Nitrogen availability alters species photosynthetic responses to elevated atmospheric CO_2

by

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Dedication

To Yvette Maes

1950-2004

My sweet mother who taught me to pursue my dreams,

Who believed in me and wanted me to finish this Ph.D.

Acknowledgements

Completing a PhD is a growing and enriching experience during which you meet many people of different ilk. This sharpens your skills scientifically, but also socially improves insights into people, ways of communication and life in general. I crossed paths with many different people, some towards whom I feel deeply grateful.

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papa, broer en zus, ik hoop dat ik de kans heb om al mijn dankbaarheid en liefde te tonen wanneer we nog eens bij elkaar zijn.
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Follow your heart and pursue your dreams,
for that it the best road to happiness.

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A_{net}	**Net Photosynthesis at growth conditions**
A_{a365}	**Area-based photosynthesis at a common CO_2 level of 365 μmol CO_2 mol^{-1}**
A_{m365}	**Mass-based photosynthesis at a common CO_2 level of 365 μmol CO_2 mol^{-1}**
Achmi	*Achillea millefolium*
Agrre	*Agropyron repens*
Anecy	*Anemone cylindrica*
Broin	*Bromus inermis*
C	**Carbon**
[CO_2]	**Carbon dioxide concentration**
FACE	**Free-Air CO_2 Enrichment**
GPP_{day}	**Daily gross primary productivity**
J_{max}	**Maximum electron transport rate**
k	**light exctinction coefficient**
Koecr	*Koeleria cristata*
LAI	**Leaf Area Index**
LMA	**Leaf Mass per area ratio**
N	**Nitrogen**
N_{area} **or** N_a	**Area-based leaf nitrogen**
N_{mass}	**Mass-based leaf nitrogen**
PAR	**Photosynthetically Active Radiation**
Pinta	*Pinus taeda*
Poapr	*Poa pratensis*
ppm	**Parts per million or μmol mol^{-1}**
Rubisco	**1,5-Ribulose biphosphate carboxylase/oxygenase**
RuBP	**Rubisco biphosphate, substrate for the Rubisco enzyme**
Solri	*Solidago rigida*
V_{cmax}	**Maximum carboxylation rate**

Abstract

Atmospheric [CO_2] and soil N availability are critical resources for plant growth, both of which are increasing due to global climate change. Therefore, it is important to understand how additional resources in the form of elevated CO_2 and increased N availability impact photosynthesis as the main driver of plant productivity. I conducted studies on pines, grasses and forbs, all grown under long-term Free-Air CO_2 Enrichment (FACE) and nitrogen fertilization, to determine how these global change factors affect plant photosynthesis and nitrogen use.

Both forbs and pines showed down-regulation of photosynthetic capacity under elevated CO_2 as changes in V_{cmax} of -23% and -17%, respectively. Grasses did not show significant photosynthetic down-regulation under elevated CO_2 compared to ambient CO_2. Grasses showed the least reduction of N_{mass} in elevated CO_2 (-7%), followed by pines (-12%) and forbs (-18%). When reductions in photosynthetic capacity occurred, as was observed in forbs, a smaller photosynthetic stimulation of 9% occurred under elevated CO_2 than when no down-regulation was observed. Compared to forb species, CO_2-induced photosynthetic stimulation was 31% to 57% for pines and grasses, respectively. A reduction in foliar N concurrent with down-regulation of photosynthetic capacity in elevated CO_2 could indicate plant N redistribution where N is allocated away from photosynthetic components. This N redistribution in response to elevated CO_2 may be a key response in adjusting plant growth to long-term elevated CO_2.

At the canopy scale, increased leaf area index (LAI) under elevated CO_2 due to photosynthetic enhancement could compensate for the effects of physiological down-regulation on canopy photosynthesis. Grasses had higher canopy photosynthesis than forbs under elevated CO_2 due to large LAI increases in combination with no photosynthetic down-regulation. Both LAI and photosynthetic down-regulation are

important in determining plant canopy productivity in elevated CO_2. Given that few models include CO_2 -induced photosynthetic adjustments such as decreased foliar N or reduced photosynthetic capacity, I conclude that much of previous experimental work on CO_2 enrichment has greatly overestimated photosynthetic enhancement in native ecosystems. Interacting effects of long-term elevated CO_2 and N fertilization may ultimately determine the magnitude of C uptake from the atmosphere and overall plant productivity.

Chapter 1

Introduction

1.1. Motivation

Human-induced changes to the atmosphere and climate are primary drivers of current global environmental change in the 20th and 21st centuries. Though increasing concentrations of carbon dioxide (CO_2) in the atmosphere have been related to global warming (IPCC, 2007), there are direct effects of rising CO_2 concentrations on plant processes given that plants capture CO_2 via photosynthesis. It is these direct effects of atmospheric CO_2 on plant that are central to this dissertation. Photosynthesis determines ecosystem productivity and the resulting ecosystem services upon which we, humans, rely. During the past century, fossil fuel use has increased the atmospheric concentration of CO_2 from about 280 μmol CO_2 mol^{-1} before the Industrial Revolution to about 385 μmol CO_2 mol^{-1} in 2007, the highest concentration on Earth during the past 400,000 years (Petit *et al.*, 1999; Canadell *et al.*, 2007). Therefore, we need to know how the process of CO_2 uptake will be affected by rising atmospheric [CO_2], how these effects will cascade through different scales and pools, and what this will mean for ecosystem functioning and C storage. As concentrations of CO_2 have increased, both land and oceans have been absorbing more CO_2 from the atmosphere. Though it is unclear exactly how much additional CO_2 is being taken up annually by each of these sinks, increased absorption of CO_2 by plants is a key part of the process (further discussed in Section 1.2.1.). Increased plant uptake may also help to mitigate higher atmospheric CO_2 levels (Kirschbaum, 2003).

In contrast to the carbon cycle with a relatively small but very dynamic carbon pool of C in the atmosphere, the nitrogen (N) cycle has its largest pool in the atmosphere,

mostly in the form of N_2, which cannot readily be used by biota due to its triple bond. The N-fixation process, mostly conducted by *Rhizobium* bacteria associated with certain plants (i.e., legumes), converts atmospheric N_2 into mineral "reactive" N-molecules that can be taken up by microbes or plants. Therefore, relatively small amounts of reactive N-molecules (e.g., NO_3^- and NH_4^+ in soils) are available to terrestrial ecosystems, and N is a major limiting element to plant productivity in most terrestrial ecosystems (Field *et al.,* 1992, Vitousek, 1994). However, the N-status of terrestrial ecosystems may be affected by several major anthropogenic factors: 1) increased rates of fossil fuel combustion that increase the amount of reactive N-molecules released via car exhaust and, 2) the production of agricultural fertilizer from the Haber-Bosch process (Galloway & Cowling, 2002). The rates of N-fixation have doubled over the past century, largely due to the production of fertilizer, to currently around 150 Tg N per year (Galloway *et al.*, 2002) significantly increasing N availability in terrestrial ecosystems. This global increase in nitrogen availability has many effects on N cycling in terrestrial ecosystems and could potentially remove N-limitation to plant productivity (see section 1.2.2.).

Increases in atmospheric CO_2 and N availability (Field *et al.*, 1992; Peterson *et al.*, 1999a) could increase plant growth because both C- and N-availability are currently limited to many plants (Reich *et al.*, 1997). Because both [CO_2] and N availability are critical resources for plant growth, it is important to understand how they affect photosynthesis to provide insight into their role in regulating productivity and understand there eventual effects on plant growth. Furthermore, if we intend to rely on plants to partly mitigate our increasing CO_2 emissions, then it is important to understand what changes in plant productivity can be expected and what other factors may affect this. The focus of this dissertation is on the effects of elevated atmospheric [CO_2] and N fertilization on potential changes in photosynthetic processes that drive plant canopy productivity in different plant species at the leaf and canopy scale.

In the following sections of this introduction, I review the individual effects of elevated CO_2 and N fertilization on photosynthesis and plant productivity. Then I discuss the role of N in plants grown in elevated CO_2, discussing potential CO_2 x N interactions, leading to my research objectives. I conclude the chapter with a brief overview of site descriptions and general methods applied in this dissertation.

1.2. Literature Review

1.2.1. Effects of elevated CO_2 on photosynthesis and plant growth

1.2.1.1. Photosynthesis

At a CO_2 concentration of 385 μmol CO_2 mol^{-1} today, most plants still operate on the steep slope of the photosynthetic CO_2 response curve, with photosynthesis rates that vary as a function of CO_2 concentration in constant saturating light conditions (Fig. 1). This occurs due to the direct catalytic effects of CO_2 in reactions at the catalytic sites of ribulose-1,5,-bisphosphate carboxylase enzyme (often referred to as the 'Rubisco' enzyme), and the efficiency of Rubisco is also enhanced due to the increase in the ratio of $[CO_2]/[O_2]$ which elicits repression of photorespiration (Drake *et al.*, 1997). Because the rate of net CO_2 assimilation in C_3 plants is not CO_2-saturated at the current atmospheric CO_2 concentration, the short-term rise in CO_2 concentration may stimulate photosynthesis. Given that photosynthesis operates on the linear portion of the CO_2 response curve (Fig. 1), a 50% increase in photosynthesis can be expected with a 50% increase in atmospheric CO_2 concentrations. Though much variation exists in species-specific responses, in time frames varying from one to several years of continuous elevated CO_2, maximum leaf photosynthesis was stimulated by 44-66% on average in woody plants, across experiments with different increases in elevated CO_2 (Gunderson & Wullschleger, 1994; Saxe *et al.*, 1998; Norby *et al.*, 1999). Across different functional groups of plants and experimental sites, photosynthesis was stimulated by an average of 25% (Nowak *et al.*, 2004). Unfortunately, this is a very large range and hence longer-term and larger-scale studies are needed. Stimulation of photosynthesis is also influenced by the physiological status of plants and by environmental factors such as climate and nutrient availability (Oren *et al.*, 2001a; Poorter & Perez-Soba, 2001). Stressful climates such as occur in cold northern hardwood plantations or the hot Mojave desert result in smaller stimulations of 38% and 22% respectively (Noormets *et al.*, 2001; Naumburg *et al.*, 2003). My study was conducted in two long-term experiments on sites with natural N-poor conditions, exposing vegetation to elevated $[CO_2]$ and N addition treatments for

up to ten years, in order to elucidate species responses and potential interactions between N availability with elevated CO_2.

