. Volume II*, pp. 143-154. CRC Press, Boca Raton, Florida.

Radin J. W., Parker L. L. & Guinn G. (1982). Water relations of cotton plants under nitrogen deficiency. V: Environmental control of absisic acid accumulation and stomatal sensitivity to absisic acid. *Plant Physiology* 70: 1066-1070.

Radoglou K. M. & Jarvis P. G. (1992). The effects of CO_2 enrichment and nutrient supply on growth morphology and anatomy of *Phaseolus vulgaris* L. seedlings. *Annals of Botany* 70: 245-256.

Radoglou K. M., Aphalo P. & Jarvis P. G. (1992). Response of photosynthesis, stomatal conductance and water use efficiency to elevated CO_2 and nutrient supply in acclimated seedlings of *Phaseolus vulgaris* L. *Annals of Botany* 70: 257-264.

Raich J. W., Rastetter E. B., Melillo M, Kicklighter D. W., Grace A. L., Moore III G. B. & Vörösmarty C. J. (1991). Potential net primary productivity in South America: application of a global model. *Ecological Applications* 1(4): 399-429.

Raven J. A. (1988). Acquisition of nitrogen by the shoots of land plants: its occurrence and implications for acid-base regulation. *New Phytologist* 109: 1-20.

Rawat S. R., Silim S. N., Kronzucker H. J., Siddiqi M. Y. & Glass A. D. M. (1999). *AtAMT1* gene expression and NH_4^+ uptake in roots of *Arabidopsis thaliana*: evidence for regulation by root glutamine levels. *The Plant Journal* 19(2): 143-152.

Reich P. B., Walters M. B. & Ellsworth D. S. (1991). Leaf age and season influence the relationships between leaf nitrogen, leaf mass per area and photosynthesis in maple and oak trees. *Plan, Cell and Environment* 14: 251-259.

Reich P. B., Walters M. B. & Ellsworth D. S. (1992). Leaf life-span in relation to leaf, plant, and stand characteristics among diverse ecosystems. *Ecological Monographs* 62(3): 365-392.

Reich P. B., Walters M. B. & Ellsworth D. S. (1997). From tropics to tundra: Global convergence in plant functioning. *Proceedings of the National Academy of Sciences* USA 94: 13730-13734.

Reich P. B., Walters M. B., Ellsworth D. S., Vose J. M., Volin J. C., Gresham C. & Bowman W. D. (1998). Relationships of leaf dark respiration to leaf nitrogen, specific leaf area and leaf life-span: a test across biomes and functional groups. *Oecologia* 114: 471-482.

Reich P. B., Ellsworth D. S., Walters M. B., Vose J. M., Gresham C., Volin J. C. & Bowman W., D. (1999). Generality of leaf trait relationships: a test across six biomes. *Ecology* 80(6): 1955-1969.

Reich P. B., Hobbie S. E., Lee T., Ellsworth D. S., West J. B., Tilman D., Knops J. M. H., Naeem S. & Trost J. (2006a). Nitrogen limitation constrains sustainability of ecosystem responses to CO2. *Nature*: 1-4.

Reich P. B., Hungate B. A. & Luo Y. (2006b). Carbon-nitrogen interactions in terrestrial ecosystems in response to rising atmospheric carbon dioxide. *Annual Review of Ecology, Evolution and Systematics* 37: 611-636.

Reynolds J. F. & Chen J. (1996). Modeling whole-plant allocation in relation to carbon and nitrogen supply: coordination versus optimization: opinion. *Plant and Soil* 185: 65-74.

Ritchie S. W., Hanway J. J. & Benson G. O. (1993): *How a corn plant develops*. Special report number 48, Iowa State University of Science and Technology. 21 pp.

Ritchie G. L., Bednarz C. W., Jost P. H. & Brown S. M. (2007). *Cotton growth and development*. University of Georgia Cooperative Extension Bulletin 1252. 16 pp.

Robinson D. (1994). The responses of plants to non-uniform supplies of nutrients. *New Phytologist* 127: 635-674.

Robinson J. M. (1996). Leaflet photosynthesis rate and carbon metabolite accumulation patterns in notrogen-limited, vegetative soybean plants. *Photosynthesis Research* 50: 133-148.

Robinson D., Linehan D. J. & Caul S. (1991). What limits nitrate uptake from soil? *Plant, Cell and Environment* 14: 77-85.

Robinson D., Linehan D. J., & Gordon D. C. (1994). Capture of nitrate from soil by wheat in relation to root length, nitrogen inflow and availability. *New Phytologist* 128: 297-305.

Roggatz U., McDonald A. J. S., Stadenberg I. & Schurr U. (1999). Effects of nitrogen deprivation on cell division and expansion in leaves of *Ricinus communis* L. *Plant, Cell and Environment* 22: 81-89.

Rosenberg M. S., Adams D. C. & Gurevitch J. (2000). *MetaWin: Statistical Software for Meta-Analysis*. Version 2. Sinauer Associates, Sunderland, Massachusetts.

Rosenzweig C. & Parry M. L. (1994). Potential impact of climate change on world food supply. *Nature* 367: 133-138.

Rowland A. J., Drew M. C. & Wellburn A. R. (1987). Foliar entry and incorporation of atmospheric

nitrogen dioxide into barley plants of different nitrogen status. *New Phytologist* 107: 357-371.

Rufty T. W., Huber S. C. & Volk R. J. (1988). Alterations in leaf carbohydrate metabolism in response to nitrogen stress. *Plant Physiology* 88: 725-730.

Runge M. (1983). Physiology and ecology of nitrogen nutrition. In: Lange O. L., Nobel P. S., Osmond C. B. & Ziegler H. (eds.). *Physiological Plant Ecology III. Responses to the chemical and biological environment*, pp. 163-200. Encyclopedia pf Plant Physiology, New Series, Vol. 12C. Springer, Berlin

Running S. W. & Coughlan J. C. (1988). A general model of forest ecosystem processes for regional applications. I Hydrological balance, canopy gas exchange and primary production processes. *Ecological Modelling* 42: 125-154.

Running S. W. & Gower S. T. (1991). FOREST-BGC, a general model of forest ecosystem processes for regional applications. II Dynamic carbon allocation and nitrogen budgets. *Tree Physiology* 9: 147-160.

Running S. W. & Hunt E. R. (1993). Generalization of a forest ecosystem process model for other biomes, BIOME-BGC, and an application for global scale models. In: Ehleringer J. R. & Field C. B. (eds.): *Scaling Physiological Processes: leaf to globe*, pp 141-158. Academic Press, San Diego.

Rustad L. (2001). Matter of time on the prairie. *Nature* 413: 578-579.

Rustad L. E., Campbell J. L, Marion G. M., Norby R. J., Mitchell M. J., Hartley A. E., Cornelissen J. H. C. & Gurevitch J. (2001). A meta-analysis of the response of soil respiration, net nitrogen mineralization, and aboveground plant growth to experimental ecosystem warming. *Oecologia* 126: 543-562.

Ryan M. G. (1991). Effects of climate change on plant respiration. *Ecological Applications* 1(2): 157-167.

Ryan M. G., Hubbard R. M., Pongracic S., Raison R. J. & McMurtrie R. E. (1996). Foliage, fine-root, woody-tissue and stand respiration in *Pinus radiata* in relation to nitrogen status. *Tree Physiology* 16: 333-343.

Sage R. (1994). Acclimation of photosynthesis to increasing atmospheric CO_2: The gas exchange perspective. *Photosynthesis Research* 39: 351-368.

Sage R. F. & Pearcy R. W. (1987a). The nitrogen use efficiency of C_3 and C_4 plants. II. Leaf nitrogen effects on the gas exchange characteristics of *Chenopodium album* (L.) and *Amaranthus retroflexus* (L.). *Plant Physiology* 84: 959-963.

Sage R. F. & Pearcy R. W (1987b). The nitrogen use efficiency of C_3 and C_4 plants. I. Leaf nitrogen, growth, and biomass partitioning in *Chenopodium album* (L.) and *Amaranthus retroflexus* (L.). *Plant Physiology* 84: 954-958.

Sakakibara H., Suzuku M., Takei K., Deji A., Taniguchi M. & Sugiyama T. (1998). A response-regulator homologue possibly involved in notrogen signal transduction mediated by cytokinin in maize. *The Plant Journal* 14(3): 337-344.

Samuelson M. E., Eliasson L. & Larsson C.-M. (1992). Nitrate-regulated growth and cytokinin responses in seminal roots of barley. *Plant Physiology* 98: 309-315.

Sarandon S. J. & Gianibelli M. C. (1990). Effect of foliar spraying and nitrogen application at sowing upon dry matter and nitrogen distribution in wheat (*Triticum aestivum* L.). *Agronomie* 10: 183-189.

Scheible W.-R., Lauerer M., Schulze E.-D., Caboche M. & Stitt M. (1997a). Accumulation of nitrate in the shoot acts as a signal to regulate shoot-root allocation in tobacco. *The Plant*

Journal 11(4): 671-691.

Scheible W.-R., González-Fontes A., Lauerer M., Müller-Röber B., Caboche M. & Stitt M. (1997b). Nitrate acts as a signal to induce organic acid metabolism and repress starch metabolism in tobacco. *The Plant Cell* 9: 783-798.

Schieving F., Werger M. J. A. & Hirose T. (1992). Canopy structure, nitrogen distribution and whole canopy photosynthetic carbon gain in growing and flowering stands of tall herbs. *Vegetatio* 102: 173-181.

Schimel D. S. (1986). Carbon and nitrogen turnover in adjacent grassland and cropland ecosystems. *Biogeochemistry* 2: 345-357.

Schuller K. A., Plaxton W. C. & Turpin D. H. (1990). Regulation of phosphoe*nol*pyruvate carboxylase from the green alga *Selenastrum minutum*. Properties associated with replenishment of tricarobxylic acid cycle intermediates during ammonium assimilation. *Plant Physiology* 93: 1303-1311.

Schultze M. & Kondorosi A. (1998). Regulation of symbiotic root nodule development. *Annual Review of Genetics* 32: 33-57.

Schulze E. D., Kelliher F. M., Körner C., Lloyd J. & Leuning R. (1994). Relationships among maximum stomatal conductance, ecosystem surface conductance, carbon assimilation rate, and plant nitrogen nutrition: A global ecology scaling exercise. *Annual Review of Ecology, Evolution, and Systematics* 25: 629-660.

Searles P. S., Flint S. D. & Caldwell M. M. (2001). A meta-analysis of plant field studies simulating stratospheric ozone depletion. *Oecologia* 127: 1-10.

Seemann J. R., Sharkey T. D., Wang J. & Osmond C. B. (1987). Environmental effects on photosynthesis, nitrogen-use efficiency, and metabolite pools in leaves of sun and shade plants. *Plant Physiology* 84: 796-802.

Seligman N. G., van Keulen H. & Goudriaan J. (1975). An elementary model of nitrogen uptake and redistribution by annual plant species. *Oecologia* 21: 243-261.

Shangguan Z. P., Shao M. A. & Dyckmans J. (2000). Nitrogen nutrition and water stress effects on leaf photosynthetic gas exchange and water use efficiency in winter wheat. *Environmental and Experimental Botany* 44: 141-149.

Shangguan Z. P., Shao M. A., Ren S. J., Zhang L. M. & Xue Q. (2004). Effect of nitrogen on root and shoot relations and gas exchange in winter wheat. *Botanical Bulletin of Academia Sinica* 45: 49-54.

Sharkey T. (1985). Photosynthesis in intact leaves of C_3 plants: Physics, physiology and rate limitations. *The Botanical Review* 51(1): 53-105.

Sheen J. (1990). Metabolic repression of transcription in higher plants. *The Plant Cell* 2: 1027-1038.

Shimshi D. (1970a). The effect of nitrogen supply on transpiration and stomatal behaviour of beans (*Phaseolus vulgaris* L.). *New Phytologist* 69: 405-412.

Shimshi D. (1970b). The effect of nitrogen supply on some indices of plant-water relations of beans (*Phaseolus vulgaris* L.). *New Phytologist* 69: 413-424.

Siddiqi M. Y., Glass A. D. M., Ruth T. J. & Fernando M. (1989). Studies of the regulation of nitrate influx by barley seedlings using $^{13}NO_3^{-1}$. *Plant Physiology* 90: 806-813.

Siddiqi M. Y., Glass A. M., Ruth T. J. & Rufty T. W. (1990). Studies of the uptake of nitrate in barley. I. Kinetics of $^{13}NO_3^-$ influx. *Plant Physiology* 93: 1426-1432.

Signora L., Smet I. C., Foyer C. H. & Zhang H. (2001). ABA plays a central role in mediating the regulatory effects of nitrate on root branching in *Arabidopsis*. *The Plant Journal* 28 (6): 655-662.

Sinclair R. R. & Horie T. (1989). Crop physiology & metabolism. Leaf nitrogen, photosynthesis, and crop radiation use efficiency: A review. *Crop Sciences* 29: 90-98.

Sitch S., Smith B., Prentice C., Arneth A., Bondeau A., Cramer W., Kaplan J. O., Levis S., Lucht W., Sykes M. T., Thonicke K. & Venevsky S. (2003). Evaluation of ecosystem dynamics, plant geography and terrestrial carbon cycling in the LPJ dynamic global vegetation model. *Global Change Biology* 9: 161-185.

Sitch S., Brovkin V., Bloh von W., Vuuren van D., Eickhout B. & Ganopolski A. (2005). Impact of future land cover changes on atmospheric Co_2 and climate. *Global Biogeochemical cycles* 19: 1-15.

Skiba U., Drewer J., Tang Y. S., Dijk van N., Helfter C., Nemith E., Famulari D., Cape J. N., Jones S. K., Twigg M., Pihlatie M., Vesala T., Larsen K. S., Carter M. S., Ambus P., Ibrom A., Beier C., Hensen A., Frumau A., Erisman J. W., Brüggemann N., Gasche R., Butterbach-Bahl K., Neftel A., Seufert G., Andersson M., Manca G., Laurila T., Aurela M., Lohila A., Zechmeister-Boltenstern S., Kitzler B., Schaufler G., Siemens J. Kindler R., Flechard C. & Sutton M. A. (2009). Biosphere-atmosphere exchange of reactive nitrogen and greenhouse gases at the NitroEurope core flux measurement sites: Measurement strategy and first data sets. *Agriculture, Ecosystems and Environment* 133: 139-149.

Sokolov A. P., Kicklighter D. W., Melillo J. M., Felzer B. S., Schlosser C. A. & Cronin T. W. (2008). Consequences of considering carbon-nitrogen interactions on the feedbacks between climate and the terrestrial carbon cycle. *Journal of Climate* 21: 3776-3796.

Somerville C. R. & Ogren W. L. (1980). Inhibition of photosynthesis in *Arabidopsis* mutants lacking leaf glutamate synthase activity. *Nature* 286: 257-259.

Son D., Kobe A. & Sugiyama T. (1992). Nitrogen-dependent regulation of the gene for alanine aminotransferase which is involved in the C_4 pathway of *Panicum miliaceum*. *Plant and Cell Physiology* 33(4): 507-509.

Sowokinos J. R. (1981). Pyrophosphorylases in *Solanum tuberosum*. *Plant Physiology* 68: 924-929.

Staswick P. E. (1994). Storage proteins of vegetative plant tissues. *Annual Reviews of Plant Physiology and Plant Molecular Biology* 45: 303-322.

Stitt M. (1991). Rising CO_2 levels and their potential significance for carbon flow in photosynthetic cells. *Plant, Cell and Environment* 14: 741-762.

Stitt M. (1999). Nitrate regulation of metabolism and growth. *Current Opinion in Plant Biology* 2: 178-186.

Stitt M. & Schulze D. (1994). Does Rubisco control the rate of photosynthesis and plant growth? An exercise in molecular ecophysiology. *Plant, Cell and Environment* 17: 465-487.

Stitt M. & Krapp A. (1999). The interaction between elevated carbon dioxide and nitrogen nutrition: the physiological and molecular background. *Plant, Cell and Environment* 22: 583-621.

Stitt M., Quick W. P., Schurr U., Schulze E.-D., Rodermel S. R. & Bogorad L. (1991). Decreased ribulose-1,5-bisphosphate carboxylase-oxygenase in transgenic tobacco transformed with ´antisense´*rbc*S. II. Flux-control coefficients for photosynthesis in varying light, CO_2, and air humidity. *Planta* 183: 555-566.

Stougaard J. (2000). Regulators and regulation of legume root nodule development. *Plant Physiology* 124: 531-540.

Strasburger E., Noll F., Schenck H. & Schimper A. F. W. (founders); newly revised by Stitte P., Weiler E. W., Kadereit J. W., Bresinsky A. & Körner C. (2002). *Lehrbuch der Botanik für Hochschulen*. 35. edition, Spektrum-Akademischer Verlag, Heidelberg, Berlin. 1124 pp.

Sugiharto B. & Sugiyama T. (1992). Effects of nitrate and ammonium on gene expression of phospho*enol*pyruvate carboxylase and nitrogen metabolism in maize leaf tissue during recovery from nitrogen stress. *Plant Physiology* 98: 1403-1408.

Sugiharto B., Miyata K., Nakamoto H., Sasakawa H. & Sugiyama T. (1990). Regulation of expression of carbon-assimilating enzymes by nitrogen in maize leaf. *Plant Physiology* 92: 963-969.

Sugiharto B, Burnell J. N. & Sugiyama T. (1992a). Cytokinin is required to induce the nitrogen-dependent accumulation of mRNAs for phospho*enol*pyruvate carboxylase and carbonic anhydrase in detached maize leaves. *Plant Physiology* 100: 153-156.

Sugiharto B., Suzuki I., Burnell J. N. & Sugiyama T. (1992b). Glutamine induces the N-dependent accumulation of mRNAs encoding phospho*enol*pyruvate carboxylase and carbonic anhydrase in detached maize leaf tissue. *Plant Physiology* 100: 2066-2070.

Sugiyama T. & Sakakibara H. (2002). Regulation of carbon and nitrogen assimilation through gene expression. In: Foyer C. H. & Noctor G. (eds.). *Photosynthetic nitrogen assimilation and associated carbon and respiratory metabolism*, pp. 227-335. Advances in Photosynthesis and Respiration, Volume 12. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Sugiyama T., Mizuno M. & Hayashi M. (1984). Partitioning of nitrogen among ribulose-1,5-bisphosphate carboxylase/oxygenase, phosphoenolpyruvate carboxylase, and pyruvate orthophosphate dikinase as related to biomass productivity in maize seedlings. *Plant Physiology* 75: 665-669.

Suzuki I., Crétin C., Omata T. & Sugiyama T. (1994). Transcriptional and posttranscriptional regulation of nitrogen-responding expression of phospho*enol*pyruvate carboxylase gene in maize. *Plant Physiology* 105: 1223-1229.

Suzuki A., Rioual S., Lemarchand S., Godfroy N., Roux Y., Boutin J.-P. & Rothstein S. (2001). Regulation by light and metabolites of ferredoxin-dependent glutamate synthase in maize. *Physiologia Plantarum* 112: 524-530.

Takano Y. & Tsunoda S. (1971). Curvilinear regression of the leaf photosynthetic rate on leaf nitrogen content among strains of *Oryza* species. *Japanese Journal of Breeding* 21(2): 69-76.

Taniguchi M., Kobe A., Kato M. & Sugiyama T. (1995). Aspartate aminotransferase isozymes in *Panicum miliaceum* L., an NAD-malic enzyme-type C_4 plant: Comparison of enzymatic properties, primary structures, an expression patterns. *Archives of Biochemistry and Biophysics* 318(2): 295-306.

Taniguchi M., Kiba T., Sakakibara H., Ueguchi C., Mizuno T. & Sugiyama T. (1998). Expression of *Arabidopsis* response regulator homologs is induced by cytokinins and nitrate. *FEBS Letters* 429: 259-262.

Temple S. J., Vance C. P. & Gantt J. S. (1998). Glutamate synthase and nitrogen assimilation. *Trends in Plant Science* 3(2): 51-56.

Terashima I. & Evans J. R. (1988). Effects of light and nitrogen nutrition on the organization of the photosynthetic apparatus in spinach. *Plant and Cell Physiology* 29(1): 143-155.

Theobald J. C., Mitchell R. A. C., Parry M. A. J. & Lawlor D. W. (1998). Estimating the excess investment in ribulose-1,5-bisphosphate carboxylase/oxygenase in leaves of spring wheat grown under elevated CO_2. *Plant Physiology* 118: 949-955.

Thomas S. M. & Thorne G. N. (1975). Effect of nitrogen fertilizer on photosynthesis and ribulose 1,5-disphosphate carboxylase activity in spring wheat in the field. *Journal of Experimental Botany* 26(90): 43-51.

Thomas S. M., Thorne G. N. & Pearman I. (1978). Effect of nitrogen on growth, yield and photorespiratory activity in spring wheat. Annals of Botany 42: 827-837.

Thornley J.H.M. (1976). *Mathematical models in plant physiology*. *A quantitative approach to problems in plant and crop physiology.* Academic Press, London, 318 pp.

Thornton P. E. & Rosenbloom N. A. (2005). Ecosystem model spin-up: Estimating steady state conditions in a coupled terrestrial carbon and nitrogen cycle model. *Ecological Modelling* 189: 25-48.

Thornton P. E. & Zimmermann N. E. (2007). An improved integration scheme for a land surface model with prognostic canopy structure. *Journal of Climate* 20: 3902-3923.

Thornton P. E., Law B. E., Gholz H. L., Clark K. L., Falge E., Ellsworth D. S., Goldstein A. H., Monson R. K., Hollinger D., Falk M., Chen J. & Sparks J. P. (2002). Modeling and measuring the effects of disturbance history and climate on carbon and water budgets in evergreen needleleaf forests. *Agricultual and Forest Meteorology* 113: 185-222.

Thornton P. E., Lamarque J.-F., Rosenbloom N. A. & Mahowald N. M. (2007). Influence of carbon-nitrogen cycle coupling on land model response to CO_2 fertilization and climate variability. *Global Biogeochemical Cycles* 21: GB4018.

Thornton P. E., Doney S. C., Lindsay K., Moore J. K., Mahowald N., Randerson J. T., Fung I., Lamarque J.-F., Feddema J.,J. & Lee Y.-H. (2009). Carbon-nitrogen interactions regulate climate-carbon cycle feedbacks: results from an atmosphere-ocean general circulation model. *Biogeosciences* 6: 2099-2120.

Thorsteinsson B., Tillberg J.-E. & Tillberg E. (1987). Carbohydrate partitioning, photosynthesis and growth in *Lemna gibba* G3. Effects of nitrogen limitation. *Physiologia Plantarium* 71: 264-270.

Tiessen A., Hendriks J. H. M., Stitt M., Branscheid A., Gibon Y., Farré E. M. & Geigenberger P. (2002). Starch synthesis in potato tubers is regulated by post-translational redox modification of ADP-glucose pyrophosphorylase: A novel regulatory mechanism linking starch synthesis to the sucrose supply. *The Plant Cell* 14: 2191-2213.

Tischner R. (2000). Nitrate uptake and reduction in higher and lower plants. *Plant, Cell and Environment* 23: 1005-1024.

Tjoelker M. G., Reich P. B. & Oleksyn J. (1999). Changes in leaf nitrogen and carbohydrates underlie temperature and CO_2 acclimation of dark respiration in five boreal tree species. *Plant, Cell and Environment* 22: 767-778.

Tummers B., van der Laan J. & Huyser K. (2008). *DataThief III*. URL: <http://www.datathief.org>

Twine T. E., Kucharik C. J. & Foley M. A. (2004). Effects of land cover change on the energy and water balance of the Mississippi river basin. *Journal of Hydrometeorology* 5: 640-655.

Van Arendonk J. J. C. M., Nieman G. J., Boon J. J. & Lambers H. (1997). Effects of nitrogen supply on the anatomy and chemical composition of leaves of four grass species belonging to the genus *Poa*, as determined by image-processing analysis and pyrolysis-mass spectrometry. *Plant, Cell and Environment* 20: 881-897.

Van den Boogaard R., Kostadinova S., Veneklaas E. & Lambers H. (1995). Association of water use efficiency and nitrogen use efficiency with photosynthetic characteristics of two wheat cultivars. *Journal of Experimental Botany* 46 Special Issue: 1429-1438.

Van der Leij M., Smith S. J. & Miller A. J. (1998). Remobilisation of vacuolar stored nitrate in barley root cells. *Planta* 205: 64-72.

Van der Werf A. & Nagel O. (1996). Carbon allocation to shoots and roots in relation to nitrogen supply is mediated by cytokinins and sucrose: Opinion. *Plant and Soil* 185: 21-32.

Van der Werf A., van den Berg G., Ravenstein H.J.L., Lambers H. & Eising R. (1992). Protein turnover: a significant component of maintenance respiration in roots? In: Lambers H. & van der Plas L.H.W. (eds.): *Molecular, biochemical and physiological aspects of plant respiration*, pp. 483-492. SPB Academic Publishing, The Hague.

Vance C. P. & Heichel G. H. (1991). Carbon in N_2 fixation: limitation or exquisite adaptation. *Annual Reviews of Plant Physiology and Plant Molecular Biology* 42: 373-392.

Vessey J. K. & Layzell D. B. (1987). Regulation of assimilate partitioning in soybean. *Plant Physiology* 83: 341-348.

Vessey J. K., Pawlowski K. & Bergman B. (2004). Root-based N_2-fixing symbioses: legumes, actinorhizal plants, *Parasponia* sp. and cycads. *Plant and Soil* 266: 205-230.

Vitousek P. M. (1994). Beyond global warming: Ecology and global change. *Ecology* 75(7): 1861-1876.

Vitousek P. M., Aber J. D., Howarth R. W., Likens G. E., Matson P. A., Schindler D. W., Schlesinger W. H. & Tilman D. G. (1997). Human alteration of the global nitrogen cycle: sources and consequences. *Ecological Applications* 7(3): 737-750.

Vitousek P. M., Cassman K., Cleveland C., Crews T., Field C. B., Grimm N. B., Howarth R. W., Marino R., Martinelli L., Rastetter E. B. & Sprent J. I. (2002). Towards an understanding of biological nitrogen fixation. *Biogeochemistry* 57/58: 1-45.

Vitousek P. M., Naylor R., Crews T., David M. B., Drinkwater L. E., Holland E., Johnes P. J., Katzenberger J., Martinelli L. A., Matson P. A., Nziguheba G., Ojima D., Palm C. A., Robertson G. P., Sanchez P. A., Townsend A. R. & Zhang F. S. (2009). Nutrient imbalances in agricultural development. *Science* 324: 1519-1520.

Von Caemmerer S. & Farquhar G. D. (1981). Some relationships between biochemistry of photosynthesis and the gas exchange of leaves. *Planta* 153: 376-387.

Vos J. & Biemond H. (1992). Effects of nitrogen on the development and growth of the potato plant. 1. Leaf appearance, expansion growth, life spans of leaves and stem branching. *Annals of Botany* 70: 27-35.

Vos J. & van der Putten P. E. L. (1998). Effect of nitrogen supply on leaf growth, leaf nitrogen economy and photosynthetic capacity in potato. *Field Crops Research* 59: 63-72.

Vos J., van der Putten P. E. L. & Birch C. J. (2005). Effect of nitrogen supply on leaf appearance, leaf growth, leaf nitrogen economy and photosynthetic capacity in maize (*Zea mays* L.). *Field Crops Research* 93: 64-73.

Walch-Liu P., Neumann G., Bangerth F. & Engels C. (2000). Rapid effects of nitrogen form on leaf morphogenesis in tobacco. *Journal of Experimental Botany* 51(343). 227-237.

Wand S. J. E., Midgley G. F., Jones M. H. & Curtis P. W. (1999). Responses of wild C_4 and C_3 grass (Poaceae) species to elevated atmospheric CO_2 concentration: a meta-analytic test of current theories and perceptions. *Global Change Biology* 5: 723-741.

Wang R., Guegler K., LaBrie S. T. & Crawford N. M. (2000). Genomic analysis of a nutrient response in Arabidopsis reveals diverse expression patterns and novel metabolic an potential regulatory genes induced by nitrate. *The Plant Cell* 12: 1491-1509.

Wang S., Grant R. F., Verseghy D. L., & Black T. A. (2001). Modelling plant carbon and nitrogen

dynamics of a boreal aspen forest in CLASS-the Canadian land surface scheme. *Ecological Modelling* 142; 135-154.

Waring R. H., Mc Donald A. J. S., Larsson S., Ericsson T., Wiren A., Arwidsson E., Ericsson A. & Lohammar T. (1985). Differences in chemical composition of plants grown at constant relative growth rates with stable mineral nutrition. *Oecologia* 66: 157-160.

Webber A. N., Nie G.-Y. & Long S. P. (1994). Acclimation of photosynthetic proteins to rising atmospheric CO_2. *Photosynthesis Research* 39: 413-425.

Weber P. & Rennenberg H. (1996). Dependency of nitrogen dioxide (NO_2) fluxes to wheat (*Triticum aestivum*) leaves from NO_2 concentration, light intensity, temperature and relative humidity determined from controlled dynamic chamber experiments. *Atmospheric Environment* 30(17): 3001-3009.

Wellburn A. R. (1990). Why are atmospheric oxides of nitrogen usually phytotoxic and not alternative fertilizers? *New Phytologist* 115: 395-429.

Werger M. J. A. & Hirose T. (1991). Leaf nitrogen distribution and whole canopy photosynthetic carbon gain in herbaceous stands. *Vegetatio* 97: 11-20.

White M. A., Thornton P. E., Running S. W. & Ramakrishna R. N. (2000). Parameterization and sensitivity analysis of the BIOME-BGC terrestrial ecosystem model: net primary production controls. *Earth Interactions* 4(3): 1-85.

Willigen P. (1991). Nitrogen turnover in the soil-crop system; comparison of fourteen simulation models. *Fertilizer Research* 27: 141-149.