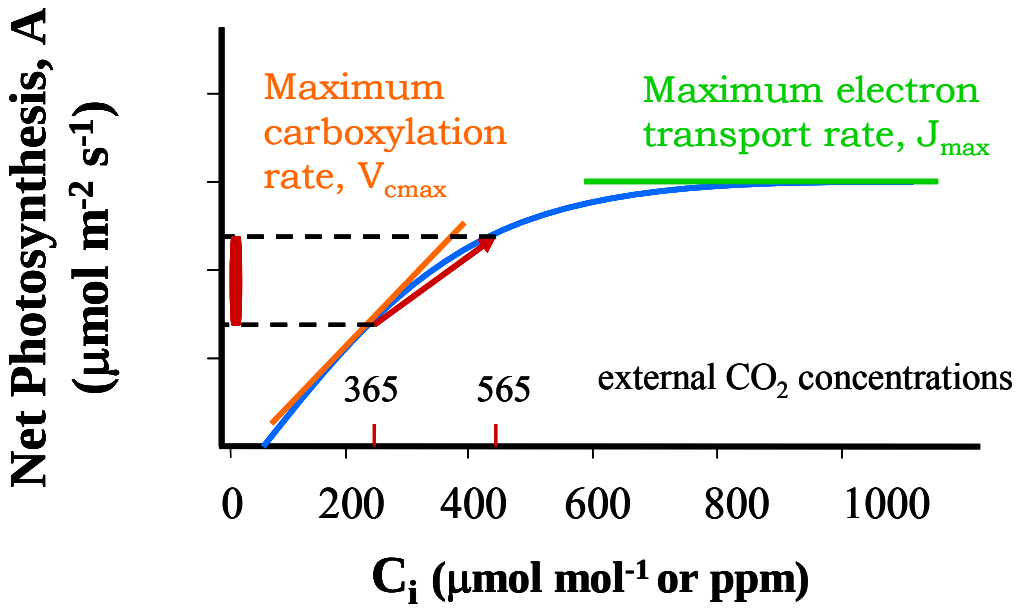

Figure 1: Diagram of a CO_2-response curve with photosynthesis as a function of internal CO_2 concentrations (Ci) (blue solid line), measured in constant saturating light conditions. The corresponding external CO_2 concentrations are indicated by the internal ticks on the X-axis showing ambient and elevated concentrations used in my measurements. The difference in photosynthesis rate represents the short-term photosynthetic enhancement in elevated CO_2 (red solid line along the Y-axis).

One of the most general responses observed in plants grown in elevated CO_2 is a reduction in leaf N concentration. This reduction in leaf N can happen in two major ways, either via a dilution response of leaf N through accumulation of carbohydrates and increased leaf mass per unit area (Rogers & Ellsworth, 2002; Ainsworth *et al.*, 2003) or, via a consistent reduction of N allocated to Rubisco (Saxe *et al.*, 1998; Yin, 2002; Ellsworth *et al.*, 2004; Bonanomi *et al.*, 2005). The reduced amount or activity of the carboxylation enzyme, ribulose bisphosphate carboxylase (Rubisco) is evoked via the expression of specific genes using signals of accumulated sucrose in the mesophyll cells (Long *et al.*, 2004). Both paths to foliar N declines have abundant support in the literature. Across 104 studies, predominantly conducted in open-top chambers, the CO_2-

induced reduction in mass-based N was 11-16% (Curtis & Wang, 1998; Luo *et al.*, 2006). In a meta-analysis of FACE (Free Air CO_2 Enrichment) studies, elevated CO_2 decreased mass-based leaf N by 13% and area-based leaf N by 5 %, whereas the maximum carboxylation rate,V_{cmax} (a measure of Rubisco content or activity) was reduced by 13-19% (Ainsworth & Long, 2005). This agrees well with the results of Medlyn et al. (1999), who found reductions of 15% in mass-based leaf N and 10% in V_{cmax}. In conclusion, reduced leaf N in elevated CO_2 occurs both via N dilution and reduced allocation to Rubisco.

When leaf N cannot be maintained under elevated CO_2, down-regulation of photosynthesis may result from reduced photosynthetic capacity (Curtis & Wang, 1998; Ellsworth *et al.*, 2004) usually expressed as a reduction of V_{cmax} and J_{max}. The down-regulation of photosynthesis is defined as an inability to sustain short-term CO_2 induced increases in photosynthetic rates with long-term CO_2 exposure (Gunderson & Wullschleger, 1994). This down-regulation of photosynthesis has been especially pronounced in N-limited ecosystems (Stitt & Krapp, 1999), for example a 22% reduction in V_{cmax} and a 12 % reduction in leaf N_{area} content were observed in FACE experiments in nutrient-poor ecosystems (Ainsworth & Long, 2005). Herbaceous species tended to exhibit larger reductions in leaf N (-14%) accompanied with smaller CO_2 enhancements (+12%) compared to woody species (+38%) where no significant differences were found in leaf N concentrations between CO_2 treatments (Nowak *et al.*, 2004). Though the magnitude of down-regulation is highly variable among species (Medlyn *et al.*, 1999; Wand *et al.*, 1999; Lee *et al.*, 2001; Sefcik *et al.*, 2007), down-regulation may occur to a greater degree in specific environmental conditions, such as where N is severely limited or in droughted environments. So far, there has not been strong evidence that down-regulation has completely offset the photosynthetic stimulation relative to ambient-grown plants, even after 6-10 years of CO_2 exposure (Ainsworth *et al.*, 2003; Crous & Ellsworth, 2004). Thus, it is unclear to what degree photosynthetic downregulation occurs in native plants grown in native, low-N soils, and if it does, to what extent this down-regulation offsets the instantaneous CO_2 enhancement effect on photosynthesis. Therefore I hypothesize that lower photosynthetic enhancement in elevated CO_2 is related to the occurrence of down-regulation and that there is a relationship between the amount

of down-regulation and the reduction in leaf N in response to elevated CO_2 (**Hypotheses H.1a-b**; Section 1.3.).

1.2.1.2. Plant growth and biomass accumulation

Do stimulated rates of photosynthesis translate into increased biomass accumulation in elevated CO_2? To assess plant growth and biomass, both photosynthesis and respiration rates need to be taken into account. Given that no significant respiratory response to elevated CO_2 was found (Amthor, 2000; Tjoelker *et al.*, 2001; Wang & Curtis, 2002), elevated [CO_2] resulted in larger plants (+14% in elevated CO_2) with larger stem diameters, more branches (+25%) and leaf area for trees (+21 %) in woody species, but no such increases in biomass were found in herbaceous species (Ainsworth & Long, 2005; McCarthy *et al.*, 2007). Biomass production increased by 20% across 29 C_3 species of different functional groups, in six different FACE experiments (Ainsworth & Long, 2005), consistent with the 19% biomass enhancement found across 16 FACE sites (Nowak *et al.*, 2004). Curtis & Wang (1998) reported a 28% increase in biomass for saplings and seedlings grown under elevated CO_2 conditions in chamber or glasshouse experiments. Thus, for field studies done at larger scales, there appear to be smaller biomass enhancements than for single-plant studies which may be the result of plant age and maturity, differences between plants grown in stands versus solitary plants (Körner, 2000), or the increasing emphasis on native, nutrient-limited ecosystems in FACE studies.

A number of these conclusions have emerged from meta-analyses of data from the multiple different open-top chamber (Curtis and Wang, 1998; Medlyn *et al.*, 1999) or FACE experiments (e.g., Ainsworth & Long, 2005; Ainsworth & Rogers, 2007). However, meta-analyses represent a *post hoc* comparison based on relative effect sizes and hence provide limited understanding of fundamental relationships between plants grown in ambient and elevated CO_2 treatments. Moreover, effect-size estimates may be correlated (e.g., not independent) if several effect-sizes are calculated from each study (Gurevitch & Hedges, 1999). Therefore, understanding plant responses and potential interactions between elevated CO_2 and N using consistent data collection approach (see

Section 1.6.) may help reconcile these results (see Section 1.2.3. on CO_2 x N interactions and **Hypothesis H.2** in Section 1.3.).

The biomass response to elevated CO_2 is affected by nutrient status of the soil (see Section 1.2.2.), but also differs among different plant functional types. Stimulation of above-ground production also differs between different functional groups with trees showing the largest response (+28%) relative to C_3 grasses (+10%). Aboveground primary productivity increases the most in response to elevated CO_2 in deserts, followed by forests and grassland ecosystems (Nowak *et al.*, 2004; Ainsworth & Long, 2005). Forests show the greatest enhancement in belowground productivity followed by grasslands and then desert ecosystems (Nowak *et al.*, 2004; Ainsworth & Long, 2005). For above- and belowground productivity combined, net primary production (NPP) is generally stimulated in elevated CO_2 by almost 12% across 11 FACE sites. Forests increase NPP more than grasslands do in response to elevated CO_2 (Nowak *et al.*, 2004; Norby *et al.*, 2005). Therefore, it is clear that different species and functional groups respond differently to elevated CO_2, which could lead to interactions between CO_2 and species (**Hypothesis H.3**.; Section 1.3.). These interactions have the potential to alter species structure and composition in elevated CO_2 (Zavaleta *et al*., 2003; Joel *et al.*, 2001).

1.2.1.3. Carbon uptake and storage at larger scales

There is considerable interest in scaling leaf-level carbon assimilation to canopy-level carbon uptake and ecosystem productivity. Scaling up photosynthesis from the leaf-level to stand or ecosystem levels requires additional information including climatological parameters and biotic parameters such as growth and the leaf area present in the canopy (Jarvis, 1995). This is a major challenge because the spatial and temporal scales of interest are much greater than the short-term and small-scale studies at which physiological measurements are made (Kull & Jarvis, 1995; Terashima & Hikosaka, 1995).

The simplest way of scaling up leaf-level photosynthesis is to treat the whole canopy as one effective ‘big leaf’. However, canopy scale enhancement of photosynthesis

by CO_2 is usually not as large as that measured for individual leaves because of self-shading effects in the tree crown and the non-linear response of photosynthetic capacity to irradiance (Hättenschwiler & Körner, 1997; Meir *et al.*, 2002). Therefore 'big leaf' models result in an overestimation of canopy photosynthesis (Sinclair *et al.*, 1976; Leuning *et al.*, 1995; Meir *et al.*, 2002). Hence, 'multi-layer' canopy models have been developed to improve the accuracy of carbon gain estimates (Reynolds *et al.*, 1992; Sellers *et al.*, 1992; Niinemets & Tenhunen, 1997). These models divide the canopy into a series of layers, representing different leaf types found in a canopy with different physiological characteristics (e.g., sun, shade and intermediate leaf types) (dePury & Farquhar, 1997), but also require more parameters to define light distribution, fraction of sunlit versus shaded leaves and canopy N for each canopy layer.

Advantages of these two major scaling approaches can be combined in a relatively simple canopy model consisting of just two layers (dePury & Farquhar, 1997). The accuracy of a simple two-layer model to predict canopy photosynthesis agrees within 5% compared to a more complicated multi-layer model (Wang & Leuning, 1998), because non-linear responses of photosynthesis to light for different leaf-types have been accounted for and therefore greatly improve the estimates compared to a 'big leaf' model (Friend, 2001). While CO_2 exchange can be measured in ambient CO_2 conditions by a variety of techniques (e.g. open-top chambers, eddy-covariance), there is currently no effective technique to predict canopy CO_2 assimilation in elevated CO_2 conditions in large statured plants (Schäfer *et al.*, 2003). Even for smaller plants, few studies have attempted whole ecosystem measurements in ambient and elevated CO_2 (Aechlimanm *et al.*, 2005; Stocker *et al.*, 1997). Therefore, modeling plays an essential role in developing an understanding of potential plant, community and ecosystem processes. In my study, I used such a two-layer model to estimate gross canopy photosynthesis of different grassland monocultures in ambient and elevated CO_2. My objective was to estimate how much C uptake increased in elevated CO_2 at the canopy level versus the leaf-level. In addition, I examined how leaf-level down-regulation affected the gross canopy photosynthesis response in elevated CO_2 (**Hypothesis H.4**.; Section 1.3.)