Wiren N., Gazzarrini S. & Frommer W. B. (1997). Regulation of mineral nitrogen uptake in plants. *Plant and Soil* 196: 191-199.

Wolt J. D. (1994). *Soil Solution Chemistry*. John Wiley & Sons, Inc. New York.

Wong S. C. (1979). Elevated atmospheric partial pressure of CO2 and plant growth. I. Interactions of nitrogen nutrition and photosynthetic capacity in C_3 and C_4 plants. *Oecologia* 44: 68-74.

Wong S.-C. (1990). Elevated atmospheric partial pressure of CO_2 and plant growth. II. Non-structural carbohydrate content in cotton plants and its effect on growth parameters *Photosynthesis Research* 23: 171-180.

Wong S.-C., Cowan I. R. & Farquhar G. D. (1985). Leaf conductance in relation to rate of CO_2 Assimilation. I. Influence of nitrogen nutrition, phosphorus nutrition, photon flux density, and ambient partial pressure of CO_2 during ontogeny. *Plant Physiology* 78: 821-825.

Woodrow I. E. (1994). Optimal acclimation of the C_3 photosynthetic system under enhanced CO_2. *Photosynthesis Research* 39: 401-412.

Woodrow I. E. & Berry J. A. (1988). Enzymatic regulation of photosynthetic CO_2 fixation in C_3 plants. *Annual Review of Plant Physiology and Plant Molecular Biology.* 39: 533-594.

Woodrow I. E., Ball J. T. & Berry J. A. (1990). Control of photosynthetic carbon dioxide fixation by the boundary layer, stomata and ribulose 1,5-biphosphate carboxylase/oxygenase. *Plant, Cell and Environment* 14: 339-347.

Worthington C. M. & Hutchinson C. M. (2005). Accumulated growing degree days as a model to determine key development stages and evaluate yield and quality of potato in northeast Florida. *Proceedings of the Florida State Horticultural Society* 118: 98-101.

Wright I. J., Reich P. B., Westoby M., Ackerly D. A.., Baruch Z, Bongers F., Cavender-Bares J., Chapin T., Cornelissen J. H. C., Diemer M., Flexas J., Garnier E., Groom P. K., Gulias J., Hikosaka K., Lamont B. B., Lee T., Lee W., Lusk C., Midgley J. J., Navas M.-L., Niinemets U., Oleksyn J., Osada N., Poorter H., Poot P., Prior L., Pyankov V., Roumet C., Thomas S. C.,

Tjoelker M. G., Veneklaas E. J., & Villar R. (2004). The worldwide leaf economics spectrum. *Nature* 428: 821-827.

Wullschleger S. D. (1993). Biochemical limitations to carbon assimilation in C_3 plants – a retrospective analysis of the A/C_i Curves from 109 species. *Journal of Experimental Botany* 44(262): 907-920.

Xu-Ri & PrenticeI. C. (2008). Terrestrial nitrogen cycle simulation with a dynamic global vegetation model. *Global Change Biology* 14: 1745-1764.

Yin Z.-H., Kaiser W. M., Heber U. & Raven J. A. (1996). Acquisition and assimilation of gaseous ammonia as revealed by intracellular pH changes in leaves of higher plants. *Planta* 200: 380-387.

Yoshida S. & Coronel V. (1976). Nitrogen nutrition, leaf resistance, and leaf photosynthetic rate of the rice plant. *Soil Science and Plant Nutrition* 22(2): 207-211.

Zadok J. C., Chang T. T. & Konzak C. F. (1974). A decimal code for the growth stages of cereals. *Weed Research* 14: 415-421.

Zaehle S., Bondeau A., Carter T. R., Cramer W., Erhard M., Prentice C., Reginster I., Rounsevell M. D. A., Sitch S., Smith B., Smith P. C., & Sykes M. (2007). Projected changes in terrestrial carbon storage in Europe under climate and land-use change, 1990-2100. *Ecosystems* 10(3): 380-401.

Zhang H. & Forde B. G. (1998). An *Arabidopsis* MADS box gene that controls nutrient-induced changes in root architecture. *Science* 279: 407-409.

Zhang H. & Forde B. G. (2000). Regulation of *Arabidopsis* root development by nitrate availability. *Journal of Experimental Botany* 51(342): 51-59.

Zhang F., Pan B. & Smith D. L. (1997). Application of gibberellic acid to the surface of soybean seed (*Glycine max* (L.) Merr.) and symbiotic nodulation, plant development, final grain and protein yield under short season conditions. *Plant and Soil* 188: 329-335.

Zhang H., Jennings A., Barlow P. W. & Forde B. G. (1999). Dual pathways for regulation of root branching by nitrate. *Proceedings of the National Academy of Sciences* USA 96: 6529-6534.

Zhen R. G. & Leigh R. A. (1990). Nitrate accumulation by wheat (*Triticum aestivum*) in relation to growth and tissue N concentrations. *Plant and Soil* 124: 157-160.

Appendix A: Abbreviations and units

Table A.1: List of abbreviations.

Section	Abbreviation	Definition
General	A	photosynthesis
	A_{max}	light-saturated photosynthesis rate
	ATP	Adenosine-5'-triphosphate
	BNF	biological N_2 fixation
	C	carbon
	CFT	crop functional type
	Chl	chlorophyll
	C_i	internal CO_2 concentration in leaves
	DGVM	dynamic global vegetation model
	GPP	gross primary production
	J_{max}	maximal electron transport rate
	LA	leaf area
	LAI	leaf area index
	LPJ	Lund-Potsdam-Jena DGVM
	LPJmL	LPJ managed Land model
	N	nitrogen
	N_L	leaf N content
	$\%N_{crit}$	critical N concentration, i.e. the minimum N content permitting maximal growth
	NPP	net primary production
	NUE	nitrogen use efficiency
	PFT	plant functional type
	P_i	inorganic phosphate
	R_G	growth respiration
	R_M	maintenance respiration
	RGR	relative growth rate
	Rubisco/Rub	Ribulose-1,5-bisphosphate-carboxylase oxygenase
	SLA	specific leaf area
	T	temperature
	TEM	Terrestrial ecosystem model
	V_{Cmax}	maximal carboxylation rate of Rubisco
Lit review	DIN	dissolved organic N
	GOGAT	Glutamine oxoglutarate aminotransferase (also known as glutamate synthase)
	GS	Glutamine synthetase
	HATS	high-affinity transport system
	iHATS	inducible HATS
	cHATS	constitutive HATS
	K_m	Michaelis-Menten constant, i.e. substrate concentration giving half-maximal enzyme activity
	LATS	low-affinity transport system
	mRNA	messenger ribonucleic acid
	NAD(P)H	Nicotinamide adenine dinucleotide (phosphate)
	NiR	Nitrite reductase
	NR	Nitrate reductase
	PEPCase	Phosphoenolpyruvate carboxylase
	PNUE	photosynthetic nitrogen use efficiency
	PPDK	Pyruvate orthophosphate dikinase
	PSI	photosystem I
	PSII	photosystem II
	R_d	leaf dark respiration
	RuBP	Ribulose-1,5-bisphosphate
	TCA	tricarboxylic acid cycle
	TPU	triosephosphate usage limitation
	V_{max}	maximal enzyme activity
Meta-analysis	AA_L	free amino acid contents in leaves
	amm	ammonium

	DAE	days after emergence
	g_s	Stomatal conductance
	k	sample size (i.e. number of effect sizes)
	N_G	N content in grains
	nit	nitrate
	Nit_L	nitrate content in leaves
	Nit_R	nitrate content in roots
	Nit_T	nitrate content in whole plant
	NSC_L	non-structural carbohydrates in leaves
	N_T	N content in whole plant
	N_{up}	N uptake rate
	$Prot_L$	soluble protein contents in leaves
	Q_B	between-group heterogeneity
	R	response ratio
	RSR	root-shoot-ratio
	$Stch_L$	starch contents in leaves
	Sug_L	sugar content in leaves
	W_R	root biomass
	W_S	shoot biomass
	W_T	whole plant biomass
Discussion	N_{atm}	atmospheric N_2
	N_{soil}	soil N concentration
	N_{inorg}	inorganic N in plant
	N_{org}	organic N in plant
	N_{res}	storage N in plant
	N_{struc}	structural N in plant
	C_{atm}	atmospheric CO_2
	C_{inorg}	inorganic C in plant
	C_{org}	organic C in plant
	C_{res}	storage C in plant
	C_{struc}	structural C in plant
	Fr	fine root compartment
	R	root compartment
	S	stem compartment
	L	leaf compartment

Table A.2: List of units.

Unit	Definition
g	gramme
µg	microgramme (10^{-6} g)
kg	kilogramme (10^{3} g)
Tg	teragramme (10^{12} g)
l	litre
mol	mole
mmol	millimol (10^{-3} mol)
µM	micromolar (µmol l^{-1})
mM	millimolar (mmol l^{-1})
Pa	pascal
mPa	millipascal (10^{-3} Pa)
ppm	parts per million
m	metre
ha	hectare (100 x 100 m)
h	hours
s	second

Appendix B: Shortlist of studies that could not be included in the meta-analysis

Table B.1: A summary of paper that could not be included in the study, stating the species studied, whether the respective maximum N supply was non-limiting for plant growth, whether sample size (n), standard deviation (SD) and/or standard error (SE) were reported, how many different N rates were supplied in the experiments, whether the authors of the study were contacted and if yes, why the data could still not be included (e.g. contacted authors did not reply or the data could not be found or was too old and could not be extracted from outdated formats).

Reference	Species	non-lim N	n + SD/SE	N rates	Contact
Barneix *et al.* (1984)	*Hordeum vulgare*	yes	no	3	yes (data too old)
Bhat *et al*. (1979)	*Brassica napus*	yes	no	5	no (E-mail not found)
Billes *et al*. (1993)	*Triticum aestivum*	no	no	2	no
Bloom & Chapin (1981)	*Hordeum vulgare*	no	yes	6	no
Brewitz *et al*. (1995)	*Hordeum vulgare*	yes	no	3	no (E-mail not found)
Colnenne *et al*. (1998)	*Brassica napus*	yes	no	3	no
Cox & Reisenauer (1973)	*Triticum aestivum*	yes	no	7	no (E-mail not found)
Delgado *et al.* (1994)	*Triticum aestivum*	yes	no	2	yes (no reply)
Fair *et al*. (1974)	*Hordeum vulgare*	yes	no	2	no (E-mail not found)
Foehse & Jungk (1983)	*Brassica napus*	?	no	4	no (E-mail not found)
Harbur & Owen (2004)	*Zea mays* *Glycine max*	?	no	2	yes (data not found)
Jackson *et al.* (1976)	*Triticum aestivum*	yes	no	3	no (E-mail not found)
Khamis *et al.* (1990)	*Zea mays*	yes	no	4	yes (no reply)
Lawlor *et al.* (1987a)	*Triticum aestivum*	no	no	2	no
Lawlor *et al.* (1987b)	*Triticum aestivum*	no	no	2	no
Lawlor *et al.* (1987c)	*Triticum aestivum*	no	no	2	no
Lee (1993)	*Hordeum vulgare*	no	yes	2	no
Lee & Rudge (1986)	*Hordeum vulgare*	no	yes	2	no
Lewis *et al.* (1982)	*Hordeum vulgare*	yes?	no	2	no (E-mail not found)
Longnecker & Robson (1994)	*Triticum aestivum*	yes	no	7	yes (no reply)
Longstreth & Nobel (1980)	*Gossypium hirsutum*	yes	no	5	yes (no reply)
Machado *et al.* (2001)	*Zea mays*			2	no
Makino & Osmond (1991)	*Triticum aestivum*	yes	no	4	yes (data too old)
Makino *et al.* (1984a)	*Oryza sativa*	yes	no	3	yes (data too old)
Makino *et al.* (1984b)	*Oryza sativa*	no	no	2	yes (data too old)
Makino *et al.* (1992)	*Triticum aestivum* *Oryza sativa* *Phaseolus vulgaris*	yes	no	4	yes (data too old)
Makino *et al.* (1994)	*Oryza sativa*	?	no	3	yes (data too old)
Masuda *et al.* (1989a)	*Glycine max*	yes	no	3	no (E-mail not found)
Masuda *et al.* (1989b)	*Glycine max*	yes	no	2	no (E-mail not found)
Mattsson *et al.* (1991)	*Hordeum vulgare*	yes?	no	7	yes (no reply)
Mattsson *et al.* (1992a)	*Hordeum vulgare*	no?	no	3	yes (no reply)

Mattsson *et al.* (1992b)	*Hordeum vulgare*	no?	no	3	yes (no reply)
Plhak (2003)	*Zea mays*	?	no	2	no (E-mail not found)
Radin (1990)	*Gossypium hirsutum*	yes	yes	2	no
Radin & Ackerson (1981)	*Gossypium hirsutum*	?	no	5	no (E-mail not found)
Radin *et al.* (1982)	*Gossypium hirsutum*	yes	no	2	no
Radoglou & Jarvis (1992)	*Phaseolus vulgaris*	?	no	2	yes (no reply)
Radoglou *et al.* (1992)	*Phaseolus vulgaris*	?	no	2	yes (no reply)
Robinson *et al.* (1991)	*Triticum aestivum*	yes	no	2	yes (data too old)
Robinson *et al.* (1994)	*Triticum aestivum*	yes	no	2	yes (data too old)
Rufty *et al.* (1988)	*Glycine max*	no	no	2	no
Samuelson *et al.* (1992)	*Hordeum vulgare*	yes	no	6	no (E-mail not found)
Sarandon & Gianibelli (1990)	*Triticum aestivum*	no	no	2	no
Seemann *et al.* (1987)	*Phaseolus vulgaris*	?	no	2	no
Shangguan *et al.* (2000)	*Triticum aestivum*	yes	no	2	yes (no reply)
Shangguan *et al.* (2004)	*Triticum aestivum*	yes	no	6	yes (no reply)
Shimshi (1970a)	*Phaseolus vulgaris*	no	yes	2	no
Shimshi (1970b)	*Phaseolus vulgaris*	no	yes	2	no
Sugiyama *et al.* (1984)	*Zea mays*	yes	no	7	no (E-mail not found)
Theobold *et al.* (1998)	*Triticum aestivum*	yes	no	2	yes (no reply)
Thomas *et al.* (1978)	*Triticum aestivum*	?	no	2	no (E-mail not found)
van den Boogaard *et al.* (1995)	*Triticum aestivum*	?	yes	2	no
Vos *et al.* (2005)	*Zea mays*	yes	no	5	yes (data too old)
Wong *et al.* (1985)	*Zea mays* *Gossypium hirsutum*	yes	no	4	no (E-mail not found)
Yoshida & Coronel (1976)	*Oryza sativa*	?	no	2	no (E-mail not found)
Zhen & Leigh (1990)	*Triticum aestivum*	yes	no	11	no

Appendix C: Datasets for parameters included in the meta-analysis

Table C.1: Study abbreviations.

Abbreviation	Study
Barn	Barneix *et al.* (1992)
Biem	Biemond & Vos (1992)
Caput	Caputo & Barneix (1997)
Carv	Carvajal *et al.* (1996)
Chap1	Chapin *et al.* (1988a)
Chap2	Chapin *et al.* (1988b)
Cram	Cramer & Lewis (1993)
Dev	Devienne *et al.* (1994)
Ev	Evans (1983)
Fr	Fricke *et al.* (1997)
Guit	Guitman *et al.* (1991)
Kham1	Khamis & Lamaze (1990)
Kham2	Khamis *et al.* (1992)
King	King *et al*. (1993)
Mit	Mitchell *et al.* (1993)
Morg	Morgan (1984)
Nakam	Nakamura *et al.* (1999)
Nakan	Nakano *et al.* (1997)
Pugn	Pugnaire & Chapin (1992)
Rad	Radin (1983)
Rob	Robinson (1996)
Sid1	Siddiqi *et al.* (1989)
Sid2	Siddiqi *et al.* (1990)
Vos1	Vos & Biemond (1992)
Vos2	Vos & van der Putten (1998)
Wo1	Wong (1979)
Wong2	Wong (1990)

Table C. 2: List of effect sizes (lnR) and associated categorical variables for the response variable leaf area (LA). Abbreviations: TA: *Triticum aestivum*; GM: *Glycine max*; GH: *Gossypium hirsutum*; ZM: *Zea mays*; ST: *Solanum tuberosum*; length lim: length of limitation; ent growth: entire growth period; nit: nitrate; amm: ammonium; freq: frequency of N application; amb: ambient CO_2 level; elev: elevated CO_2 level; pot: pot size; dev: development stage; veg: vegetative; repr: reproductive; leg: leguminous; non: non-leguminous; nod: leguminous, nodulating; non-nod: leguminous, non-nodulating; Var: variance. The shorthand for studies is as follows: study/species/(interaction)/experiment

study	N rate	species	length lim	N source	freq	pH	[CO2]	stress	pot	medium	dev	C3/C4	leg	crop	lnR	Var (lnR)	%change
EvTa1	very low	TA	ent growth	nit	3-7	no	amb	none	medium	soil	-	C3	no	cereal	-2.74	0.02	-93.52
EvTa2	very low	TA	ent growth	nit	3-7	no	amb	none	medium	soil	-	C3	no	cereal	-2.75	0.03	-93.64
EvTa3	very low	TA	ent growth	nit	3-7	no	amb	none	medium	soil	-	C3	no	cereal	-1.91	0.03	-85.23
EvTa4	low	TA	ent growth	nit	3-7	no	amb	none	medium	soil	-	C3	no	cereal	-0.85	0.03	-57.04
RobGm1	low	GM	ent growth	nit + amm	1-2	yes	amb	none	medium	inert	veg	C3	non nod	oilseed	-0.02	0.00	-2.02
Wo1GhE1	medium	GH	ent growth	nit	1-2	no	elev	none	medium	soil	veg	C3	no	fibre	-0.39	0.01	-32.56
Wo1GhE2	low	GH	ent growth	nit	1-2	no	elev	none	medium	soil	veg	C3	no	fibre	-0.87	0.01	-58.14
Wo1GhE3	very low	GH	ent growth	nit	1-2	no	elev	none	medium	soil	veg	C3	no	fibre	-1.56	0.05	-79.07
Wo1GhA1	medium	GH	ent growth	nit	1-2	no	amb	none	medium	soil	veg	C3	no	fibre	-0.30	0.00	-25.93
Wo1GhA2	low	GH	ent growth	nit	1-2	no	amb	none	medium	soil	veg	C3	no	fibre	-0.81	0.00	-55.55
Wo1GhA3	very low	GH	ent growth	nit	1-2	no	amb	none	medium	soil	veg	C3	no	fibre	-1.35	0.02	-74.07
Wo1ZmE1	medium	ZM	ent growth	nit	1-2	no	elev	none	medium	soil	veg	C4	no	cereal	-0.17	0.01	-15.79
Wo1ZmE2	low	ZM	ent growth	nit	1-2	no	elev	none	medium	soil	veg	C4	no	cereal	-0.50	0.00	-39.47
Wo1ZmE3	very low	ZM	ent growth	nit	1-2	no	elev	none	medium	soil	veg	C4	no	cereal	-1.15	0.01	-68.42
Wo1ZmA1	medium	ZM	ent growth	nit	1-2	no	amb	none	medium	soil	veg	C4	no	cereal	-0.30	0.02	-25.64
Wo1ZmA2	low	ZM	ent growth	nit	1-2	no	amb	none	medium	soil	veg	C4	no	cereal	-0.67	0.02	-48.72
Wo1ZmA3	very low	ZM	ent growth	nit	1-2	no	amb	none	medium	soil	veg	C4	no	cereal	-1.18	0.01	-69.23
Vos1St1	medium	ST	ent growth	?	>7	no	amb	none	big	sand	repr	C3	no	tuber	-0.14	0.00	-12.71
Vos1St2	low	ST	ent growth	?	>7	no	amb	none	big	sand	repr	C3	no	tuber	-1.39	0.00	-75.08
NakamGm1E	very low	GM	ent growth	amm	?	no	elev	none	small	soil	repr	C3	nod	oilseed	-0.20	0.00	-17.89
NakamGm1A	very low	GM	ent growth	amm	?	no	amb	none	small	soil	repr	C3	nod	oilseed	-0.06	0.01	-5.83
NakamGm2E	very low	GM	ent growth	amm	?	no	elev	none	small	soil	repr	C3	non nod	oilseed	-0.26	0.03	-23.02
NakamGm2A	very low	GM	ent growth	amm	?	no	amb	none	small	soil	repr	C3	non nod	oilseed	-0.32	0.00	-27.65

Table C.3: List of effect sizes (lnR) and associated categorical variables for the response variable whole plant biomass (W_T). Abbreviations: HV: *Hordeum vulgare*; hydro: hydroponic; mat: maturity. Further abbreviations as before.

study	N rate	species	length lim	N source	freq	pH	[CO2]	stress	pot	medium	dev	C3/C4	leg	crop	lnR	VAR (lnR)	%change
CramTaNit	medium	TA	ent growth	nit	3-7	yes	amb	none	big	hydro	veg	C3	no	cereal	0.12	0.01	12.38
CramTaAm	medium	TA	ent growth	amm	3-7	yes	amb	none	big	hydro	veg	C3	no	cereal	0.08	0.04	8.47
CramZmNit	medium	ZM	ent growth	nit	3-7	yes	amb	none	big	hydro	veg	C4	no	cereal	0.25	0.03	28.62
CramZmAm	medium	ZM	ent growth	amm	3-7	yes	amb	none	big	hydro	veg	C4	no	cereal	-0.24	0.02	-21.26
Wo1ZmE1	medium	ZM	ent growth	nit	1-2	no	elev	none	medium	soil	veg	C4	no	cereal	-0.17	0.01	-15.53
Wo1ZmE2	low	ZM	ent growth	nit	1-2	no	elev	none	medium	soil	veg	C4	no	cereal	-0.48	0.01	-38.29
Wo1ZmE3	very low	ZM	ent growth	nit	1-2	no	elev	none	medium	soil	veg	C4	no	cereal	-1.16	0.01	-68.63
Wo1ZmA1	medium	ZM	ent growth	nit	1-2	no	amb	none	medium	soil	veg	C4	no	cereal	-0.23	0.02	-20.78
Wo1ZmA2	low	ZM	ent growth	nit	1-2	no	amb	none	medium	soil	veg	C4	no	cereal	-0.52	0.01	-40.30
Wo1ZmA3	very low	ZM	ent growth	nit	1-2	no	amb	none	medium	soil	veg	C4	no	cereal	-1.11	0.02	-66.94
Wo1GhE1	medium	GH	ent growth	nit	1-2	no	elev	none	medium	soil	veg	C3	no	fibre	-0.22	0.00	-19.70
Wo1GhE2	low	GH	ent growth	nit	1-2	no	elev	none	medium	soil	veg	C3	no	fibre	-0.59	0.01	-44.72
Wo1GhE3	very low	GH	ent growth	nit	1-2	no	elev	none	medium	soil	veg	C3	no	fibre	-1.04	0.00	-64.75
Wo1GhA1	medium	GH	ent growth	nit	1-2	no	amb	none	medium	soil	veg	C3	no	fibre	-0.24	0.01	-21.49
Wo1GhA2	low	GH	ent growth	nit	1-2	no	amb	none	medium	soil	veg	C3	no	fibre	-0.56	0.01	-42.99
Wo1GhA3	very low	GH	ent growth	nit	1-2	no	amb	none	medium	soil	veg	C3	no	fibre	-1.04	0.02	-64.48
PugnHv1	low	HV	ent growth	nit	1-2	no	amb	none	small	inert	mat	C3	no	cereal	-0.84	0.00	-56.99
BiemSt1	low	ST	ent growth	?	>7	no	amb	none	big	sand	mat	C3	no	tuber	-1.23	0.00	-70.74
BiemSt2	medium	ST	ent growth	?	>7	no	amb	none	big	sand	mat	C3	no	tuber	-0.24	0.00	-21.22
Chap2Hv1	very low	HV	< ½	nit	3-7	no	amb	none	small	hydro	veg	C3	no	cereal	-0.43	0.01	-34.86
DevTa1	very low	TA	ent growth	nit	1-2	no	amb	none	big	hydro	veg	C3	no	cereal	-0.78	0.04	-53.94
DevTa2	very low	TA	ent growth	nit	1-2	no	amb	none	big	hydro	veg	C3	no	cereal	-0.55	0.05	-42.53
DevTa3	very low	TA	ent growth	nit	1-2	no	amb	none	big	hydro	veg	C3	no	cereal	-0.43	0.05	-35.07
DevTa4	low	TA	ent growth	nit	1-2	no	amb	none	big	hydro	veg	C3	no	cereal	-0.39	0.04	-32.26
DevTa5	low	TA	ent growth	nit	1-2	no	amb	none	big	hydro	veg	C3	no	cereal	-0.23	0.04	-20.59
DevTa6	medium	TA	ent growth	nit	1-2	no	amb	none	big	hydro	veg	C3	no	cereal	0.04	0.05	4.39
DevTa7	high	TA	ent growth	nit	1-2	no	amb	none	big	hydro	veg	C3	no	cereal	0.05	0.03	4.60
GuitTa1	very low	TA	ent growth	nit + amm	3-7	no	amb	none	medium	soil	mat	C3	no	cereal	-0.03	0.00	-2.66
MitTaAT	low	TA	ent growth	nit	>7	no	amb	none	medium	inert	mat	C3	no	cereal	-0.51	0.00	-40.17
MtTaAT+	low	TA	ent growth	nit	>7	no	amb	yes	medium	inert	mat	C3	no	cereal	-0.42	0.01	-34.57

MitTaET	low	TA	ent growth	nit	>7	no	elev	none	medium	inert	mat	C3	no	cereal	-0.55	0.00	-42.36
MitTaET+	low	TA	ent growth	nit	>7	no	elev	yes	medium	inert	mat	C3	no	cereal	-0.46	0.00	-37.06
NakamGm1E	very low	GM	ent growth	amm	?	no	elev	none	small	soil	repr	C3	nod	oilseed	-0.37	0.01	-30.81
NakamGm1A	very low	GM	ent growth	amm	?	no	amb	none	small	soil	repr	C3	nod	oilseed	-0.15	0.00	-13.53
NakamGm2E	very low	GM	ent growth	amm	?	no	elev	none	small	soil	repr	C3	non nod	oilseed	-0.55	0.01	-42.47
NakamGm2A	very low	GM	ent growth	amm	?	no	amb	none	small	soil	repr	C3	non nod	oilseed	-0.18	0.01	-16.57
Wong2GhA1	medium	GH	ent growth	nit	1-2	no	amb	none	medium	soil	veg	C3	no	fibre	-0.14	0.04	-12.87
Wong2GhA2	low	GH	ent growth	nit	1-2	no	amb	none	medium	soil	veg	C3	no	fibre	-0.23	0.04	-20.42
Wong2GhA3	very low	GH	ent growth	nit	1-2	no	amb	none	medium	soil	veg	C3	no	fibre	-1.06	0.05	-65.37
Wong2GhE1	medium	GH	ent growth	nit	1-2	no	elev	none	medium	soil	veg	C3	no	fibre	0.25	0.01	28.01
Wong2GhE2	low	GH	ent growth	nit	1-2	no	elev	none	medium	soil	veg	C3	no	fibre	-0.12	0.01	-11.45
Wong2GhE3	very low	GH	ent growth	nit	1-2	no	elev	none	medium	soil	veg	C3	no	fibre	-1.19	0.01	-69.57
Kham1Zm1	very low	ZM	> ½	nit	<1	yes	amb	none	small	sand	veg	C4	no	cereal	-0.28	0.03	-24.22
Kham1Zm2	low	ZM	> ½	nit	<1	yes	amb	none	small	sand	veg	C4	no	cereal	0.22	0.02	24.53
Kham1Zm3	medium	ZM	> ½	nit	<1	yes	amb	none	small	sand	veg	C4	no	cereal	0.07	0.01	6.92
RobGm1	low	GM	ent growth	nit + amm	1-2	yes	amb	none	medium	inert	veg	C3	non nod	oilseed	-0.17	0.00	-15.65

Table C. 4: List of effect sizes (lnR) and associated categorical variables for the response variable shoot biomass (W_S). Abbreviations: seedl: seedling. Further abbreviations as before.