1.2.2. Effects of N fertilization on photosynthesis and plant growth

Nitrogen is an essential nutrient for plants because it is needed to build proteins and nucleic acids. Nitrogen is of particular importance in mediating long-term responses to elevated CO_2 because most N in leaves is invested in Rubisco (Evans, 1989). However, nitrogen limits productivity in the majority of terrestrial ecosystems (Vitousek & Howarth, 1991; LeBauer & Treseder, 2008). A nutrient limitation is recognized by an increase in growth in response to the addition of the limiting nutrient because site fertility to individual plants is controlled by the availability of the nutrient in shortest supply (Von Liebig's Law of the Minimum (Von Liebig, 1840)). Therefore, one of the most consistent responses of plants to N addition is increased growth usually combined with an increase in foliar N (Bauer *et al.*, 2004; Magill *et al.*, 2004; Xia & Wan, 2008). Increased growth and C gain with increased leaf N concentration is predicted via the relationship between photosynthetic capacity and foliar N (Field & Mooney, 1986; Evans, 1989; Reich *et al.*, 1997). Thus, increased N availability via fertilization typically increases leaf N concentrations, photosynthetic rates, growth rates and biomass accumulation (Field & Mooney, 1986; Aerts & Chapin, 2000; Magill *et al.*, 2000).

LeBauer and Treseder (2008) did a literature study to assess global patterns of N limitation. Using data from 126 studies in different biomes across the globe, they tested the hypothesis that N limitation would increase with latitude, consistent with a temperature or precipitation limitation of N mineralization. This hypothesis was based on higher N:C and N:P ratios in tropical plants (Reich & Oleksyn, 2004) and increased N mineralization rates in wetter and warmer climates (Schlesinger & Andrews, 2000). They found that N limitation is widespread among biomes and influenced by geography and climate. The response ratio of aboveground plant productivity to N addition was 29% with significant differences in response in different biomes. Forests, tropical and temperate, responded 20% to N regardless of latitude, whereas grasslands showed an increased response with latitude from 26% in tropical grasslands to 53% response to N fertilization in temperate grasslands (LeBauer & Treseder, 2008). Synergetic responses were also observed when N addition was combined with other resources, exceeding the response to N alone (Elser *et al.*, 2007; Harpole & Tilman, 2007). Thus, if plants are

jointly limited by soil N and also current concentrations of atmospheric CO_2, I expect a synergistic effect between rising atmospheric CO_2 and N (**Hypothesis H.2.**; Section 1.3.).

Whereas the natural source of available N for plants is mineralized N, in the form of nitrate (NO_3^-) and ammonium (NH_4^+), anthropogenic N fertilization (> 160 Tg N per year globally) has now overtaken natural N fixation (~110 Tg N per year) as the first N source for plants (Galloway *et al.*, 2008). Moreover, N deposition rates are expected to double or triple before leveling out (Vitousek *et al.*, 1997; Galloway & Cowling, 2002). The effects of this additional N availability on ecosystem processes are not well understood (Gruber & Galloway, 2008). Alleviating limitation through N additions may have important consequences for N cycling. Whether N has positive or negative effects on plant productivity depends on the N status of the system (Aber, 1992) and on how N is distributed in the ecosystem. It is possible that increased rates of N deposition are not absorbed by plants. Nadelhoffer *et al.* (2004) found that soils were the dominant N sinks with increased deposition, assimilating 3-10 times more N deposition than trees did. Labeling studies using ^{15}N recovered on average less than 25% of ^{15}N additions in trees when inputs were smaller than 5 g N m^{-2} yr^{-1} (Tietema *et al.,* 1998; Zak *et al.*, 2004; Perakis & Hedin, 2001; Nadelhoffer *et al*., 1992). This suggests that only small fractions of N inputs (<10%) were likely to be assimilated in woody biomass, though N concentrations in plant biomass may be higher with N-addition than in unamended conditions for many years. Magill *et al.* (2004) found higher foliar N and double the amount of N in fine roots after 4 years of N-addition in two forest ecosystems of Harvard Forest (Magill *et al.*, 2004). However, N deposition may only have a small effect on temperate forest C balance suggesting that increased N-addition inputs will likely not significantly stimulate C uptake in tree biomass in some systems (Nadelhoffer *et al.,* 1999).

Moreover, current rates of N-addition in the two long-term experiments described here are higher (40-110 kg N ha^{-1}) than current or predicted N-deposition rates for those ecosystems. As such, plant responses in these experiments may not realistically reflect plant responses to increased N deposition rates predicted for these regions. However, results from N-addition experiments are not designed to mimic future N deposition rates, but rather to analyze plant mechanisms of response when exposed to increased N

availability. Labeled ^{15}N-addition treatments can provide information on the partitioning of N inputs among forest ecosystem components and the rates of N-fluxes between pools. Results from high N-addition treatments can help assess growth responses in agricultural crops and can be used as a management tool. More importantly, **N-addition experiments can increase our understanding of plant responses to this perturbation on its own or in combination with other factors such as elevated CO_2 or species diversity**. My study will be able to evaluate the relative strength of species responses of physiological variables to elevated CO_2 and N addition. Based on evidence discussed here, I expect a stronger CO_2 effect than an N-addition effect on photosynthesis across species, though individual species responses may vary.

Individual species adjust to their environment by responding to climatic and abiotic perturbations (Field & Mooney, 1983; Field *et al.*, 1992; Lambers & Poorter, 1992), which affects ecosystem productivity and species diversity. N-addition affects species diversity (Gress *et al.*, 2007) and species composition (Wedin & Tilman, 1996; Gough *et al.*, 2000; Zavaleta *et al.*, 2003; Suding *et al.*, 2005). Given the positive relationship between biodiversity and ecosystem stability (Tilman *et al.*, 2006), reductions in species diversity in response to N addition may result in greater variability in ecosystem functions under environmental perturbations. Moreover, changes in species composition affect ecosystem growth responses and competition for resources, which in turn could influence ecosystem productivity (Xia & Wan, 2008). Different species responses to N addition are expected and my study examines how different species and functional groups differ in response to both elevated CO_2 and N addition (**Hypothesis H.3**.; Section 1.3.) indicating potential changes in species composition.

Ultimately, the balance between production and decomposition determines the impact of N on the amount of C uptake by terrestrial ecosystems (Shaver *et al.*, 1992). Whereas the response of plant productivity to N is more consistently positive , the response of decomposition to N addition is less consistent (Fog, 1988). It depends on environmental conditions, substrate quality and microbial physiology (Schlesinger & Andrews, 2000; Knorr *et al.*, 2005). A CO_2-induced increase in N immobilization may reduce N release to soils, decreasing N mineralization over time (Hungate *et al.*, 2004). This in turn may eventually lead to a decline in productivity with lower C sequestration.

Alternatively, increased litter input in elevated CO_2 may increase N-mineralization rates (Zak *et al*., 1993, 2000). These potential CO_2 x N interactions in soils are not well understood but are important to understand how anthropogenic impacts will affect N and C cycling belowground. Overall, ecosystem productivity will mainly depend on the degree to which N limits NPP in both temperate and tropical ecosystems. Only an integrative approach with multi-factorial experiments can provide insight regarding the response of ecosystems to global change (Körner, 2000) and how resource availability and species composition affect this response (Field *et al.*, 1992). These interactions between CO_2 and N are discussed at different scales in the next section.

1.2.3. Interactions between elevated CO_2 and N fertilization: the role of N in plants grown in elevated CO_2

1.2.3.1. C-N interactions at the point of CO_2 capture

In recent years, we have gained a fairly solid understanding of the independent effects of elevated CO_2 and N on plant physiology and ecosystem functioning (Ainsworth *et al.*, 2003; Galloway *et al.*, 2004; Magill *et al.*, 2004; Nowak *et al.*, 2004; Ainsworth & Rogers, 2007). However, interactions among ecological factors cannot always be predicted based upon the responses of organisms to single factor manipulations (Mikkelsen *et al.*, 2008). Given that foliar N concentrations respond in opposite directions to elevated CO_2 (decrease) and N addition (increase), any interactions between N availability and atmospheric CO_2 concentration may determine in part how ecosystem functioning is affected by elevated CO_2 and increased N availability together. Because biogeochemical cycling of C and N are linked, interactive effects are likely, and may limit the magnitude of photosynthetic enhancement under elevated CO_2 (McMurtrie & Comins, 1996; Rastetter *et al.*, 1997; Luo *et al.*, 2004).

Recently, studies in forests as well as in grasslands have shown that the initial enhanced growth response of plants in elevated CO_2 can only be sustained with sufficient N supply (Oren *et al.*, 2001; Grünzweig & Körner, 2003; Schneider *et al.*, 2004; Reich *et al.*, 2006). Recent studies have found substantial growth responses in elevated CO_2

because additional N was foraged from deeper soil layers (Zak *et al*., 2007; Iversen *et al.*, 2008). If C sequestration is indeed constrained by N availability (Poorter, 1998; Lüscher *et al.*, 2000; Oren *et al.*, 2001; Hungate *et al.*, 2003; Reich *et al.*, 2006), potentially weakening the buffer of terrestrial ecosystems against rising atmospheric CO_2 concentrations, it is crucial to understand interactive effects of CO_2 and N on plant physiological processes. Unfortunately, our current ability to assess these long-term interactions between CO_2 and N over meaningful ecosystem time frames is severely limited due to the low number of realistic long-term experiments, such as Free-Air CO_2 Enrichment (FACE) experiments (section 2.3). My study aims to determine long-term effects of elevated CO_2 and N fertilization and their potential interactions on photosynthesis of diverse C_3 plant species from different FACE sites. Insight into the underlying physiological mechanisms at the leaf-level scale may enable us to better understand biomass accumulation and species-specific responses to elevated CO_2 and N fertilization (**Hypotheses H.1-3**; Section 1.3).

One tool to evaluate these physiological mechanisms is via the photosynthesis-nitrogen relationship. Because most leaf N is used to build photosynthetic enzymes (Evans, 1989; Takashima *et al.*, 2004), there is generally a strong positive relationship between leaf photosynthesis and leaf nitrogen both among (Reich *et al.*, 1997; Reich *et al.*, 1998a) and within species (Ellsworth & Reich, 1993; Reich & Walters, 1994; Reich *et al.*, 1998b; Crous & Ellsworth, 2004). This photosynthesis-leaf N relationship is a central ecophysiological paradigm of the last decade (Field *et al.*, 1983; Field & Mooney, 1986; Reich *et al.*, 1997) because it reflects the leaf-level manifestation of coupling between the carbon and nitrogen cycles (Vitousek, 1994). However, excess N from additional N inputs may decouple the photosynthesis-nitrogen relationship via large changes in N partitioning away from the photosynthetic machinery, weakening the relationship. Lower leaf N in elevated CO_2 may eventually translate into reduced photosynthetic rates and reduced plant productivity (Norby & Cotrufo, 1998; Peterson *et al.*, 1999b) (**Hypotheses H.1** and **H.4**.). If the photosynthesis-nitrogen relationship is affected by elevated [CO_2] or N-addition, then our predictions of C uptake at higher scales may be inaccurate because the connection between foliar N and photosynthetic capacity of the leaf is the central basis of a number of models that predict plant and

ecosystem carbon balance (Aber & Federer, 1992; McMurtrie & Wang, 1993; Luo *et al.*, 1994; Katul *et al.*, 2000; Cramer *et al.*, 2001; Baldocchi *et al.*, 2002). The strong relationship between photosynthesis and leaf N emphasizes the importance of key physiological processes at the leaf level in order to accurately model canopy growth dynamics and potential feedbacks with regard to global change.

1.2.3.2. C-N interactions at the whole plant level: biomass accumulation and plant productivity

CO_2 and N interactions can also occur at the whole plant level and can be examined by the theory of multiple-resource limitation (Field *et al.*, 1992; Rastetter & Shaver, 1992). When plants experience multiple resource limitations (e.g., such as both C-limitation and N-limitation in plants), interactions between CO_2 and N supply could limit biomass accumulation and plant productivity (Oren *et al.*, 1993; Schneider *et al.*, 2004; Reich *et al.*, 2006a). According to the multiple-resource limitation concept, biomass increase in elevated CO_2 concentration is greater at higher N supply (filled diamonds in Fig. 2) than lower N supply rates (open diamonds).

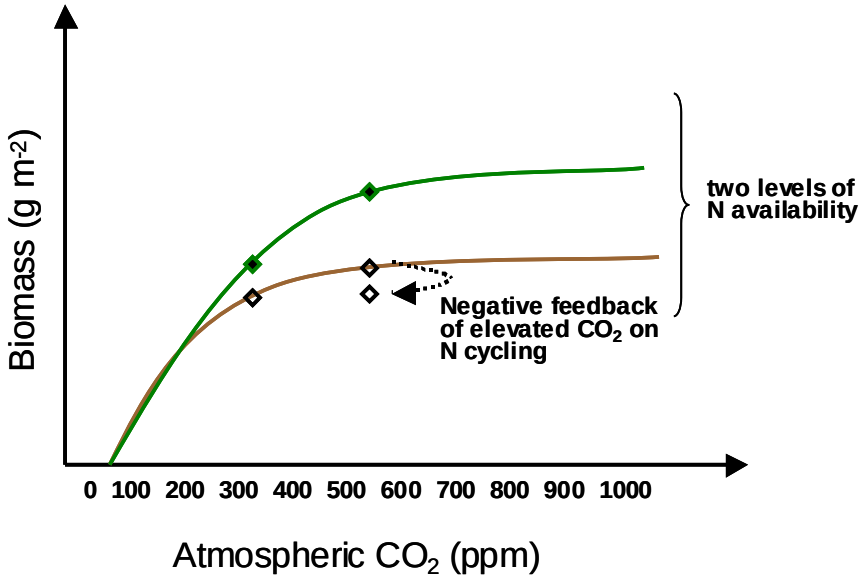

Figure 2: Conceptual diagram of the multiple limitation theory on plant growth as a function of CO_2 at two different levels of N availability (after (Reich *et al.*, 2006b). Biomass increase in elevated CO_2 concentration (ppm or μmol mol^{-1}) is greater at higher N supply (filled diamonds) than lower N supply rates (open diamonds).

Most studies have indeed found higher biomass accumulation in response to elevated CO_2 with high N supply than elevated CO_2 with low N supply. For example, trees grown under nutrient limitations showed 14% less enhanced biomass accumulation in elevated CO_2 (Ainsworth & Long, 2005)(see Section 1.4). A recent analysis across four forest FACE sites found a consistent 23% enhancement in NPP (Norby *et al.*, 2005), which was decreased to 19% under low N availability, whereas under intermediate and high N availability the percent CO_2 stimulation was 27% (Finzi *et al.*, 2002). Poplar seedlings and saplings showed higher aboveground biomass enhancement in elevated CO_2 at higher N supply than at low N supply (Zak *et al.*, 2000; Sigurdsson *et al.*, 2002; Liberloo *et al.*, 2005). The same effect was found in 15-year-old loblolly pine trees after two years of CO_2 and N manipulation (Oren *et al.*, 2001). However, young ponderosa pine and spruce-beech forests had similar responses to elevated CO_2 at both high and low N supply rates after six and four years in open top chambers (Johnson *et al.*, 1997; Spinnler *et al.*, 2002).

In herbaceous grassland systems exposed to both elevated CO_2 and N fertilization, plant responses to elevated CO_2 did not differ with variation in N supply either in wheat (Ainsworth & Long, 2005) or natural annual grasslands in California (Dukes *et al.*, 2005). Studies of other grassland ecosystems have reported various degrees of higher biomass enhancement with high N supply compared to low N supply (ryegrass in (Schneider *et al.*, 2004), perennial grassland in (Reich *et al.*, 2006a)). Based on these experimental studies conducted for at least 2 years in forest and herbaceous communities, there seems to be some evidence for a general N-limitation to elevated CO_2 effects on plants, although responses are not ubiquitous nor is the role of species in this response understood. I studied eight different species from three different functional groups to examine potential patterns in elevated CO_2 responses across species (**Hypothesis H.3**. and Chapter 3).

1.2.3.3. C-N interactions at the ecosystem level: C sequestration and storage

Given that plant biomass production linked to ecosystem carbon uptake, the higher CO_2-induced biomass enhancement with N addition may potentially stimulate the

C uptake response at the ecosystem level (e.g. increased net ecosystem productivity). This depends on whether other processes such as heterotrophic respiration and N mineralization processes also respond to elevated CO_2 and N supply. Because N mineralization largely controls N availability to plants, potential feedback effects of elevated CO_2 on N uptake and plant growth could affect overall ecosystem carbon sequestration (van Groenigen *et al.*, 2006). For example, recent meta-analyses suggest that soil carbon only increases with additional N but soil C is insensitive to elevated CO_2 in the absence of N supplements (Luo *et al.*, 2006; van Groenigen *et al.*, 2006), though the small sample sizes in meta-analyses could result in inaccurate confidence intervals (Hedges *et al.* 1999).

When N-limited ecosystems are exposed to long-term elevated [CO_2], negative feedbacks in N cycling (connected open diamonds in Fig. 2) may determine the potential for C storage in elevated CO_2, as postulated in the Progressive Nitrogen Limitation concept (Luo *et al.*, 2004). The progressive nitrogen limitation concept describes the reduction of N availability over time in elevated CO_2, either via increased N immobilization or by increased plant N sequestration. Carbon uptake by plants may be limited if there is a negative feedback from elevated CO_2 due to sequestration of N in soil organic matter (increased N immobilization). Alternatively, if N is sequestered in plant biomass, it will reduce the labile N availability to plants (Rastetter *et al.*, 1992; Field & Fung, 1999; Gill *et al.*, 2006), eventually constraining plant productivity unless N losses are reduced or N inputs increased (Oren *et al.*, 2001; Gill *et al.*, 2002; Luo *et al.*, 2004). Evidence reported to test the concept of progressive N limitation has been only partially supportive (Finzi *et al.*, 2006; Gill *et al.*, 2006; Hungate *et al.*, 2006). This may be because the theory of progressive N limitation predicts that initial stimulation of NPP in elevated CO_2 will decline through time. Plants in N-poor ecosystems can exhibit several mechanisms to delay N-limitation such as increased C:N ratios via reduced leaf N concentration, increased N use efficiency, shifts in N allocation or increased N uptake from the soil via increased root exploration (Zak *et al.*, 2000; Luo *et al.*, 2004; Gill *et al.*, 2006). Hungate *et al.* (2006) found that reduced N concentrations were able to support the increased C accumulation in aboveground biomass for at least seven years in a Scrub Oak ecosystem exposed to elevated CO_2. Though there is some evidence of N-limitations

in elevated CO_2, there is a lack of concrete support that reduced N-cycling also occurs or is responsible for the increases in N-limitation over time.

Whether N-cycling is increased, decreased or stays equal in elevated CO_2 is still unclear. Increased litter inputs in elevated CO_2 could stimulate N mineralization (Zak *et al.*, 1993), but increased C:N in leaves grown in elevated CO_2 may mean increased N immobilization (Diaz *et al*., 1993). Despite reduced N concentration in leaves grown in elevated CO_2 and its potential consequences for changes in litter quality and decomposition rates, recent tests have suggested little influence of elevated CO_2 on the C:N ratio of litter or on the rate of plant litter decomposition (Norby *et al.*, 2001), nor any dependence thereof on soil N supply (Henry *et al.*, 2005; de Graaff *et al.*, 2006b). Currently, there is little evidence that elevated CO_2 alters gross N mineralization (de Graaff *et al.*, 2006a) or net N mineralization (Matamala & Drake, 1999; Finzi & Schlesinger, 2003; Johnson *et al.*, 2003). However, studies in some nutrient-poor ecosystems have found that elevated CO_2 reduces net N mineralization under field conditions in a cold perennial grassland (Reich *et al.*, 2001b; Reich *et al.*, 2006a), a warm perennial grassland (Gill *et al.*, 2002) and a temperate pine forest (Finzi *et al.*, 2006). Together, these findings indicate that under ambient soil conditions, elevated CO_2 has neutral or negative effects on net N mineralization rates. This trend cannot easily be generalized as ecosystems contain different species with different effects on litter fall and decomposition rates (de Graaff *et al.*, 2006b; Dijkstra *et al.*, 2006). Elevated CO_2 is likely to have a larger effect on litter decomposition by altering species composition (Dukes *et al.*, 2005; Gill *et al.*, 2006), though this indirect mechanism would be ecosystem specific (Henry *et al.*, 2005).

1.3. General Research Hypotheses

Based on the background provided, there is a clear research need to further elucidate how elevated atmospheric CO_2 and N interact in affecting plant eco-physiological processes. My study examines the potential interactions between long-term elevated CO_2 and N-addition at the leaf-level scale and canopy scale based on the following overarching hypotheses:

H.1a.: The magnitude of photosynthetic enhancement in elevated CO_2 is higher when no down-regulation occurs.

With a 50% increase in atmospheric CO_2 concentrations, I expect a photosynthetic enhancement of ~50%. The amount of enhancement lower than 50% indicates some degree of down-regulation of photosynthesis (Section 1.2.1.1.).

H.1b.: Leaf N reduction in elevated CO_2 is reflected in reductions in photosynthetic capacity (e.g. carboxylation rates, V_{cmax} and electron transport rates, J_{max}) of similar magnitude.

If reduced leaf N and reduced carboxylation capacity occurs, then this might be due to N allocated away from photosynthesis, causing some degree of down-regulation in elevated CO_2.

H.2.: The amount of down-regulation is stronger in low soil N that in soils with N-addition.

Based on the multiple limitation theory, I expect that if both C and N are limiting plant growth then **the response to elevated CO_2 will be larger with higher N supply than with low N supply**. Working at two FACE sites with natural N-poor soil conditions and N-addition treatments can help elucidate potential interactions between elevated CO_2 responses and N-addition responses (Section 1.4.).

H.3.: The amount of CO_2-induced enhancement in photosynthesis will be stronger in trees compared to herbaceous species.

Though herbaceous species have higher absolute photosynthesis rates, I expect that the relative increases in photosynthesis in elevated CO_2 will be higher in trees compared to herbaceous species and therefore CO_2-enhancement will be stronger in trees compared to grasses and forb species.

H.4.: Increased photosynthesis at the leaf-level will lead to increased C uptake at the canopy scale, though the effects of down-regulation at the leaf-scale will also reduce C uptake at the canopy scale.

I expect to see a consistent pattern at the canopy scale compared to the leaf-level though smaller in magnitude due self-shading of leaves deeper in the canopy.

My dissertation work aims to examine the mechanism by which plants adjust to environmental perturbations such as elevated CO_2 and N fertilization by quantifying fundamental eco-physiological relationships, addressed in Section 1.6. It is my goal to determine potential changes in this photosynthesis-nitrogen relationship under elevated CO_2 concentrations using data collected in a consistent manner at two long-running FACE sites (Section 1.4. and 1.5.). These changes in the relationship with photosynthetic capacity and nitrogen allow me to evaluate mechanisms of down-regulation at the leaf-level scale and how these leaf-level effects change plant productivity at the whole-plant scale.

Given that individual species responses to elevated CO_2 can vary substantially, I purposefully included species of different growth forms and functional groups to elucidate species-specific responses and evaluate if these responses are consistent within their functional group.