study	N rate	species	length lim	N source	freq	pH	[CO2]	stress	pot	medium	dev	C3/C4	leg	crop	lnR	Var (lnR)	%change
CaputTa1	very low	TA	ent growth	nit	1-2	no	amb	none	small	sand	seedl	C3	no	cereal	-0.49	0.01	-38.80
CaputTa2	low	TA	ent growth	nit	1-2	no	amb	none	small	sand	seedl	C3	no	cereal	-0.20	0.01	-18.10
CaputTa3	low	TA	ent growth	nit	1-2	no	amb	none	small	sand	seedl	C3	no	cereal	0.13	0.01	13.91
CaputTa4	medium	TA	ent growth	nit	1-2	no	amb	none	small	sand	seedl	C3	no	cereal	0.21	0.01	23.71
CaputTa5	high	TA	ent growth	nit	1-2	no	amb	none	small	sand	seedl	C3	no	cereal	0.24	0.01	27.25
Chap2Hv1	very low	HV	< ½	nit	3-7	no	amb	none	small	hydro	veg	C3	no	cereal	-0.71	0.01	-50.70
DevTa1	very low	TA	ent growth	nit	1-2	no	amb	none	big	hydro	veg	C3	no	cereal	-0.85	0.05	-57.36
DevTa2	very low	TA	ent growth	nit	1-2	no	amb	none	big	hydro	veg	C3	no	cereal	-0.66	0.06	-48.15
DevTa3	very low	TA	ent growth	nit	1-2	no	amb	none	big	hydro	veg	C3	no	cereal	-0.63	0.05	-46.49
DevTa4	low	TA	ent growth	nit	1-2	no	amb	none	big	hydro	veg	C3	no	cereal	-0.48	0.06	-38.27
DevTa5	low	TA	ent growth	nit	1-2	no	amb	none	big	hydro	veg	C3	no	cereal	-0.33	0.04	-28.04
DevTa6	medium	TA	ent growth	nit	1-2	no	amb	none	big	hydro	veg	C3	no	cereal	0.03	0.05	3.24
DevTa7	high	TA	ent growth	nit	1-2	no	amb	none	big	hydro	veg	C3	no	cereal	0.05	0.03	5.51
Kham1Zm1	very low	ZM	> ½	nit	>1	yes	amb	none	small	sand	veg	C4	no	cereal	-0.47	0.05	-37.74
Kham1Zm2	low	ZM	> ½	nit	>1	yes	amb	none	small	sand	veg	C4	no	cereal	0.17	0.02	18.06
Kham1Zm3	medium	ZM	> ½	nit	>1	yes	amb	none	small	sand	veg	C4	no	cereal	0.05	0.01	5.34
Kham2Zm1	very low	ZM	> ½	nit	>1	no	amb	none	medium	sand	veg	C4	no	cereal	-0.06	0.03	-6.25
Kham2Zm2	very low	ZM	> ½	nit	>1	no	amb	none	medium	sand	veg	C4	no	cereal	-0.83	0.04	-56.25
Kham2Zm3	very low	ZM	> ½	nit	>1	no	amb	none	medium	sand	veg	C4	no	cereal	-1.29	0.03	-72.50
RobGm1	low	GM	ent growth	nit + amm	1-2	yes	amb	none	medium	inert	veg	C3	non nod	oilseed	-0.30	0.01	-26.01
PugnHv1	low	HV	ent growth	nit	1-2	no	amb	none	small	inert	mat	C3	no	cereal	-1.15	0.00	-68.18
BiemSt1	low	ST	ent growth	?	<7	no	amb	none	big	sand	mat	C3	no	tuber	-1.60	0.00	-79.82
BiemSt2	medium	ST	ent growth	?	<7	no	amb	none	big	sand	mat	C3	no	tuber	-0.52	0.00	-40.38

Table C. 5: List of effect sizes (lnR) and associated categorical variables for the response variable root biomass (W_R). Abbreviations as before.

study	N rate	species	length lim	N source	freq	pH	[CO2]	stress	pot	medium	dev	C3/C4	leg	crop	lnR	Var (lnR)	%change
Chap2Hv1	very low	HV	< ½	nit	3-7	no	amb	none	small	hydro	veg	C3	no	cereal	0.27	0.01	30.63
DevTa1	very low	TA	ent growth	nit	1-2	no	amb	none	big	hydro	veg	C3	no	cereal	-0.65	0.03	-48.02
DevTa2	very low	TA	ent growth	nit	1-2	no	amb	none	big	hydro	veg	C3	no	cereal	-0.39	0.02	-32.46
DevTa3	very low	TA	ent growth	nit	1-2	no	amb	none	big	hydro	veg	C3	no	cereal	-0.10	0.03	-9.90
DevTa4	low	TA	ent growth	nit	1-2	no	amb	none	big	hydro	veg	C3	no	cereal	-0.23	0.04	-20.71
DevTa5	low	TA	ent growth	nit	1-2	no	amb	none	big	hydro	veg	C3	no	cereal	-0.05	0.02	-5.06
DevTa6	medium	TA	ent growth	nit	1-2	no	amb	none	big	hydro	veg	C3	no	cereal	0.04	0.04	3.79
DevTa7	high	TA	ent growth	nit	1-2	no	amb	none	big	hydro	veg	C3	no	cereal	0.02	0.04	2.16
Kham1Zm1	very low	ZM	> ½	nit	>1	yes	amb	none	small	sand	veg	C4	no	cereal	0.68	0.01	96.66
Kham1Zm2	low	ZM	> ½	nit	>1	yes	amb	none	small	sand	veg	C4	no	cereal	0.60	0.02	82.41
Kham1Zm3	medium	ZM	> ½	nit	>1	yes	amb	none	small	sand	veg	C4	no	cereal	0.19	0.03	21.09
RobGm1	low	GM	ent growth	nit + amm	1-2	yes	amb	none	medium	inert	veg	C3	non nod	oilseed	0.32	0.01	37.51
PugnHv1	low	HV	ent growth	nit	1-2	no	amb	none	small	inert	mat	C3	no	cereal	-1.18	0.01	-69.23

Table C. 6: List of effect sizes (lnR) and associated categorical variables for the response variable root-shoot ratio (RSR). Abbreviations as before.

study	N rate	species	length lim	N source	freq	pH	[CO2]	stress	pot	medium	dev	C3/C4	leg	crop	lnR	Var (lnR)	%change
CramTaNit	medium	TA	ent growth	nit	3-7	yes	amb	none	big	hydro	veg	C3	no	cereal	0.08	0.00	8.73
CramTaAm	medium	TA	ent growth	amm	3-7	yes	amb	none	big	hydro	veg	C3	no	cereal	0.01	0.01	0.72
CramZmNit	medium	ZM	ent growth	nit	3-7	yes	amb	none	big	hydro	veg	C4	no	cereal	0.07	0.01	7.29
CramZmAm	medium	ZM	ent growth	amm	3-7	yes	amb	none	big	hydro	veg	C4	no	cereal	0.22	0.00	24.45
RobGm1	low	GM	ent growth	nit + amm	1-2	yes	amb	none	medium	inert	veg	C3	non nod	oilseed	0.69	0.00	99.99
PugnHv1	low	HV	ent growth	nit	1-2	no	amb	none	small	inert	mat	C3	no	cereal	0.07	0.00	7.14
Chap2Hv1	very low	HV	< ½	nit	3-7	no	amb	none	small	hydro	veg	C3	no	cereal	0.92	0.03	150.00
DevTa1	very low	TA	ent growth	nit	1-2	no	amb	none	big	hydro	veg	C3	no	cereal	0.20	0.08	21.90
DevTa2	very low	TA	ent growth	nit	1-2	no	amb	none	big	hydro	veg	C3	no	cereal	0.26	0.09	30.26
DevTa3	very low	TA	ent growth	nit	1-2	no	amb	none	big	hydro	veg	C3	no	cereal	0.52	0.08	68.34
DevTa4	low	TA	ent growth	nit	1-2	no	amb	none	big	hydro	veg	C3	no	cereal	0.25	0.10	28.44
DevTa5	low	TA	ent growth	nit	1-2	no	amb	none	big	hydro	veg	C3	no	cereal	0.28	0.06	31.94
DevTa6	medium	TA	ent growth	nit	1-2	no	amb	none	big	hydro	veg	C3	no	cereal	0.01	0.08	0.52
DevTa7	high	TA	ent growth	nit	1-2	no	amb	none	big	hydro	veg	C3	no	cereal	-0.03	0.07	-3.17
Kham1Zm1	very low	ZM	> ½	nit	>1	yes	amb	none	small	sand	veg	C4	no	cereal	1.15	0.07	215.72
Kham1Zm2	low	ZM	> ½	nit	>1	yes	amb	none	small	sand	veg	C4	no	cereal	0.43	0.04	54.42
Kham1Zm3	medium	ZM	> ½	nit	>1	yes	amb	none	small	sand	veg	C4	no	cereal	0.14	0.04	14.92
Wong2GhA1	medium	GH	ent growth	nit	1-2	no	amb	none	medium	soil	veg	C3	no	fibre	0.11	0.01	11.23
Wong2GhA2	low	GH	ent growth	nit	1-2	no	amb	none	medium	soil	veg	C3	no	fibre	0.28	0.00	31.93
Wong2GhA3	very low	GH	ent growth	nit	1-2	no	amb	none	medium	soil	veg	C3	no	fibre	0.54	0.00	71.39
Wong2GhE1	medium	GH	ent growth	nit	1-2	no	elev	none	medium	soil	veg	C3	no	fibre	0.01	0.01	1.31
Wong2GhE2	low	GH	ent growth	nit	1-2	no	elev	none	medium	soil	veg	C3	no	fibre	0.42	0.00	52.56
Wong2GhE3	very low	GH	ent growth	nit	1-2	no	elev	none	medium	soil	veg	C3	no	fibre	0.88	0.01	141.94

Table C.7: List of effect sizes (lnR) and associated categorical variables for the response variable specific leaf area (SLA). Abbreviations as before.

study	N rate	species	length lim	N source	freq	pH	[CO2]	stress	pot	medium	dev	C3/C4	leg	crop	lnR	Var (lnR)	%change
Vos1St1	medium	ST	ent growth	?	<7	no	amb	none	big	sand	repr	C3	no	tuber	0.28	0.00	32.56
Vos2St1	medium	ST	ent growth	nit + amm	<7	no	amb	none	big	soil	veg	C3	no	tuber	0.02	0.00	2.11
Vos2St2	medium	ST	ent growth	nit + amm	<7	no	amb	none	big	soil	repr	C3	no	tuber	-0.04	0.00	-4.36
Vos2St3	medium	ST	ent growth	nit + amm	<7	no	amb	none	big	soil	repr	C3	no	tuber	-0.10	0.00	-9.42
RobGm1	low	GM	ent growth	nit + amm	1-2	yes	amb	none	medium	inert	veg	C3	non nod	oilseed	-0.19	0.00	-17.51
PugnHv1	low	HV	ent growth	nit	1-2	no	amb	none	small	inert	mat	C3	no	cereal	0.27	0.01	31.22
Wong2GhA1	medium	GH	ent growth	nit	1-2	no	amb	none	medium	soil	veg	C3	no	fibre	-0.05	0.00	-5.22
Wong2GhA2	low	GH	ent growth	nit	1-2	no	amb	none	medium	soil	veg	C3	no	fibre	-0.16	0.00	-14.79
Wong2GhA3	very low	GH	ent growth	nit	1-2	no	amb	none	medium	soil	veg	C3	no	fibre	-0.47	0.00	-37.34
Wong2GhE1	medium	GH	ent growth	nit	1-2	no	elev	none	medium	soil	veg	C3	no	fibre	-0.02	0.00	-2.44
Wong2GhE2	low	GH	ent growth	nit	1-2	no	elev	none	medium	soil	veg	C3	no	fibre	-0.11	0.00	-10.77
Wong2GhE3	very low	GH	ent growth	nit	1-2	no	elev	none	medium	soil	veg	C3	no	fibre	-0.27	0.00	-23.65
Chap2Hv1	very low	HV	< ½	nit	3-7	no	amb	none	small	hydro	veg	C3	no	cereal	0.19	0.00	20.78
NakamGm1E	very low	GM	ent growth	amm	?	no	elev	none	small	soil	repr	C3	nod	oilseed	0.13	0.00	14.17
NakamGm1A	very low	GM	ent growth	amm	?	no	amb	none	small	soil	repr	C3	nod	oilseed	0.08	0.01	8.09
NakamGm2E	very low	GM	ent growth	amm	?	no	elev	none	small	soil	repr	C3	non nod	oilseed	-0.02	0.00	-1.80
NakamGm2A	very low	GM	ent growth	amm	?	no	amb	none	small	soil	repr	C3	non nod	oilseed	-0.04	0.00	-3.77

Table C.8: List of effect sizes (lnR) and associated categorical variables for the response variable leaf sugar content (Sug_L). Abbreviations: OS: *Oryza sativa*. Further abbreviations as before.

unit	study	N rate	species	length lim	N source	freq	pH	[CO2]	stress	pot	medium	dev	C3/C4	leg	crop	lnR	Var (lnR)	%change
weight	CaputTa1	very low	TA	ent growth	nit	1-2	no	amb	none	small	sand	seedl	C3	no	cereal	1.44	0.01	320.17
weight	CaputTa2	low	TA	ent growth	nit	1-2	no	amb	none	small	sand	seedl	C3	no	cereal	0.58	0.02	77.82
weight	CaputTa3	low	TA	ent growth	nit	1-2	no	amb	none	small	sand	seedl	C3	no	cereal	-0.26	0.10	-23.12
weight	CaputTa4	medium	TA	ent growth	nit	1-2	no	amb	none	small	sand	seedl	C3	no	cereal	-0.10	0.02	-9.88
weight	CaputTa5	high	TA	ent growth	nit	1-2	no	amb	none	small	sand	seedl	C3	no	cereal	-0.17	0.13	-15.32
weight	GuitTa1	very low	TA	ent growth	nit + amm	3-7	no	amb	none	medium	soil	mat	C3	no	cereal	-0.32	0.00	-27.66
weight	Kham1Zm1	very low	ZM	< ½	nit	>1	yes	amb	none	small	sand	veg	C4	no	cereal	0.11	0.07	11.58
weight	Kham1Zm2	low	ZM	< ½	nit	>1	yes	amb	none	small	sand	veg	C4	no	cereal	0.17	0.06	18.76
weight	Kham1Zm3	medium	ZM	< ½	nit	>1	yes	amb	none	small	sand	veg	C4	no	cereal	0.08	0.05	8.27
area	NakanOsA1	very low	OS	< ½	nit + amm	3-7	yes	amb	none	medium	hydro	veg	C3	no	cereal	-0.48	0.05	-38.29
area	NakanOsA2	low	OS	< ½	nit + amm	3-7	yes	amb	none	medium	hydro	veg	C3	no	cereal	-0.04	0.04	-3.46
area	NakanOsE1	very low	OS	< ½	nit + amm	3-7	yes	elev	none	medium	hydro	veg	C3	no	cereal	-0.69	0.02	-50.05
area	NakanOsE2	low	OS	< ½	nit + amm	3-7	yes	elev	none	medium	hydro	veg	C3	no	cereal	-0.21	0.01	-19.21
area	RobGm1	low	GM	ent growth	nit + amm	1-2	yes	amb	none	medium	inert	veg	C3	non nod	oilseed	0.26	0.01	29.05

Table C.9: List of effect sizes (lnR) and associated categorical variables for the response variable leaf starch content ($Stch_L$). Abbreviations as before.

unit	study	N rate	species	length lim	N source	freq	pH	[CO2]	stress	pot	medium	dev	C3/C4	leg	crop	lnR	Var (lnR)	%change
area	RobGm1	low	GM	ent growth	nit + amm	1-2	yes	amb	none	medium	inert	veg	C3	non nod	oilseed	0.58	0.01	78.28
weight	Kham1Zm1	very low	ZM	< ½	nit	>1	yes	amb	none	small	sand	veg	C4	no	cereal	1.39	0.24	303.13
weight	Kham1Zm2	low	ZM	< ½	nit	>1	yes	amb	none	small	sand	veg	C4	no	cereal	0.82	0.25	127.66
weight	Kham1Zm3	medium	ZM	< ½	nit	>1	yes	amb	none	small	sand	veg	C4	no	cereal	0.35	0.27	42.50
area	NakanOsA1	very low	OS	< ½	nit + amm	3-7	yes	amb	none	medium	hydro	veg	C3	no	cereal	0.69	0.14	99.11
area	NakanOsA2	low	OS	< ½	nit + amm	3-7	yes	amb	none	medium	hydro	veg	C3	no	cereal	0.38	0.26	46.43
area	NakanOsE1	very low	OS	< ½	nit + amm	3-7	yes	elev	none	medium	hydro	veg	C3	no	cereal	1.11	0.12	204.87
area	NakanOsE2	low	OS	< ½	nit + amm	3-7	yes	elev	none	medium	hydro	veg	C3	no	cereal	1.05	0.19	184.51