1.4. Site Description

My work focuses on the effects of N on elevated CO_2 responses with field work at two research sites. One site is a stand of mature loblolly pine (*Pinus taeda*) located in Duke Forest in North Carolina (http://c-h2oecology.env.duke.edu/Duke-FACE/main.cfm) and the other site (BIOCON) is a prairie grassland LTER site located in Cedar Creek, Minnesota (http://www.lter.umn.edu/biocon/). At Cedar Creek, there are 16 plant species grown in different combinations of 1, 4, 9 and 16 species; in my work, I restricted measurements to only monoculture plots of several C_3 grasses and non-leguminous forbs characteristic of prairie grasslands.

Both sites have long-term elevated CO_2 treatments using free-air CO_2 enrichment (FACE) technology (see below). The year 2006 was the 10^{th} growing season of elevated CO_2 exposure for Duke Forest and the 9^{th} growing season for BioCON. Both sites also have N addition treatments. The N addition in Duke Forest began in March 2005, whereas N addition at BioCON has been maintained since the beginning of the experiment in 1998. Each experiment has a split-plot design, with three replicated plots for the elevated CO_2 treatment and subplots being the two N treatments within each plot.

Both N addition and elevated CO_2 treatments allow me to examine the relationships between photosynthetic components and nitrogen as well as the possible interactions of these two treatment factors. More information can be found for Duke Forest in Hendrey *et al.* (1999) and (Ellsworth *et al.*, 1995), and for BioCON in Lee *et al.* (2001).

1.5. Brief overview of the Free-Air CO_2 Enrichment (FACE) design

Free-Air CO_2 Enrichment (FACE) experiments are state-of-the-art integrated, ecological experiments involving interdisciplinary investigators from many universities. These experiments expose vegetation to predicted CO_2 concentrations for mid-century (2050), but it is important to understand that they do not mimic a specific environment. Rather, FACE experiments are designed to examine the mechanisms of plant and

ecosystem responses to high atmospheric [CO_2] in the hope to provide a scientific basis understanding rising atmospheric [CO_2].

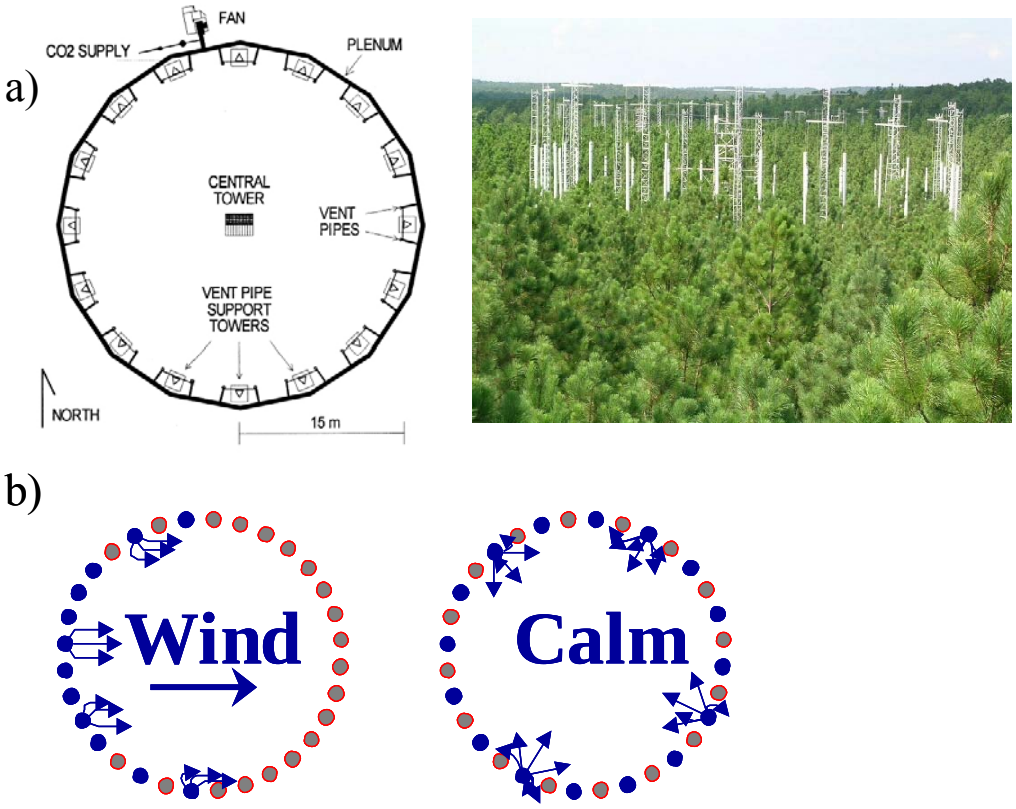

Figure 3: Schematic layout of a FACE plot. (a) Overhead view showing location of central and supporting towers at Duke FACE (left, from Hendrey *et al.,* 1999) and the result (side view) in the forest itself (right). (b) Different operation of the vent pipes depending on wind speed and direction where blue cicles show actived/opened vent pines and gray circles are inactive (e.g. not blowing air into the plot).

The FACE approach developed by Brookhaven National Laboratory is a unique technique to study the effects of CO_2 enrichment on vegetation and natural ecosystems in an open-air setting without containment. The FACE system aims at a target of 200 µmol CO_2 mol^{-1} CO_2 above ambient and provides stable control of that target (e.g. 550 µmol CO_2 mol^{-1} ± 10% over 90% of operational time in one-minute averages). Because of these well-controlled CO_2 concentrations (Hendrey *et al.*, 1993), FACE can use open field conditions without creating the markedly different micro-environmental conditions

characteristic of enclosed or open-top chambers (Olszyk *et al.*, 1980; McLeod *et al.*, 1985). Therefore this technique is an excellent tool to study responses to elevated CO_2 at a number of different scales, from the leaf up to the ecosystem including potential feedbacks and interactions that may occur.

The FACE system (Fig. 3a) consists of a circular array of vertical vent pipes connected to a toroidal plenum (30 m diameter) through which CO_2 enriched air is released. Each vent pipe is individually controlled via a pneumatically actuated quarter-turn ball valve, which is activated depending on the wind direction (only upwind directions are opened, Fig. 3b). Liquid CO_2, stored in an on-site tank, is vaporized via a heat-exchange element and transported to each FACE plot after the gas pressure has been decreased. A fan is used to run ambient air through the plenum torus and out the vent pipes. Where CO_2 treatment is applied, this air is mixed with pure CO_2 carefully controlled by an algorithm that includes terms for wind speed and current CO_2 concentration in the center of the plot. The CO_2 concentration within each plot is controlled by drawn air samples from the center of the plot to an infra-red gas analyzer feeding back to the CO_2 control algorithm. Higher wind speeds decrease the variance around the target concentration whereas poorest control occurs at low wind velocities (<0.4 m/s) (Hendrey *et al.*, 1993). Basically, within each FACE plot, there is about 380 m^2 of space with optimal [CO_2] control where experiments can be conducted (Hendrey *et al.*, 1993; Hendrey *et al.*, 1999).

1.6. General research approach

1.6.1. Measurement protocol

Leaf samples were collected from each replicated treatment of CO_2 x N level. All gas exchange measurements were conducted in the form of CO_2 response curves with a portable infrared gas analyzer (Li-Cor 6400, Li-Cor Inc., Lincoln, NE) using the following conditions: saturating light of 1800 μmol m^{-2} s^{-1}, constant air flow and temperature controlled at 28-30°C. The different CO_2 levels used for each curve were 60, 150, 230, 295, ambient (365), elevated (565), 900 and 1500 μmol CO_2 mol^{-1}. These

levels were selected to maximize the accuracy of calculations from the Farquhar photosynthesis model (Farquhar *et al.* 1980). Farquhar *et al*. (1980) proposed that net leaf photosynthesis, A_n, could be modelled as the minimum of two limiting rates:

$$A_n = \min (A_c, A_j) - R_d$$

A_c is the rate of photosynthesis when Rubisco activity is limiting and A_j the rate when RuBP-regeneration is limiting. R_d is the rate of mitochondrial respiration. Rubisco-limited photosynthesis is given by

$$A_c = V_{cmax} (C_i - \Gamma*) / (C_i + K_c (1 + O_i/K_o))$$

where V_{cmax} is the maximum rate of Rubisco activity, C_i and O_i are the intercellular concentrations of CO_2 and O_2, respectively, K_c and K_o are the Michaelis-Menten coefficients of Rubisco activity for CO_2 and O_2, respectively, and $\Gamma*$ is the photosynthetic CO_2 compensation point without mitochondrial respiration contributions. The rate of photosynthesis when RuBP regeneration is limiting is given by

$$A_j = J_{max} / 4 \ (C_i - \Gamma*) / (C_i + 2\Gamma*)$$

where J_{max} is the rate of maximal electron transport. Via this model, several variables among which the V_{cmax} from the linear part of the CO_2 response curve (Fig. 1; orange line) and J_{max} from the saturating part of the CO_2 response curve (Fig. 1; green line) are calculated independently (Farquhar *et al.*, 1980b). The model was fit to the data using specialized software (Crous and Ellsworth (2004).

Each measured leaf and additional leaves from the same plant were sampled for elemental C and N analysis. Segments of a known area were cut from the leaf and put in the drying oven (70°C), after which they were weighed. The leaf mass per area (LMA) was calculated by taking the ratio of mass to the area of the leaf. Leaves on which gas exchange measurements were performed were dried and ground to a fine homogenous powder. A subsample of 6-8 mg was combusted in an elemental gas analyzer (EA Flash Carlo-Erba, Milan, Italy) for total C and N content in the leaf. These basic measurements allow me to assess photosynthetic relationships as a function of total N.

1.6.2. Statistical approach

Two main modes of statistical analysis were employed here. A first analysis employed regression techniques to determine differences between CO_2 treatments in the relationships of photosynthetic components (electron transport capacity and carboxylation capacity) as a function of total leaf N between treatments. Standard assumptions such as normally distributed residuals and equal variance were checked via box plots and histograms, and residual plots were consulted. To evaluate treatment effects on slopes and intercepts of the relationships, such as elevated CO_2 effects and N-fertilization effects, I included dummy variable(s) as factors in the linear regression model (see first data chapter for more details, section 2.3.2.).

The second major analysis technique was Analysis of Variance (ANOVA) to determine the effects of global change factors incorporated in the designed experiments described above. Separate ANOVA were conducted for each site because of inherent site differences such as length of growing season, climate and soil texture. Ratios such as J_{max}/V_{cmax} and the fraction of N in Rubisco or other subprocesses were transformed prior to analysis to conform to the assumption of normally distributed data.

1.6.3. Modeling

In order to understand the implications of changes in leaf N partitioning on estimated whole-canopy CO_2 exchange with the atmosphere, I modeled canopy CO_2 exchange for a simplified system using the grass canopy at BioCON, avoiding the complexity of clumping that occurs in a pine canopy (Law *et al.*, 2001). The canopy is divided into two layers, which correspond to the sunlit and shaded portions of the canopy. The plant height of the herbaceous vegetation facilitates two-layer measurements of light and nitrogen in the canopy rather than a full gradient through a tall canopy because there are no intermediates between the sun and shade leaves.

I used a physiological model that relied on these two leaf classes (sunlit and shaded) and a light submodel to calculate gross canopy photosynthesis as a function of summed leaf canopy N (see Appendix A of Medlyn *et al.*, 2000). The model includes a mechanistic regulation of photosynthesis via the Farquhar photosynthesis model

(Farquhar *et al.,* 1980) and parameterizations of N partitioning to photosynthetic capacity using V_{cmax}-*N* and J_{max}-*N* relationships. While there may be minor disadvantages of this approach due to simplification and minimal meteorological information for the site, the model should be robust because it will reflect accurately the different foliage classes and the radiation incident upon them (Medlyn *et al.*, 2000).

To assess CO_2 effects on canopy photosynthesis, I modeled canopy photosynthesis and N partitioning according to two 'what-if' scenarios: 1) what happens if the N content is reduced but not the V_{cmax} or J_{max} and 2) what happens if V_{cmax} or J_{max} are reduced but not N content in the canopy? These two scenarios follow straight from the leaf-level measurements and leaf chemistry and allow me to assess whether changes in N partitioning among photosynthetic components at the leaf-level can be expected to affect CO_2 assimilation of the whole canopy and to what magnitude. Therefore, the model is directly consistent with most of my measurements and is appropriate for scaling those results to understand whole-canopy processes (See section 4.3 for more details).

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Chapter 2

Elevated CO_2 concentration affects leaf photosynthesis-nitrogen relationships in *Pinus taeda* over nine years in Free-Air CO_2 Enrichment (FACE)

2.1. Summary

Carbon dioxide-induced enhancements of leaf net photosynthesis may be reduced when trees are grown in infertile soils. To understand if long-term elevated CO_2 concentration causes declines in photosynthetic enhancement and leaf nitrogen, I measured photosynthesis, carboxylation capacity and leaf nitrogen concentration on an area basis (N_{area}) in needles in the canopy of *Pinus taeda* under long-term Free-air CO_2 enrichment (FACE) at a nitrogen-limited site. We also determined how the underlying components governing net photosynthesis such as rates of carboxylation (V_{cmax}) and electron transport (J_{max}) varied with leaf N_{area} under elevated CO_2 in *Pinus taeda*. The slope of the relationship between leaf photosynthetic capacity ($A_{net\text{-}Ca}$) and leaf N_{area} in one-year-old needles was significantly reduced by 37% after five to nine years of elevated CO_2 exposure, whereas current-year needles were unaffected in this regard. There was evidence for decreases in relationships between both V_{cmax} and J_{max} as a function of leaf N_{area} in one-year old needles after up to nine years of growth in long-term elevated CO_2. These decreases were associated with a 15% reduction in nitrogen allocation to the carboxylating enzyme with long-term CO_2 exposure. Nitrogen fertilization (110 kg N ha^{-1}) in the ninth year of elevated CO_2 restored V_{cmax}-N_{area} as well as J_{max}-N_{area} relationships to levels indistinguishable from control trees. The ratio of J_{max}:V_{cmax} is highly conserved with environmental manipulations such as long-term elevated CO_2 or fertilization. Fundamental relationships between photosynthesis or its component

processes with leaf N_{area} may be altered in aging pine needles after >5 years of exposure to elevated atmospheric CO_2. We conclude that photosynthetic down-regulation in one-year old pine foliage in elevated CO_2 was mainly driven by reductions in apparent nitrogen allocation toward active Rubisco enzyme. This suggests that changes in the apparent allocation of nitrogen to photosynthetic components may be an important adjustment in pines on low-fertility sites in elevated CO_2.

2.2. Introduction

Forests store large amounts of CO_2 from the atmosphere, with feedback effects on atmospheric CO_2 concentrations (Dixon *et al.*, 1994; Barford *et al.*, 2001). Because atmospheric CO_2 concentrations have been steadily rising by nearly 2 μmol mol^{-1} $year^{-1}$ in the last decade (Keeling & Whorf, 2005), there is a strong need to understand how CO_2 enrichment affects photosynthetic processes driving C storage. Given the abundance of pines (i.e. *Pinus spp*.) worldwide, understanding how pine species will respond to atmospheric CO_2 enrichment can be important in developing policy and management practices such as afforestation aimed at C sequestration (House *et al.*, 2003). There have been numerous studies addressing the photosynthetic responses of various coniferous species to elevated atmospheric CO_2 concentrations over the past decade (Wang *et al.*, 1996; Jach & Ceulemans, 2000; Ellsworth *et al.*, 2004; Handa *et al.*, 2005) and reviews on elevated [CO_2] responses of trees (e.g.,(Curtis & Wang, 1998; Nowak *et al.*, 2004; Ainsworth & Long, 2005). However, the sustainability of coniferous forest sinks for atmospheric CO_2 over the next century remains uncertain (White *et al.*, 2000; Oren *et al.*, 2001). To understand this sustainability, experimental elevated CO_2 exposures of trees have increased in scale from single branches (Teskey, 1997) to entire trees (Maier et al. 1998) to stands and ecosystems (Crous & Ellsworth, 2004; Körner *et al.*, 2005; Liberloo *et al.*, 2007), and have increased in duration from a single year up to a decade of continuous exposure to CO_2 enrichment, as in this study.

I report on effects of long-term elevated CO_2 exposure on photosynthetic capacity in the longest running forest Free-Air CO_2 Enrichment experiment to date, conducted in a pine forest ecosystem (Hendrey *et al.*, 1999; Oren *et al.*, 2001). It is well-known that

early enhancement effects of elevated atmospheric [CO_2] on leaf photosynthesis may not necessarily be sustained over time (Sage, 1994; Poorter & Pérez-Soba, 2001; Rogers & Ellsworth, 2002). The lack of sustained photosynthetic enhancement in ecosystems under elevated [CO_2] is especially apparent in low-nutrient sites (Oren *et al.*, 2001; Norby *et al.*, 2005; Reich *et al.*, 2006a), and is strongly related to the availability and root exploitation of limiting nutrients such as nitrogen (Zak *et al.*, 2000; Oren *et al.*, 2001; Finzi *et al.*, 2002; Luo *et al.*, 2004). As such, it would be expected that additions of nitrogen to a nitrogen-limited system could be expected to lead to recovery of initial, high CO_2 enhancement of photosynthesis and also growth (Oren *et al,.* 2001; Finzi *et al*., 2006).

Conifer species such as pines naturally occur on strongly N-limited soils (Aerts & Chapin, 2000), resulting in low productivity and growth rates relative to the amount of nitrogen invested in photosynthetic components. The evergreen habit of pines is considered to increase the efficiency of nutrient-use by maintaining photosynthetic activity over longer foliage lifetimes (Reich *et al.*, 1992). Because of the high proportion of old foliage in pines and its importance to sustain photosynthetic nitrogen-use efficiency over several years, photosynthetic activity in one-year-old needles may also serve to maintain photosynthetic and growth enhancement in elevated [CO_2] (Finzi *et al.*, 2002; Norby *et al.*, 2005; Finzi *et al.*, 2006).

Elevated [CO_2] has the potential to decrease photosynthetic nitrogen-use efficiency (PNUE) via reduced photosynthetic capacity in aging foliage. Because photosynthesis serves as the first major coupling point between canopy carbon and nitrogen cycles, understanding effects of long-term CO_2 enrichment on the well-known photosynthesis-nitrogen relationship are critical (Peterson *et al.*, 1999b). A decrease in PNUE due to reduced photosynthetic capacity in elevated [CO_2] could result in a weaker relationship between photosynthesis and leaf nitrogen. Thus, the interaction between nitrogen availability and plant productivity in conditions of elevated atmospheric [CO_2] has the potential to affect the fundamental relationship between photosynthesis and nitrogen within forest canopies (Reich *et al.*, 2006b). The nutrient-poor Piedmont plateau of central NC, USA in which the experiment is located is an ideal system for exploring how nitrogen availability affects plant responses in elevated [CO_2].

Given questions about the effect of elevated [CO_2] on the photosynthesis-nitrogen relationship and the ability of trees under low nutrient availability to sustain enhanced growth rates in high [CO_2] over time periods longer than a few years (Poorter, 1998; Oren *et al.*, 2001; Norby & Iversen, 2006), I examined the response of *P. taeda* foliage to elevated CO_2 exposure for up to nine growing seasons in the Duke Forest FACE facility. In contrast to earlier work (Crous & Ellsworth, 2004), here I report on a long-term dataset with nine years of elevated CO_2 exposure, and focus on how fundamental photosynthetic metabolism as a function of leaf nitrogen is affected in elevated atmospheric [CO_2] in combination with fertilization. My present objectives were 1) to quantify functional relationships of photosynthetic capacity and leaf nitrogen in ambient and elevated [CO_2], 2) to examine if and how these relationships are affected in one-year old mature *P. taeda* needles exposed to long-term elevated [CO_2] on a low-nutrient site, and 3) to understand how increased nitrogen availability may affect the response to long-term elevated CO_2 in aging foliage.

2.3. Methods

The measurements were conducted at the Duke Forest FACE facility (35° 58.6' N, 70° 05.6' W) in the North Carolina piedmont plateau, which has been described in detail elsewhere (Ellsworth, 1999; Hendrey *et al.*, 1999; Schäfer, KVR *et al.*, 2003). Briefly, the growing season in the vicinity of Duke Forest is from early March to mid-October with mean annual temperature of 15.5°C and mean annual precipitation of 1154 mm. Loblolly pine (*P. taeda)* forests generally occur on acidic, nutrient-poor soils in the region which are considered to be N-limited (Oren *et al*., 2001). Since August 1996, planted pine trees have been exposed to elevated [CO_2] via the Free-Air CO_2 Enrichment (FACE) technique (Hendrey *et al*., 1999). The Duke forest FACE experiment consists of six 30-m diameter plots, with 3 replicates at ambient CO_2 and 3 replicates at an elevated CO_2 target [CO_2] of ambient +200 μmol CO_2 mol^{-1}. Daytime exposure to elevated atmospheric [CO_2] was nearly continuous throughout the year except when temperatures were less than 5^oC or windspeeds were greater than 6 m s^{-1}, which together represented < 5% of the possible running time. In each plot, canopy access was gained by upright

personal platform lifts (UL48, Upright, Charlotte, NC) or a walk-up tower in the center of each plot.

To examine potential interactions of plant responses to both enhanced carbon and enhanced nitrogen supply, plots were divided in half using a two meter deep root barrier and half the plot was fertilized with NH_4NO_3 (ammonium nitrate) in March 2005. The rate of fertilization was 110 kg N ha^{-1} yr^{-1} compared to the ambient nitrogen mineralization rates at the site of about 30 kg N ha^{-1} yr^{-1} (Matamala & Schlesinger, 2000; Finzi *et al.*, 2002) and a background nitrogen deposition of about 6.5 kg N ha^{-1} yr^{-1} (Oren *et al.* 2001). This fertilization rate is what is currently used in fertilized commercial stands of loblolly pines. I continued the same measurement protocol as described below when fertilization began, hence one or two of the designated measurement trees per plot were located in the sector that received nitrogen fertilizer. The fertilization treatment was analyzed only in the first year of nitrogen fertilization.