Table C.10: List of effect sizes (lnR) and associated categorical variables for the response variable non-structural carbohydrate content (NSC_L). Abbreviations as before.

unit	study	N rate	species	length lim	N source	freq	pH	[CO2]	stress	pot	medium	dev	C3/C4	leg	crop	lnR	Var (lnR)	%change
weight	Wong2GhA1	medium	GH	ent growth	nit	1-2	no	amb	none	medium	soil	veg	C3	no	fibre	1.02	0.03	177.51
weight	Wong2GhA2	low	GH	ent growth	nit	1-2	no	amb	none	medium	soil	veg	C3	no	fibre	1.65	0.03	420.02
weight	Wong2GhA3	very low	GH	ent growth	nit	1-2	no	amb	none	medium	soil	veg	C3	no	fibre	2.22	0.02	824.98
weight	Wong2GhE1	medium	GH	ent growth	nit	1-2	no	elev	none	medium	soil	veg	C3	no	fibre	0.05	0.02	5.42
weight	Wong2GhE2	low	GH	ent growth	nit	1-2	no	elev	none	medium	soil	veg	C3	no	fibre	0.09	0.01	9.82
weight	Wong2GhE3	very low	GH	ent growth	nit	1-2	no	elev	none	medium	soil	veg	C3	no	fibre	0.29	0.00	33.34
area	RobGm1	low	GM	ent growth	nit + amm	1-2	yes	amb	none	medium	inert	veg	C3	non nod	oilseed	0.56	0.01	75.93
area	NakanOsA1	very low	OS	< ½	nit + amm	3-7	yes	amb	none	medium	hydro	veg	C3	no	cereal	-0.14	0.04	-12.77
area	NakanOsA2	low	OS	< ½	nit + amm	3-7	yes	amb	none	medium	hydro	veg	C3	no	cereal	0.06	0.04	5.80
area	NakanOsE1	very low	OS	< ½	nit + amm	3-7	yes	elev	none	medium	hydro	veg	C3	no	cereal	0.01	0.03	0.61
area	NakanOsE2	low	OS	< ½	nit + amm	3-7	yes	elev	none	medium	hydro	veg	C3	no	cereal	0.19	0.04	21.29
weight	Kham1Zm1	very low	ZM	< ½	nit	>1	yes	amb	none	small	sand	veg	C4	no	cereal	0.83	0.07	129.03
weight	Kham1Zm2	low	ZM	< ½	nit	>1	yes	amb	none	small	sand	veg	C4	no	cereal	0.49	0.07	62.63
weight	Kham1Zm3	medium	ZM	< ½	nit	>1	yes	amb	none	small	sand	veg	C4	no	cereal	0.20	0.07	22.07

Table C.11: List of effect sizes (lnR) and associated categorical variables for the response variable relative growth rate (RGR). Abbreviations as before.

study	N rate	species	length lim	N source	freq	pH	[CO2]	stress	pot	medium	dev	C3/C4	leg	crop	lnR	Var (lnR)	%change
Wong2GhA1	medium	GH	ent growth	nit	1-2	no	amb	none	medium	soil	veg	C3	no	fibre	0	0.62	0
Wong2GhA2	low	GH	ent growth	nit	1-2	no	amb	none	medium	soil	veg	C3	no	fibre	0.14	0.61	15.38
Wong2GhA3	very low	GH	ent growth	nit	1-2	no	amb	none	medium	soil	veg	C3	no	fibre	-0.96	0.63	-61.54
Wong2GhE1	medium	GH	ent growth	nit	1-2	no	elev	none	medium	soil	veg	C3	no	fibre	0.54	0.05	71.43
Wong2GhE2	low	GH	ent growth	nit	1-2	no	elev	none	medium	soil	veg	C3	no	fibre	0.36	0.03	42.86
Wong2GhE3	very low	GH	ent growth	nit	1-2	no	elev	none	medium	soil	veg	C3	no	fibre	-0.34	0.06	-28.57
PugnHvW+1	low	HV	ent growth	nit	1-2	no	amb	none	small	inert	mat	C3	no	cereal	-1.10	0.42	-66.67
PugnHvW-1	low	HV	ent growth	nit	1-2	no	amb	yes	small	inert	mat	C3	no	cereal	-1.20	0.67	-69.84
Rad1Gh1	very low	GH	ent growth	nit	?	no	amb	none	?	soil	veg	C3	no	fibre	-0.49	0.01	-38.89
Rad1Hv1	very low	HV	ent growth	nit	?	no	amb	none	?	soil	veg	C3	no	cereal	-0.29	0.03	-25.00
FrHv1	low	HV	ent growth	nit + amm	<1	no	amb	none	medium	hydro	mat	C3	no	cereal	-0.15	0.00	-14.29
FrHv2	very low	HV	ent growth	nit + amm	<1	no	amb	none	medium	hydro	mat	C3	no	cereal	-0.81	0.00	-55.50

Table C.12: List of effect sizes (lnR) and associated categorical variables for the response variable leaf N content (N_L). Abbreviations as before.

unit	study	N rate	species	length lim	N source	freq	pH	[CO2]	stress	pot	medium	dev	C3/C4	leg	crop	lnR	Var (lnR)	%change
weight	NakamGm1E	very low	GM	ent growth	amm	?	no	elev	none	small	soil	repr	C3	nod	oilseed	-0.10	0.00	-9.21
weight	NakamGm1A	very low	GM	ent growth	amm	?	no	amb	none	small	soil	repr	C3	nod	oilseed	-0.60	0.00	-45.14
weight	NakamGm2E	very low	GM	ent growth	amm	?	no	elev	none	small	soil	repr	C3	non nod	oilseed	-1.24	0.01	-70.97
weight	NakamGm2A	very low	GM	ent growth	amm	?	no	amb	none	small	soil	repr	C3	non nod	oilseed	-1.18	0.00	-69.16
area	NakanOsA1	very low	OS	< ½	nit + amm	3-7	yes	amb	none	medium	hydro	veg	C3	no	cereal	-0.56	0.02	-42.98
area	NakanOsA2	low	OS	< ½	nit + amm	3-7	yes	amb	none	medium	hydro	veg	C3	no	cereal	-0.32	0.01	-27.37
area	NakanOsE1	very low	OS	< ½	nit + amm	3-7	yes	elev	none	medium	hydro	veg	C3	no	cereal	-0.76	0.02	-53.27
area	NakanOsE2	low	OS	< ½	nit + amm	3-7	yes	elev	none	medium	hydro	veg	C3	no	cereal	-0.45	0.02	-36.06
weight	GuitTa1	very low	TA	ent growth	nit + amm	3-7	no	amb	none	medium	soil	mat	C3	no	cereal	-0.94	0.03	-60.98
area	EvTa1	very low	TA	ent growth	nit	3-7	no	amb	none	medium	soil	-	C3	no	cereal	-0.51	0.10	-40.00
area	EvTa2	very low	TA	ent growth	nit	3-7	no	amb	none	medium	soil	-	C3	no	cereal	-0.45	0.09	-36.29
area	EvTa3	very low	TA	ent growth	nit	3-7	no	amb	none	medium	soil	-	C3	no	cereal	-0.50	0.10	-39.26
area	EvTa4	low	TA	ent growth	nit	3-7	no	amb	none	medium	soil	-	C3	no	cereal	-0.10	0.05	-9.63
area	Vos2St1	medium	ST	ent growth	nit + amm	<7	no	amb	none	big	soil	veg	C3	no	tuber	-0.31	0.00	-26.72
area	Vos2St2	medium	ST	ent growth	nit + amm	<7	no	amb	none	big	soil	repr	C3	no	tuber	-0.21	0.00	-19.25
area	Vos2St3	medium	ST	ent growth	nit + amm	<7	no	amb	none	big	soil	repr	C3	no	tuber	-0.17	0.01	-15.56
area	Wong2GhA1	medium	GH	ent growth	nit	1-2	no	amb	none	medium	soil	veg	C3	no	fibre	-0.07	0.00	-6.57
area	Wong2GhA2	low	GH	ent growth	nit	1-2	no	amb	none	medium	soil	veg	C3	no	fibre	-0.47	0.00	-37.31
area	Wong2GhA3	very low	GH	ent growth	nit	1-2	no	amb	none	medium	soil	veg	C3	no	fibre	-0.64	0.00	-47.18
area	Wong2GhE1	medium	GH	ent growth	nit	1-2	no	elev	none	medium	soil	veg	C3	no	fibre	-0.16	0.00	-14.56
area	Wong2GhE2	low	GH	ent growth	nit	1-2	no	elev	none	medium	soil	veg	C3	no	fibre	-0.42	0.00	-34.58
area	Wong2GhE3	very low	GH	ent growth	nit	1-2	no	elev	none	medium	soil	veg	C3	no	fibre	-0.73	0.00	-51.77
weight	PugnHv1	low	HV	ent growth	nit	1-2	no	amb	none	small	inert	mat	C3	no	cereal	-0.17	0.03	-15.22
area	PugnHv1	low	HV	ent growth	nit	1-2	no	amb	none	small	inert	mat	C3	no	cereal	-0.57	0.06	-43.66

Table C.13: List of effect sizes (lnR) and associated categorical variables for the response variable grain N content (N_G). Abbreviations as before.

study	N rate	species	length lim	N source	freq	pH	[CO2]	stress	pot	medium	dev	C3/C4	leg	crop	lnR	Var (lnR)	%change
GuitTa1	very low	TA	ent growth	nit + amm	3-7	no	amb	none	medium	soil	mat	C3	no	cereal	-0.30	0.01	-25.85
PugnHv1	low	HV	ent growth	nit	1-2	no	amb	none	small	inert	mat	C3	no	cereal	-0.48	0.00	-38.20
BarTa1_1	very low	TA	< ½	nit + amm	1-2	no	amb	none	medium	inert	mat	C3	no	cereal	-0.09	0.00	-8.72
BarTa1_2	low	TA	ent growth	nit + amm	1-2	no	amb	none	medium	inert	mat	C3	no	cereal	-0.19	0.00	-17.43
BarTa1_3	very low	TA	ent growth	nit + amm	1-2	no	amb	none	medium	inert	mat	C3	no	cereal	-0.31	0.00	-26.55
BarTa2L	very low	TA	< ½	nit + amm	1-2	no	amb	none	medium	inert	mat	C3	no	cereal	-0.36	0.01	-30.48
BarTa2S	very low	TA	< ½	nit + amm	1-2	no	amb	light	medium	inert	mat	C3	no	cereal	-0.08	0.00	-7.97
BarTa3L	very low	TA	< ½	nit + amm	1-2	no	amb	none	medium	inert	mat	C3	no	cereal	-0.20	0.00	-17.81
BarTa3S	very low	TA	< ½	nit + amm	1-2	no	amb	light	medium	inert	mat	C3	no	cereal	-0.18	0.00	-16.46
BarTa4	very low	TA	< ½	nit + amm	1-2	no	amb	none	medium	soil	mat	C3	no	cereal	-0.23	0.00	-20.36

Table C.14: List of effect sizes (lnR) and associated categorical variables for the response variable whole plant N content (N_T). Abbreviations as before.

study	N rate	species	length lim	N source	freq	pH	[CO2]	stress	pot	medium	dev	C3/C4	leg	crop	lnR	Var (lnR)	%change
DevTa1	very low	TA	ent growth	nit	1-2	no	amb	none	big	hydro	veg	C3	no	cereal	-0.08	0.00	-7.87
DevTa2	very low	TA	ent growth	nit	1-2	no	amb	none	big	hydro	veg	C3	no	cereal	-0.10	0.00	-9.83
DevTa3	very low	TA	ent growth	nit	1-2	no	amb	none	big	hydro	veg	C3	no	cereal	-0.04	0.00	-4.10
DevTa4	low	TA	ent growth	nit	1-2	no	amb	none	big	hydro	veg	C3	no	cereal	-0.07	0.00	-6.82
DevTa5	low	TA	ent growth	nit	1-2	no	amb	none	big	hydro	veg	C3	no	cereal	-0.03	0.00	-2.64
DevTa6	medium	TA	ent growth	nit	1-2	no	amb	none	big	hydro	veg	C3	no	cereal	-0.04	0.00	-3.84
DevTa7	high	TA	ent growth	nit	1-2	no	amb	none	big	hydro	veg	C3	no	cereal	-0.02	0.00	-2.00
GuitTa1	very low	TA	ent growth	nit + amm	3-7	no	amb	none	medium	soil	mat	C3	no	cereal	-0.38	0.00	-31.50
CaputTa1	very low	TA	ent growth	nit	1-2	no	amb	none	small	sand	seedl	C3	no	cereal	-1.41	0.01	-75.59
CaputTa2	low	TA	ent growth	nit	1-2	no	amb	none	small	sand	seedl	C3	no	cereal	-0.53	0.03	-41.35
CaputTa3	low	TA	ent growth	nit	1-2	no	amb	none	small	sand	seedl	C3	no	cereal	-0.31	0.00	-26.77
CaputTa4	medium	TA	ent growth	nit	1-2	no	amb	none	small	sand	seedl	C3	no	cereal	-0.12	0.02	-10.93
CaputTa5	high	TA	ent growth	nit	1-2	no	amb	none	small	sand	seedl	C3	no	cereal	0.00	0.01	-0.36
Wo1GhE1	medium	GH	ent growth	nit	1-2	no	elev	none	medium	soil	veg	C3	no	fibre	-0.52	0.02	-40.56
Wo1GhE2	low	GH	ent growth	nit	1-2	no	elev	none	medium	soil	veg	C3	no	fibre	-0.91	0.02	-59.84
Wo1GhE3	very low	GH	ent growth	nit	1-2	no	elev	none	medium	soil	veg	C3	no	fibre	-1.20	0.02	-69.88
Wo1GhA1	medium	GH	ent growth	nit	1-2	no	amb	none	medium	soil	veg	C3	no	fibre	-0.24	0.00	-21.12
Wo1GhA2	low	GH	ent growth	nit	1-2	no	amb	none	medium	soil	veg	C3	no	fibre	-0.58	0.01	-43.90
Wo1GhA3	very low	GH	ent growth	nit	1-2	no	amb	none	medium	soil	veg	C3	no	fibre	-1.07	0.01	-65.73
Wo1ZmE1	medium	ZM	ent growth	nit	1-2	no	elev	none	medium	soil	veg	C4	no	cereal	-0.28	0.00	-24.78
Wo1ZmE2	low	ZM	ent growth	nit	1-2	no	elev	none	medium	soil	veg	C4	no	cereal	-0.70	0.00	-50.15
Wo1ZmE3	very low	ZM	ent growth	nit	1-2	no	elev	none	medium	soil	veg	C4	no	cereal	-1.16	0.01	-68.51
Wo1ZmA1	medium	ZM	ent growth	nit	1-2	no	amb	none	medium	soil	veg	C4	no	cereal	-0.21	0.01	-18.75
Wo1ZmA2	low	ZM	ent growth	nit	1-2	no	amb	none	medium	soil	veg	C4	no	cereal	-0.67	0.00	-48.86
Wo1ZmA3	very low	ZM	ent growth	nit	1-2	no	amb	none	medium	soil	veg	C4	no	cereal	-1.00	0.01	-63.35