2.3.1. Photosynthetic measurements

To quantify photosynthetic performance in ambient and elevated CO_2 concentrations, net CO_2 assimilation (A_{net}) of pine needles at different atmospheric CO_2 concentrations was measured in each treatment with a Li-Cor 6400 (Licor Inc., Lincoln, NE) portable photosynthesis system. Measurements were made on leaves at the top of the canopy crown (upper locations; > 90% of total tree height) and the lowest living branch of the canopy (lower locations) as described previously in Crous and Ellsworth (2004). Given the importance of the one-year old needles to year-round photosynthesis (Ellsworth, 2000; Schäfer *et al.*, 2003), one-year old needles were measured in both early and late summer, while current-year needles were only sufficiently developed to measure alongside one-year old needles in late summer. Within each plot, two to three candidate trees were chosen to be measured. Leaves were carefully positioned into the leaf chamber where they were exposed to a saturating quantum flux density of 1800 μmol m^{-2} s^{-1}, similar to full sunlight conditions at the site. Leaf temperatures were controlled at 28°C in early summer and 30°C in late summer, reflecting prevailing temperatures at these times

of year. Measurements were made between the second growing season (1998) and the ninth growing season (2005) of elevated CO_2 exposure in the experiment, inclusive.

At least seven [CO_2] levels were used in the stepwise photosynthetic CO_2 response curve, including the ambient and elevated [CO_2] that were the control and treatment targets in the experiment, respectively. Hence, net CO_2 assimilation of all needles was measured at a common [CO_2] of 365 μmol mol^{-1}, which I refer to as $A_{net\text{-}Ca}$. The net CO_2 assimilation rate of trees grown in an ambient or elevated CO_2 atmosphere was analyzed by comparing A_{net} measured at the appropriate [CO_2] corresponding to the treatment target [CO_2]. Thus, treatment comparisons of A_{net} represent the overall response to increased atmospheric [CO_2], whereas $A_{net\text{-}Ca}$ represents photosynthetic capacity at a common [CO_2] for trees grown in ambient and elevated CO_2 concentrations. The photosynthetic relationships in this study are reported on a needle surface area basis (all-sided).

After measuring each CO_2 response curve, needles were removed and stored at 0°C and later analyzed for surface area and dry mass as described previously (Crous and Ellsworth 2004). Homogenized subsamples of dried and ground needle tissue were analyzed for nitrogen concentration with an NA-1500 elemental analyzer (Carlo-Erba, Milan, Italy).

2.3.2. Statistical analyses

The dataset comprises over 310 photosynthetic CO_2 response curves taken from 1998-2005, the second through the ninth full season of elevated CO_2 exposure in FACE. Data for this study were collected by D.S. Ellsworth during the first five years of CO_2 exposure, data in years six to nine were collected by K.Y. Crous. In order to assess the response of pines to long-term elevated CO_2 exposure, and potentially elucidate the role of nitrogen in this response, I focused my analysis on the relationship between leaf photosynthesis and leaf N_{area}. This relationship has been hypothesized to be general (Field & Mooney, 1986a; Reich *et al.*, 1997) and any potential changes in this relationship identified in separate-slopes analysis in regression could be considered robust and hence useful in modeling photosynthesis on the basis of leaf nitrogen (Ollinger *et al.*, 2002).

The N_{area} of leaves at upper and lower canopy represent the nitrogen range throughout the canopy crown, which is used to examine photosynthesis-nitrogen relationships.

I fit the model of Farquhar *et al.* (1980) to the photosynthetic CO_2 response curve data as described by Ellsworth *et al.* (2004), using the temperature parameters in (Medlyn *et al.*, 2002) and (Bernacchi *et al.*, 2001). From this, I could analyze long-term trends in V_{cmax}, the maximum carboxylation capacity of leaves, and J_{max}, the maximum electron transport rate of leaves, as key biochemical components driving A_{net} (Farquhar *et al.*, 1980; Niinemets & Tenhunen, 1997). This was expected to provide insight into the specific components of the photosynthetic apparatus that were responsive to long-term elevated CO_2 exposure (Rogers & Ellsworth, 2002). To minimize artifacts of the fitting procedure for V_{cmax} and J_{max} in the data, I removed data with apparent problems such as a leaky chamber (Pons & Welschen, 2002), e.g., day respiration > 1.5 mmol m^{-2} s^{-1}), low stomatal conductance (stomatal conductance < 3 mmol m^{-2} s^{-1} during measurements), or failure to meet nutrient analysis standards using blind pine standards (National Institute of Standards and Technology, Boulder, CO USA). Less than 10% of the dataset failed these criteria. I predicted the fraction of nitrogen allocated to active-state Rubisco (fN_{rub}) as described previously (Niinemets & Tenhunen, 1997). This assumes that all of the activated Rubisco participates in carboxylation, and is an estimate of the nitrogen-use efficiency for carboxylation in the absence of mesophyll diffusional limitations.

All statistical analyses were performed with JMP (version 5, SAS Institute, Cary NC USA). Least-squares linear regression was used for statistical analyses because there is a strong precedent for linear leaf photosynthesis-nitrogen relationships, and because differences in regression relationships between leaf N_{area} and biochemical parameters underlying photosynthesis (V_{cmax}, J_{max}) could provide basic insights into photosynthetic biochemistry. A series of linear regressions were conducted to determine relationships between V_{cmax}, J_{max}, and leaf nitrogen concentration per unit area (N_{area}) with respect to CO_2 treatment, needle age, and time in the experiment. Based on previous work by Crous and Ellsworth (2004), analyses focused on one-year old needles in the canopy.

To test the effect of the duration of elevated CO_2 treatment on photosynthetic parameters in one-year old needles, the dataset was divided into three periods: an early period, e.g. the second and third years of complete elevated [CO_2]; a middle period, e.g.

the fifth to seventh year of elevated [CO_2]; and a late period, e.g. near the end of the experiment with eight to nine years of cumulative elevated [CO_2]. The results were not sensitive to varying these year-groupings by inclusion or removal of a year so long as the sample size was not unduly restricted. Differences in slope between each CO_2 treatment or each fertilization treatment were tested via a dummy variable representing the interaction term between the independent (N_{area}) and [CO_2] in the regression analyses.

2.4. Results

After almost ten years of elevated CO_2 exposure, A_{net} was still stimulated in elevated CO_2 compared to ambient CO_2 in both current-year (Fig. 4a) and one-year-old needles (Fig. 4b) across a two-fold range in leaf N_{area}. Photosynthetic enhancement in elevated CO_2 was characterized by an increase in the intercept of the A_{net} versus N_{area} relationship for both current- and one-year old needles (Fig. 4a-b). In addition to a difference in x-intercept, there was a significant increase in slope of A_{net} versus N_{area} for current-year needles in elevated CO_2 (Table 1, Fig. 4a-b). For the ambient [CO_2], the A_{net} versus N_{area} relationship between needle age classes had similar slopes and intercepts ($P >$ 0.1, Table 1). In contrast, for the elevated [CO_2] one-year old needles had lower slopes of A_{net} as a function of N_{area} than current-year needles ($P = 0.003$, Fig. 4a-b). To illustrate the magnitude of this [CO_2] effect, at a standardized mean N_{area} of 0.9 g m^{-2}, enhancement averaged +68 ± 6% (mean ± 95% confidence interval) for current-year needles and only 40 ± 3% for one-year-old needles.

The effect of elevated CO_2 on A_{net} (Fig. 4a-b) is the result of a combination of direct CO_2 stimulation of photosynthesis and offsetting reductions in photosynthetic capacity from enzyme down-regulation. In turn, changes in photosynthetic capacity could be due to changes in leaf N_{area}, changes in capacity per leaf N_{area}, or both. Changes in capacity can be estimated by changes in photosynthetic capacity for leaves developed in elevated vs. ambient CO_2 but measured at a common ambient [CO_2] of 365 μmol mol^{-1} ($A_{net\text{-}Ca}$). Significantly lower intercepts of $A_{net\text{-}Ca}$ as a function of N_{area} (Table 1) indicate that growth in elevated [CO_2] diminished photosynthetic capacity for one-year-old foliage (Fig. 4c,d). In addition, a nearly-significant difference in slopes ($P = 0.058$, Table

1) for one-year old foliage suggests that elevated CO_2-induced reductions in $A_{net\text{-}Ca}$ are greater at higher N_{area}. Leaf N_{area} did not vary with treatment [CO_2] for any of the periods examined (Table 2; $P > 0.10$). In combination, these results indicate that elevated CO_2 decreased photosynthetic capacity due to decreased photosynthesis per unit N_{area} rather than decreased leaf N_{area}.

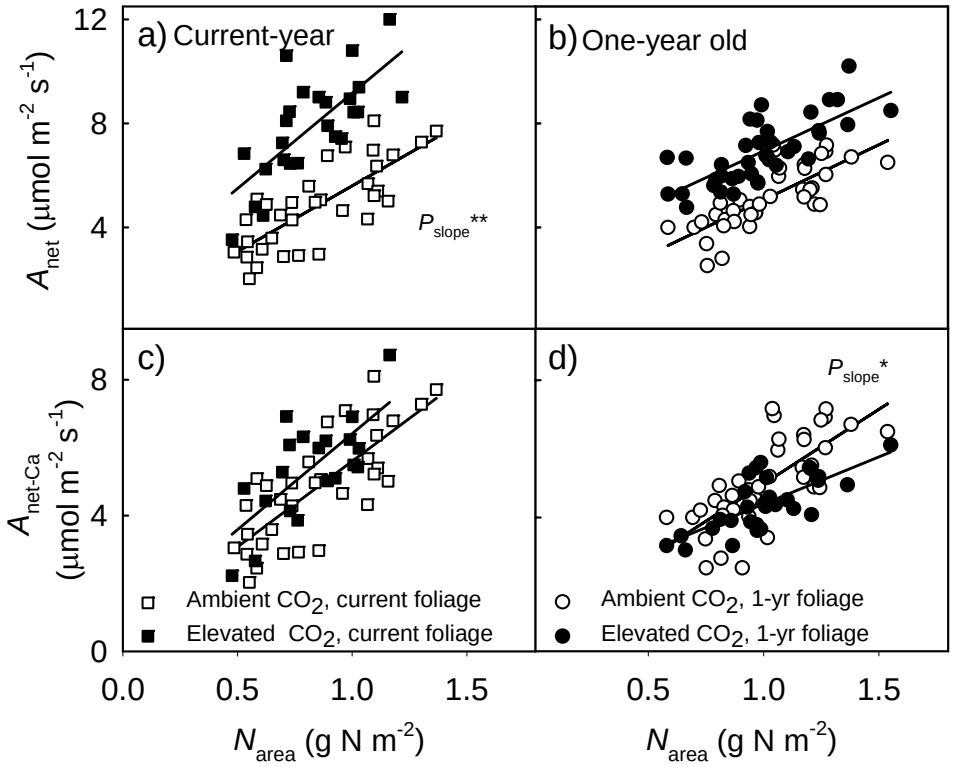

Figure 4: Relationships of photosynthesis in current growth conditions (A_{net}) (top panels) and photosynthesis at a common CO_2 ($A_{net\text{-}Ca}$) (bottom panels) with leaf nitrogen concentration on an area basis (N_{area}). The left panels represent the relationship for current-year foliage (squares) whereas the right panels represent the relationships for one-year old foliage (circles; 1-yr) in ambient (open symbols) and elevated (filled symbols) [CO_2] across the fifth to ninth growing seasons of elevated [CO_2] exposure in unfertilized conditions. Differences in slope between CO_2 treatments are indicated as P_{slope} * for $P \leq 0.10$ and P_{slope} ** for $P \leq 0.05$. Regression equations and statistics are in Table 1.