Table C.15: List of effect sizes (lnR) and associated categorical variables for the response variable leaf nitrate content (Nit_L). Abbreviations as before.

unit	study	N rate	species	length lim	N source	freq	pH	[CO2]	stress	pot	medium	dev	C3/C4	leg	crop	lnR	Var (lnR)	%change
weight	Chap2Hv1	very low	HV	< ½	nit	3-7	no	amb	none	small	hydro	veg	C3	no	cereal	-2.60	0.02	-92.57
weight	Kham1Zm1	very low	ZM	< ½	nit	>1	yes	amb	none	small	sand	veg	C4	no	cereal	-3.33	2.29	-96.43
weight	Kham1Zm2	low	ZM	< ½	nit	>1	yes	amb	none	small	sand	veg	C4	no	cereal	-1.52	0.16	-78.07
weight	Kham1Zm3	medium	ZM	< ½	nit	>1	yes	amb	none	small	sand	veg	C4	no	cereal	-0.43	0.08	-34.82
area	Vos2St1	medium	ST	ent growth	nit + amm	<7	no	amb	none	big	soil	veg	C3	no	tuber	-1.13	2.00	-67.57
area	Vos2St2	medium	ST	ent growth	nit + amm	<7	no	amb	none	big	soil	repr	C3	no	tuber	-1.32	2.00	-73.22
area	Vos2St3	medium	ST	ent growth	nit + amm	<7	no	amb	none	big	soil	repr	C3	no	tuber	no effect size could be calculated		
weight	Kham2Zm1	very low	ZM	> ½	nit	>1	no	amb	none	medium	sand	veg	C4	no	cereal	-3.16	0.02	-95.74
weight	Kham2Zm2	very low	ZM	< ½	nit	>1	no	amb	none	medium	sand	veg	C4	no	cereal	-3.16	0.01	-95.74
weight	Kham2Zm3	very low	ZM	< ½	nit	>1	no	amb	none	medium	sand	veg	C4	no	cereal	-3.85	0.02	-97.87

Table C.16: List of effect sizes (lnR) and associated categorical variables for the response variable root nitrate content (Nit_R). Abbreviations as before.

study	N rate	species	length lim	N source	freq	pH	[CO2]	stress	pot	medium	dev	C3/C4	leg	crop	lnR	Var (lnR)	R	%change
Chap2Hv1	very low	HV	< ½	nit	3-7	no	amb	none	small	hydro	veg	C3	no	cereal	-2.23	0.04	0.11	-89.20
DevTa1	very low	TA	ent growth	nit	1-2	no	amb	none	big	hydro	veg	C3	no	cereal	0.33	0.00	1.39	38.76
DevTa2	very low	TA	ent growth	nit	1-2	no	amb	none	big	hydro	veg	C3	no	cereal	0.16	0.00	1.18	17.68
DevTa3	very low	TA	ent growth	nit	1-2	no	amb	none	big	hydro	veg	C3	no	cereal	-0.12	0.00	0.89	-11.20
DevTa4	low	TA	ent growth	nit	1-2	no	amb	none	big	hydro	veg	C3	no	cereal	0.34	0.00	1.40	40.30
DevTa5	low	TA	ent growth	nit	1-2	no	amb	none	big	hydro	veg	C3	no	cereal	0.06	0.00	1.06	6.04
DevTa6	medium	TA	ent growth	nit	1-2	no	amb	none	big	hydro	veg	C3	no	cereal	-0.06	0.00	0.94	-5.59
DevTa7	high	TA	ent growth	nit	1-2	no	amb	none	big	hydro	veg	C3	no	cereal	-0.01	0.00	0.99	-1.37
Kham1Zm1	very low	ZM	< ½	nit	>1	yes	amb	none	small	sand	veg	C4	no	cereal	-2.87	0.05	0.06	-94.34
Kham1Zm2	low	ZM	< ½	nit	>1	yes	amb	none	small	sand	veg	C4	no	cereal	-1.67	0.39	0.19	-81.18
Kham1Zm3	medium	ZM	< ½	nit	>1	yes	amb	none	small	sand	veg	C4	no	cereal	-0.47	0.06	0.63	-37.29
Sid1Hv1	very low	HV	> ½	nit	>1	no	amb	none	big	hydro	seedl	C3	no	cereal	-0.73	0.02	0.48	-51.83
Sid1Hv2	very low	HV	< ½	nit	>1	no	amb	none	big	hydro	seedl	C3	no	cereal	-0.22	0.02	0.81	-19.44
Sid1Hv3	low	HV	< ½	nit	>1	no	amb	none	big	hydro	seedl	C3	no	cereal	-0.17	0.02	0.85	-15.41

Table C.17: List of effect sizes (lnR) and associated categorical variables for the response variable whole plant nitrate content (Nit_T). Abbreviations as before.

study	N rate	species	length lim	N source	freq	pH	[CO2]	stress	pot	medium	dev	C3/C4	leg	crop	lnR	Var (lnR)	%change
CaputTa1	very low	TA	ent growth	nit	1-2	no	amb	none	small	sand	seedl	C3	no	cereal	-2.07	0.32	-87.34
CaputTa2	low	TA	ent growth	nit	1-2	no	amb	none	small	sand	seedl	C3	no	cereal	-1.86	0.08	-84.39
CaputTa3	low	TA	ent growth	nit	1-2	no	amb	none	small	sand	seedl	C3	no	cereal	-1.42	0.04	-75.93
CaputTa4	medium	TA	ent growth	nit	1-2	no	amb	none	small	sand	seedl	C3	no	cereal	-0.40	0.02	-32.77
CaputTa5	high	TA	ent growth	nit	1-2	no	amb	none	small	sand	seedl	C3	no	cereal	-0.02	0.01	-2.25
DevTa1	very low	TA	ent growth	nit	1-2	no	amb	none	big	hydro	veg	C3	no	cereal	0.07	0.00	6.94
DevTa2	very low	TA	ent growth	nit	1-2	no	amb	none	big	hydro	veg	C3	no	cereal	0.02	0.00	2.35
DevTa3	very low	TA	ent growth	nit	1-2	no	amb	none	big	hydro	veg	C3	no	cereal	-0.06	0.00	-5.37
DevTa4	low	TA	ent growth	nit	1-2	no	amb	none	big	hydro	veg	C3	no	cereal	0.21	0.00	23.32
DevTa5	low	TA	ent growth	nit	1-2	no	amb	none	big	hydro	veg	C3	no	cereal	0.33	0.00	39.06
DevTa6	medium	TA	ent growth	nit	1-2	no	amb	none	big	hydro	veg	C3	no	cereal	-0.17	0.00	-15.23
DevTa7	high	TA	ent growth	nit	1-2	no	amb	none	big	hydro	veg	C3	no	cereal	-0.13	0.00	-11.84
Kham1Zm1	very low	ZM	< ½	nit	>1	yes	amb	none	small	sand	veg	C4	no	cereal	-3.10	0.48	-95.51
Kham1Zm2	low	ZM	< ½	nit	>1	yes	amb	none	small	sand	veg	C4	no	cereal	-1.58	0.12	-79.44
Kham1Zm3	medium	ZM	< ½	nit	>1	yes	amb	none	small	sand	veg	C4	no	cereal	-0.44	0.04	-35.91

Table C.18: List of effect sizes (lnR) and associated categorical variables for the response variable leaf free amino acid content (AA_L). Abbreviations as before.

study	N rate	species	length lim	N source	freq	pH	[CO2]	stress	pot	medium	dev	C3/C4	leg	crop	lnR	Var (lnR)	%change
CaputTa1	very low	TA	ent growth	nit	1-2	no	amb	none	small	sand	seedl	C3	no	cereal	-1.47	0.03	-76.97
CaputTa2	low	TA	ent growth	nit	1-2	no	amb	none	small	sand	seedl	C3	no	cereal	-0.51	0.03	-39.66
CaputTa3	low	TA	ent growth	nit	1-2	no	amb	none	small	sand	seedl	C3	no	cereal	-0.17	0.01	-15.95
CaputTa4	medium	TA	ent growth	nit	1-2	no	amb	none	small	sand	seedl	C3	no	cereal	-0.04	0.01	-4.20
CaputTa5	high	TA	ent growth	nit	1-2	no	amb	none	small	sand	seedl	C3	no	cereal	0.00	0.01	0.13
GuitTa1	very low	TA	ent growth	nit + amm	3-7	no	amb	none	medium	soil	repr	C3	no	cereal	-1.71	0.09	-81.90
Kham1Zm1	very low	ZM	< ½	nit	>1	yes	amb	none	small	sand	veg	C4	no	cereal	-0.38	0.01	-31.27
Kham1Zm2	low	ZM	< ½	nit	>1	yes	amb	none	small	sand	veg	C4	no	cereal	0.11	0.00	11.19
Kham1Zm3	medium	ZM	< ½	nit	>1	yes	amb	none	small	sand	veg	C4	no	cereal	0.08	0.00	8.11
Chap2Hv1	very low	HV	< ½	nit	3-7	no	amb	none	small	hydro	veg	C3	no	cereal	-0.63	0.00	-46.54

Table C.19: List of effect sizes (lnR) and associated categorical variables for the response variable leaf soluble protein content ($Prot_L$). Abbreviations as before.

unit	study	N rate	species	length lim	N source	freq	pH	[CO2]	stress	pot	medium	dev	C3/C4	leg	crop	lnR	Var (lnR)	%change
weight	GuitTa1	very low	TA	ent growth	nit + amm	3-7	no	amb	none	medium	soil	repr	C3	no	cereal	-1.99	0.32	-86.37
weight	Kham1Zm1	very low	ZM	< ½	nit	>1	yes	amb	none	small	sand	veg	C4	no	cereal	-0.95	0.04	-61.24
weight	Kham1Zm2	low	ZM	< ½	nit	>1	yes	amb	none	small	sand	veg	C4	no	cereal	-0.31	0.01	-26.29
weight	Kham1Zm3	medium	ZM	< ½	nit	>1	yes	amb	none	small	sand	veg	C4	no	cereal	-0.02	0.01	-1.86
area	RobGm1	low	GM	ent growth	nit + amm	1-2	yes	amb	none	medium	inert	veg	C3	non nod	oilseed	-0.42	0.00	-34.05
area	Wo1GhE1	medium	GH	ent growth	nit	1-2	no	elev	none	medium	soil	veg	C3	no	fibre	-0.32	0.01	-27.17
area	Wo1GhE2	low	GH	ent growth	nit	1-2	no	elev	none	medium	soil	veg	C3	no	fibre	-0.54	0.01	-41.85
area	Wo1GhE3	very low	GH	ent growth	nit	1-2	no	elev	none	medium	soil	veg	C3	no	fibre	-0.55	0.02	-42.37
area	Wo1GhA1	medium	GH	ent growth	nit	1-2	no	amb	none	medium	soil	veg	C3	no	fibre	-0.17	0.01	-15.50
area	Wo1GhA2	low	GH	ent growth	nit	1-2	no	amb	none	medium	soil	veg	C3	no	fibre	-0.39	0.01	-32.40
area	Wo1GhA3	very low	GH	ent growth	nit	1-2	no	amb	none	medium	soil	veg	C3	no	fibre	-0.66	0.01	-48.55
area	Wo1ZmE1	medium	ZM	ent growth	nit	1-2	no	elev	none	medium	soil	veg	C4	no	cereal	-0.09	0.01	-9.00
area	Wo1ZmE2	low	ZM	ent growth	nit	1-2	no	elev	none	medium	soil	veg	C4	no	cereal	-0.28	0.01	-24.63
area	Wo1ZmE3	very low	ZM	ent growth	nit	1-2	no	elev	none	medium	soil	veg	C4	no	cereal	-0.37	0.01	-30.85
area	Wo1ZmA1	medium	ZM	ent growth	nit	1-2	no	amb	none	medium	soil	veg	C4	no	cereal	-0.23	0.00	-20.30
area	Wo1ZmA2	low	ZM	ent growth	nit	1-2	no	amb	none	medium	soil	veg	C4	no	cereal	-0.20	0.00	-18.38
area	Wo1ZmA3	very low	ZM	ent growth	nit	1-2	no	amb	none	medium	soil	veg	C4	no	cereal	-0.36	0.01	-30.07

Table C.20: List of effect sizes (lnR) and associated categorical variables for the response variable leaf Chl content (Chl). Abbreviations as before.

unit	study	N rate	species	length lim	N source	freq	pH	[CO2]	stress	pot	medium	dev	C3/C4	leg	crop	lnR	Var (lnR)	%change
weight	GuitTa1	very low	TA	ent growth	nit + amm	3-7	no	amb	none	medium	soil	repr	C3	no	cereal	-1.25	0.11	-71.36
weight	Kham1Zm1	very low	ZM	< ½	nit	>1	yes	amb	none	small	sand	veg	C4	no	cereal	-0.18	0.03	-16.66
weight	Kham1Zm2	low	ZM	< ½	nit	>1	yes	amb	none	small	sand	veg	C4	no	cereal	0.05	0.01	5.56
weight	Kham1Zm3	medium	ZM	< ½	nit	>1	yes	amb	none	small	sand	veg	C4	no	cereal	0.10	0.01	10.55
area	NakanOsA1	very low	OS	< ½	nit + amm	3-7	yes	amb	none	medium	hydro	veg	C3	no	cereal	-0.59	0.01	-44.58
area	NakanOsA2	low	OS	< ½	nit + amm	3-7	yes	amb	none	medium	hydro	veg	C3	no	cereal	-0.36	0.01	-30.12
area	NakanOsE1	very low	OS	< ½	nit + amm	3-7	yes	elev	none	medium	hydro	veg	C3	no	cereal	-0.79	0.02	-54.55
area	NakanOsE2	low	OS	< ½	nit + amm	3-7	yes	elev	none	medium	hydro	veg	C3	no	cereal	-0.43	0.02	-35.07
area	EvTa1	very low	TA	ent growth	nit	3-7	no	amb	none	medium	soil	-	C3	no	cereal	-0.54	0.00	-41.93
area	EvTa2	very low	TA	ent growth	nit	3-7	no	amb	none	medium	soil	-	C3	no	cereal	-0.39	0.01	-32.26
area	EvTa3	very low	TA	ent growth	nit	3-7	no	amb	none	medium	soil	-	C3	no	cereal	-0.39	0.01	-32.26
area	EvTa4	low	TA	ent growth	nit	3-7	no	amb	none	medium	soil	-	C3	no	cereal	-0.18	0.00	-16.13
area	RobGm1	low	GM	ent growth	nit + amm	1-2	yes	amb	none	medium	inert	veg	C3	non nod	oilseed	-0.33	0.00	-27.98
area	Wo1GhE1	medium	GH	ent growth	nit	1-2	no	elev	none	medium	soil	veg	C3	no	fibre	-0.22	0.00	-19.97
area	Wo1GhE2	low	GH	ent growth	nit	1-2	no	elev	none	medium	soil	veg	C3	no	fibre	-0.36	0.00	-30.04
area	Wo1GhE3	very low	GH	ent growth	nit	1-2	no	elev	none	medium	soil	veg	C3	no	fibre	-0.53	0.01	-40.84
area	Wo1GhA1	medium	GH	ent growth	nit	1-2	no	amb	none	medium	soil	veg	C3	no	fibre	-0.15	0.01	-13.59
area	Wo1GhA2	low	GH	ent growth	nit	1-2	no	amb	none	medium	soil	veg	C3	no	fibre	-0.24	0.01	-21.38
area	Wo1GhA3	very low	GH	ent growth	nit	1-2	no	amb	none	medium	soil	veg	C3	no	fibre	-0.45	0.00	-36.43
area	Wo1ZmE1	medium	ZM	ent growth	nit	1-2	no	elev	none	medium	soil	veg	C4	no	cereal	-0.17	0.00	-15.77
area	Wo1ZmE2	low	ZM	ent growth	nit	1-2	no	elev	none	medium	soil	veg	C4	no	cereal	-0.59	0.00	-44.61
area	Wo1ZmE3	very low	ZM	ent growth	nit	1-2	no	elev	none	medium	soil	veg	C4	no	cereal	-1.18	0.00	-69.21
area	Wo1ZmA1	medium	ZM	ent growth	nit	1-2	no	amb	none	medium	soil	veg	C4	no	cereal	-0.12	0.00	-10.93
area	Wo1ZmA2	low	ZM	ent growth	nit	1-2	no	amb	none	medium	soil	veg	C4	no	cereal	-0.51	0.00	-39.70
area	Wo1ZmA3	very low	ZM	ent growth	nit	1-2	no	amb	none	medium	soil	veg	C4	no	cereal	-0.78	0.01	-54.13

Table C.21: List of effect sizes (lnR) and associated categorical variables for the response variable Rubisco activity (Rub). Abbreviations as before.

study	N rate	species	length lim	N source	freq	pH	[CO2]	stress	pot	medium	dev	C3/C4	leg	crop	lnR	Var (lnR)	%change
EvTa1	very low	TA	ent growth	nit	3-7	no	amb	none	medium	soil	-	C3	no	cereal	-0.40	0.03	-32.79
EvTa2	very low	TA	ent growth	nit	3-7	no	amb	none	medium	soil	-	C3	no	cereal	-0.47	0.01	-37.71
EvTa3	very low	TA	ent growth	nit	3-7	no	amb	none	medium	soil	-	C3	no	cereal	-0.61	0.03	-45.90
EvTa4	low	TA	ent growth	nit	3-7	no	amb	none	medium	soil	-	C3	no	cereal	-0.21	0.01	-19.13
Wo1GhE1	medium	GH	ent growth	nit	1-2	no	elev	none	medium	soil	veg	C3	no	fibre	-0.51	0.03	-39.97
Wo1GhE2	low	GH	ent growth	nit	1-2	no	elev	none	medium	soil	veg	C3	no	fibre	-1.26	0.06	-71.73
Wo1GhE3	very low	GH	ent growth	nit	1-2	no	elev	none	medium	soil	veg	C3	no	fibre	-2.16	0.02	-88.43
Wo1GhA1	medium	GH	ent growth	nit	1-2	no	amb	none	medium	soil	veg	C3	no	fibre	-0.36	0.01	-30.09
Wo1GhA2	low	GH	ent growth	nit	1-2	no	amb	none	medium	soil	veg	C3	no	fibre	-0.75	0.03	-52.94
Wo1GhA3	very low	GH	ent growth	nit	1-2	no	amb	none	medium	soil	veg	C3	no	fibre	-1.76	0.02	-82.80
Wo1ZmE1	medium	ZM	ent growth	nit	1-2	no	elev	none	medium	soil	veg	C4	no	cereal	-0.45	0.01	-36.25
Wo1ZmE2	low	ZM	ent growth	nit	1-2	no	elev	none	medium	soil	veg	C4	no	cereal	-0.86	0.01	-57.50
Wo1ZmE3	very low	ZM	ent growth	nit	1-2	no	elev	none	medium	soil	veg	C4	no	cereal	-1.18	0.01	-69.39
Wo1ZmA1	medium	ZM	ent growth	nit	1-2	no	amb	none	medium	soil	veg	C4	no	cereal	-0.37	0.01	-31.25
Wo1ZmA2	low	ZM	ent growth	nit	1-2	no	amb	none	medium	soil	veg	C4	no	cereal	-0.80	0.01	-55.01
Wo1ZmA3	very low	ZM	ent growth	nit	1-2	no	amb	none	medium	soil	veg	C4	no	cereal	-1.11	0.01	-67.05
Kham2Zm1	very low	ZM	> ½	nit	>1	no	amb	none	medium	sand	veg	C4	no	cereal	0.06	0.01	6.16
Kham2Zm2	very low	ZM	> ½	nit	>1	no	amb	none	medium	sand	veg	C4	no	cereal	-0.18	0.01	-16.11
Kham2Zm3	very low	ZM	> ½	nit	>1	no	amb	none	medium	sand	veg	C4	no	cereal	-0.89	0.01	-58.77

Table C.22: List of effect sizes (lnR) and associated categorical variables for the response variable photosynthesis rate (A). PAR denotes the photosynthetically active radiation under which measurement were made (light is light-saturated, growth is growth PAR). Further abbreviations as before.

PAR	unit	study	species	length lim	N source	freq	pH	[CO2]	stress	pot size	medium	dev	C3/C4	leg	crop	lnR	Var (lnR)	%change
light	area	EvTa1	TA	ent growth	nit	3-7	no	amb	none	medium	soil	-	C3	no	cereal	-0.14	0.00	-13.47
light	area	EvTa2	TA	ent growth	nit	3-7	no	amb	none	medium	soil	-	C3	no	cereal	-0.19	0.00	-17.38
light	area	EvTa3	TA	ent growth	nit	3-7	no	amb	none	medium	soil	-	C3	no	cereal	-0.19	0.00	-17.02
light	area	EvTa4	TA	ent growth	nit	3-7	no	amb	none	medium	soil	-	C3	no	cereal	0.03	0.00	3.19
light	area	Kham2Zm1	ZM	> ½	nit	>1	no	amb	none	medium	sand	veg	C4	no	cereal	0.03	0.01	3.28
light	area	Kham2Zm2	ZM	> ½	nit	>1	no	amb	none	medium	sand	veg	C4	no	cereal	-0.19	0.01	-17.49
light	area	Kham2Zm3	ZM	> ½	nit	>1	no	amb	none	medium	sand	veg	C4	no	cereal	-0.85	0.01	-57.38
growth	area	CramTaNit	TA	ent growth	nit	3-7	yes	amb	none	big	hydro	veg	C3	no	cereal	0.02	0.00	2.02
growth	area	CramTaAm	TA	ent growth	amm	3-7	yes	amb	none	big	hydro	veg	C3	no	cereal	0.17	0.00	18.40
growth	area	CramZmNit	ZM	ent growth	nit	3-7	yes	amb	none	big	hydro	veg	C4	no	cereal	-0.13	0.01	-11.75
growth	area	CramZmAm	ZM	ent growth	amm	3-7	yes	amb	none	big	hydro	veg	C4	no	cereal	-0.06	0.01	-6.26
growth	area	RobGm1	GM	ent growth	nit + amm	1-2	yes	amb	none	medium	inert	veg	C3	non nod	oilseed	-0.14	0.00	-12.65
light	area	RobGm2	GM	ent growth	nit + amm	1-2	yes	amb	none	medium	inert	veg	C3	non nod	oilseed	-0.17	0.01	-16.02
growth	area	Wo1GhE1	GH	ent growth	nit	1-2	no	elev	none	medium	soil	veg	C3	no	fibre	-0.19	0.01	-17.63
growth	area	Wo1GhE2	GH	ent growth	nit	1-2	no	elev	none	medium	soil	veg	C3	no	fibre	-0.51	0.01	-39.76
growth	area	Wo1GhE3	GH	ent growth	nit	1-2	no	elev	none	medium	soil	veg	C3	no	fibre	-0.91	0.01	-59.91
growth	area	Wo1GhA1	GH	ent growth	nit	1-2	no	amb	none	medium	soil	veg	C3	no	fibre	-0.16	0.01	-14.62
growth	area	Wo1GhA2	GH	ent growth	nit	1-2	no	amb	none	medium	soil	veg	C3	no	fibre	-0.25	0.01	-22.08
growth	area	Wo1GhA3	GH	ent growth	nit	1-2	no	amb	none	medium	soil	veg	C3	no	fibre	-0.53	0.00	-41.29
growth	area	Wo1ZmE1	ZM	ent growth	nit	1-2	no	elev	none	medium	soil	veg	C4	no	cereal	-0.15	0.00	-13.72
growth	area	Wo1ZmE2	ZM	ent growth	nit	1-2	no	elev	none	medium	soil	veg	C4	no	cereal	-0.61	0.00	-45.71
growth	area	Wo1ZmE3	ZM	ent growth	nit	1-2	no	elev	none	medium	soil	veg	C4	no	cereal	-1.08	0.01	-65.92
growth	area	Wo1ZmA1	ZM	ent growth	nit	1-2	no	amb	none	medium	soil	veg	C4	no	cereal	-0.11	0.00	-10.77
growth	area	Wo1ZmA2	ZM	ent growth	nit	1-2	no	amb	none	medium	soil	veg	C4	no	cereal	-0.35	0.00	-29.19
growth	area	Wo1ZmA3	ZM	ent growth	nit	1-2	no	amb	none	medium	soil	veg	C4	no	cereal	-0.63	0.00	-46.87
growth	area	Chap2Hv1	HV	< ½	nit	3-7	no	amb	none	small	hydro	veg	C3	no	cereal	-1.25	0.07	-71.31
growth	weight	Kham1Zm1	ZM	< ½	nit	>1	yes	amb	none	small	sand	veg	C4	no	cereal	-0.16	0.02	-14.79
growth	weight	Kham1Zm2	ZM	< ½	nit	>1	yes	amb	none	small	sand	veg	C4	no	cereal	-0.07	0.01	-6.87
growth	weight	Kham1Zm3	ZM	< ½	nit	>1	yes	amb	none	small	sand	veg	C4	no	cereal	0.05	0.01	5.52
light	area	NakamGm1E	GM	ent growth	amm	?	no	elev	none	small	soil	repr	C3	nod	oilseed	-0.10	0.02	-9.28

light	area	NakamGm1A	GM	ent growth	amm	?	no	amb	none	small	soil	repr	C3	nod	oilseed	-0.23	0.03	-20.70
light	area	NakamGm2E	GM	ent growth	amm	?	no	elev	none	small	soil	repr	C3	non nod	oilseed	-2.64	1.79	-92.90
light	area	NakamGm2A	GM	ent growth	amm	?	no	amb	none	small	soil	repr	C3	non nod	oilseed	-1.81	0.38	-83.64
light	area	NakanOsA1	OS	< ½	nit + amm	3-7	yes	amb	none	medium	hydro	veg	C3	no	cereal	-0.74	0.01	-52.24
light	area	NakanOsA2	OS	< ½	nit + amm	3-7	yes	amb	none	medium	hydro	veg	C3	no	cereal	-0.51	0.06	-39.73
light	area	NakanOsE1	OS	< ½	nit + amm	3-7	yes	elev	none	medium	hydro	veg	C3	no	cereal	-0.97	0.00	-61.91
light	area	NakanOsE2	OS	< ½	nit + amm	3-7	yes	elev	none	medium	hydro	veg	C3	no	cereal	-0.66	0.01	-48.33

Table C.23: List of effect sizes (lnR) and associated categorical variables for the response variable stomatal conductance (g_s). Abbreviations as before.

PAR	study	N rate	species	length lim	N source	freq	pH	[CO2]	stress	pot	medium	dev	C3/C4	leg	crop	lnR	Var (lnR)	%change
growth	Chap2Hv1	very low	HV	< ½	nit	3-7	no	amb	none	small	hydro	veg	C3	no	cereal	-0.05	0.06	-4.93
?	CarvTa1	very low	TA	< ½	nit	<7	no	amb	none	?	?	seedl	C3	no	cereal	-0.87	0.02	-57.95
growth	CramTaNit	medium	TA	ent growth	nit	3-7	yes	amb	none	big	hydro	veg	C3	no	cereal	0.20	0.01	22.02
growth	CramTaAm	medium	TA	ent growth	amm	3-7	yes	amb	none	big	hydro	veg	C3	no	cereal	0.58	0.01	79.21
growth	CramZmNit	medium	ZM	ent growth	nit	3-7	yes	amb	none	big	hydro	veg	C4	no	cereal	-0.33	0.04	-28.24
growth	CramZmAm	medium	ZM	ent growth	amm	3-7	yes	amb	none	big	hydro	veg	C4	no	cereal	-0.43	0.05	-35.11
growth	RobGm1	low	GM	ent growth	nit + amm	1-2	yes	amb	none	medium	inert	veg	C3	non nod	oilseed	-0.58	0.03	-44.13
light	RobGm2	low	GM	ent growth	nit + amm	1-2	yes	amb	none	medium	inert	veg	C3	non nod	oilseed	-0.57	0.01	-43.41
light	MorgTa1	low	TA	ent growth	nit + amm	3-7	yes	amb	yes	medium	hydro	veg	C3	no	cereal	0.67	0.02	95.70

Table C.24: List of effect sizes (lnR) and associated categorical variables for the response variable N uptake (N_{up}). Abbreviations as before.

study	N rate	species	length lim	N source	freq	pH	[CO2]	stress	pot	medium	dev	C3/C4	leg	crop	lnR	Var (lnR)	%change
KingHv1	very low	HV	< ½	nit	1-2	yes	amb	none	big	hydro	seedl	C3	no	cereal	1.46	0.00	330.34
KingHv2	low	HV	< ½	nit	1-2	yes	amb	none	big	hydro	seedl	C3	no	cereal	0.90	0.03	146.90
Chap1Hv1	very low	HV	< ½	nit	3-7	no	amb	none	small	hydro	veg	C3	no	cereal	0.83	0.00	130.11
Rad1Gh1	very low	GH	ent growth	nit	?	no	amb	none	?	soil	veg	C3	no	fibre	-1.34	0.37	-73.68
Rad1Hv1	very low	HV	ent growth	nit	?	no	amb	none	?	soil	veg	C3	no	cereal	-1.39	0.57	-75.00
Sid2HvL	very low	HV	> ½	nit	>1	no	amb	none	big	hydro	seedl	C3	no	cereal	-0.81	0.01	-55.63
Sid2HvH	very low	HV	> ½	nit	>1	no	amb	none	big	hydro	seedl	C3	no	cereal	-0.72	0.01	-51.16

This study represents original work by the author and has not been previously submitted in any form for any degree or diploma thesis to any University. Where use has been made of the work of others it is duly acknowledged in the text.

Verena Seufert

Stuttgart, December 2009