Table 1: Linear regression statistics for relationships between photosynthetic variables and leaf N_{area} in *P. taeda* across different time frames of the experiment with different CO_2 treatments. The variables used to describe the relationships and equations are the photosynthesis measured at the [CO_2] target for long-term growth (A_{net}, μmol m^{-2} s^{-1}), photosynthesis at a common [CO_2] level ($A_{net\text{-}Ca}$, μmol m^{-2} s^{-1}), maximal carboxylation rate (V_{cmax}, μmol m^{-2} s^{-1}), maximal electron transport rate (J_{max}, μmol m^{-2} s^{-1}), and leaf nitrogen content expressed on an area basis (N_{area}, g N m^{-2}). Significance levels for individual regression relationships are ^{ns} when not significant, ** for $P < 0.05$, *** for $P < 0.001$. Differences in intercept and slope between CO_2 treatments (Ambient vs. Elevated) within years of the experiment (Yrs) and age class are indicated in the appropriate columns. Fig. shows the numbered Figure where the relationships are displayed.

Relationship	[CO_2] Treat	N Treat	Age Class	Yrs	Equation	R^2	[CO_2] effect on intercept	[CO_2] effect on slope	Fig.
A_{net}-N_{area}	Ambient	Amb N	Current-year	5-9	$A_{net} = 0.55 + 5.05*N_{area}$	0.60^{***}			4
	Elevated	Amb N	Current-year	5-9	$A_{net} = 1.13 + 8.28*N_{area}$	0.57^{***}	<0.0001	0.048	4
	Ambient	Amb N	One-year old	5-9	$A_{net} = 0.87 + 4.21*N_{area}$	0.58^{***}			4
	Elevated	Amb N	One-year old	5-9	$A_{net} = 3.04 + 3.87 *N_{area}$	0.58^{***}	<0.0001	0.68	4
A_{net-Ca}-N_{area}	Ambient	Amb N	Current-year	5-9	$A_{net-Ca} = 0.55 + 5.05*N_{area}$	0.60^{***}			4
	Elevated	Amb N	Current-year	5-9	$A_{net-Ca} = 0.76 + 5.65*N_{area}$	0.51^{***}	0.022	0.69	4
	Ambient	Amb N	One-year old	5-9	$A_{net-Ca} = 0.87 + 4.21*N_{area}$	0.58^{***}			4
	Elevated	Amb N	One-year old	5-9	$A_{net-Ca} = 1.74 + 2.66*N_{area}$	0.59^{***}	0.0001	0.058	4
V_{cmax}-N_{area}	Ambient	Amb N	One-year old	2-3	$V_{cmax} = 21.11 + 13.49*N_{area}$	0.18^{ns}			5
	Elevated	Amb N	One-year old	2-3	$V_{cmax} = 11.05 + 17.15*N_{area}$	0.30^{ns}	--	--	5
	Ambient	Amb N	One-year old	5-7	$V_{cmax} =12.76 + 15.99*N_{area}$	0.31^{**}			5
	Elevated	Amb N	One-year old	5-7	$V_{cmax} =14.24 + 12.25*N_{area}$	0.44^{***}	0.10	0.57	5
	Ambient	Amb N	One-year old	8-9	$V_{cmax} = 5.61 + 27.06*N_{area}$	0.52^{***}			5
	Elevated	Amb N	One-year old	8-9	$V_{cmax} =16.67 + 9.81*N_{area}$	0.22^{**}	0.0003	0.028	5
J_{max}-N_{area}	Ambient	Amb N	One-year old	2-3	$J_{max} = 40.40 + 26.61*N_{area}$	0.11^{ns}			5
	Elevated	Amb N	One-year old	2-3	$J_{max} = 31.71 + 25.05*N_{area}$	0.45^{ns}	--	--	5
	Ambient	Amb N	One-year old	5-7	$J_{max} = 17.49 + 39.37*N_{area}$	0.43^{***}			5
	Elevated	Amb N	One-year old	5-7	$J_{max} = 33.75 + 19.60*N_{area}$	0.40^{***}	0.18	0.11	5
	Ambient	Amb N	One-year old	8-9	$J_{max} = 17.57 + 39.77*N_{area}$	0.70^{***}			5
	Elevated	Amb N	One-year old	8-9	$J_{max} = 32.48 + 19.25*N_{area}$	0.30^{**}	0.0055	0.044	5

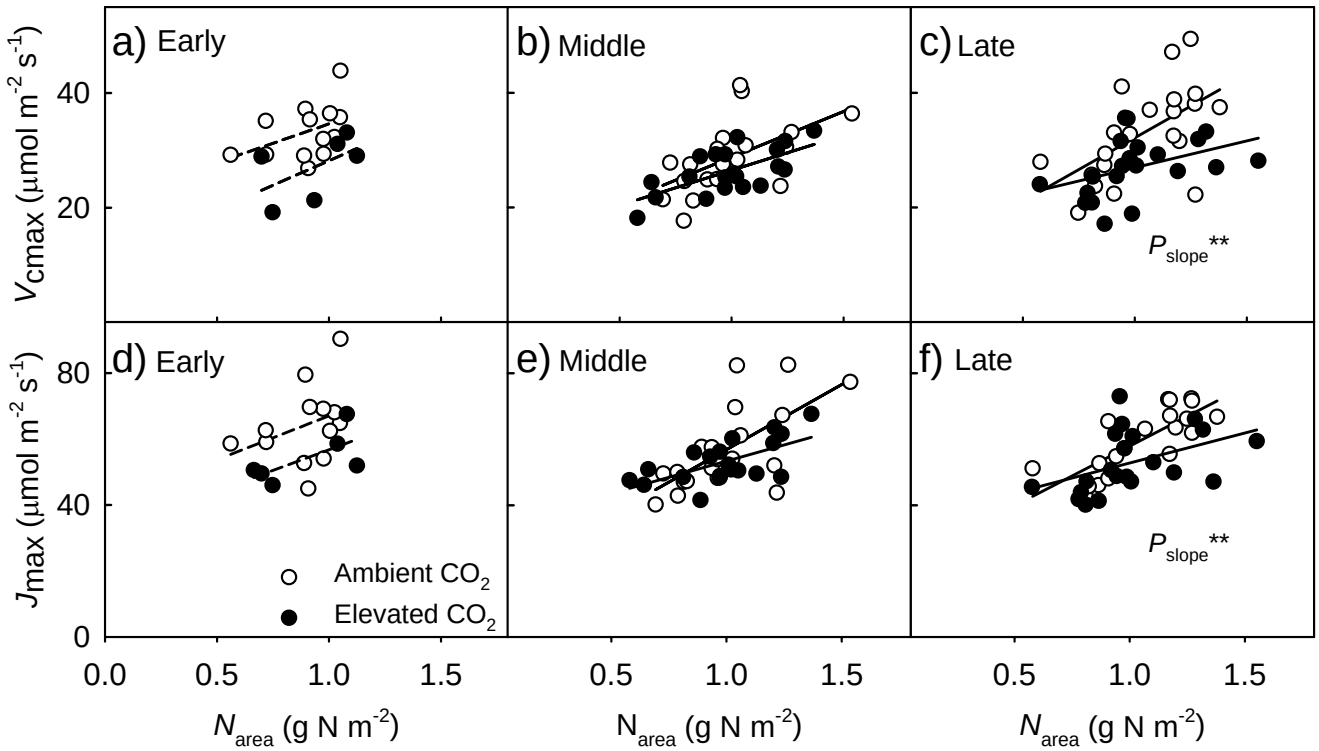

Figure 5: Relationships for the physiological parameters V_{cmax} (upper panels a-c) and J_{max} (lower panels d-f) as a function of leaf nitrogen (N_{area}) by CO_2 treatment in early (2nd and 3rd years of elevated CO_2 treatment, left panels), middle (5th to 7th year of elevated CO_2 treatment, middle panels) and late (8th to 9th year of CO_2 treatment, right panels) time periods of the experiment in unfertilized, one-year old foliage. Open circles represent ambient [CO_2], filled circles represent the elevated [CO_2]. Significant differences in slopes between CO_2 treatments are indicated as in Fig. 4. Regression equations and statistics are in Table 1.

Lower photosynthetic capacity at elevated CO_2 could be due to lower electron transport capacity, lower capacity for enzymatic CO_2 fixation, or both. Similar to relationships for $A_{net\text{-}Ca}$, both V_{cmax} (a measure of Rubisco activity for CO_2 fixation) and J_{max} (a measure of electron transport) were generally strongly correlated with leaf N_{area} in one-year old needles ($P < 0.001$; Table 1, Fig. 5). The exception was that neither V_{cmax} nor J_{max} were related to N_{area} early in the experiment (2nd and 3rd years; Fig. 5 a,d) where variation in N_{area} in the canopy was small. Similar to relationships for $A_{net\text{-}Ca}$, trees grown in ambient CO_2 conditions showed no significant variation in V_{cmax}-N_{area} and J_{max}-N_{area} relationships across time (Table 1) and no significant time-dependent change in slopes, based on pairwise comparisons of slopes across the time

periods (Fig. 7 and data not shown). Growth in elevated [CO_2] did affect V_{cmax}-N_{area} and J_{max}-N_{area} relationships. In general, compared to ambient CO_2 needles, elevated CO_2 needles tended toward lower V_{cmax} and J_{max} at a given N_{area}. Such differences were greater at greater needle N_{area} (e.g., slope term; Table 1) and with longer duration of CO_2 exposure (Table 4, Fig. 5, Fig. 6). In the 8th and 9th years of the experiment this difference resulted in a 64% reduction in the V_{cmax}-N_{area} slope with elevated versus ambient CO_2 and a 52% reduction in the J_{max}-N_{area} slope (Fig. 6, Table 1).

Table 2: Analysis of variance of predicted fraction of nitrogen allocated to Rubisco (fN_{rub}) and total leaf nitrogen (N_{area}) for upper canopy pine foliage at the Duke FACE site over three different periods of years (Period) in the 9-year experiment. The early, middle and late periods of the experiment are defined in the Methods section. MS is mean square error, d.f. denotes degrees of freedom and N.S. denotes non-significant variables ($P > 0.1$).

		fN_{rub}		N_{area}	
Source	d.f.	MS	*P*-value	MS	*P*-value
Ageclass	1	0.0236	<0.0001	0.32077	0.0036
CO_2 Treatmentt	1	0.00044	N.S.	0.00280	N.S.
Period	2	0.0041	0.0332	0.06586	N.S.
Ageclass*Period	2	0.00079	N.S.	0.03452	N.S.
Ageclass*CO_2 Treatment	1	0.0052	0.0036	0.00733	N.S.
Period *CO_2 Treatment	2	0.0007	N.S.	0.06456	N.S.
Ageclass* Period *CO_2 Treatment	2	0.0011	0.0866	0.04658	N.S.
Residual error	83	0.00032	-	0.03578	-

To further examine allocation to carboxylating capacity I calculated the fraction of leaf nitrogen apparently allocated to Rubisco (fN_{rub}) from V_{cmax} and needle N_{area} (Ellsworth *et al.*, 2004). Apparent fN_{rub} was significantly different between different foliage age classes ($P < 0.0001$, Table 2). However, there was also a significant needle age class × CO_2 concentration interaction ($P = 0.0036$). Moreover, a weak 3-way interaction of needle age class × CO_2 concentration × Period ($P < 0.09$, Table 2) indicated lower fN_{rub} in one-year-old needles under elevated [CO_2] in years 5-9 of the elevated [CO_2] treatment, consistent with the reduction in the V_{cmax}-N_{area} slope observed in the later years of the experiment (Fig. 5c, Fig. 6a). The apparent fN_{rub} in one-year old foliage in the upper crown of mature *P. taeda* trees declined 15% in elevated [CO_2], from 10.3 ± 0.6 (mean ± s.e.) to 8.7 ± 0.5 with eight to nine years of elevated [CO_2] in FACE.

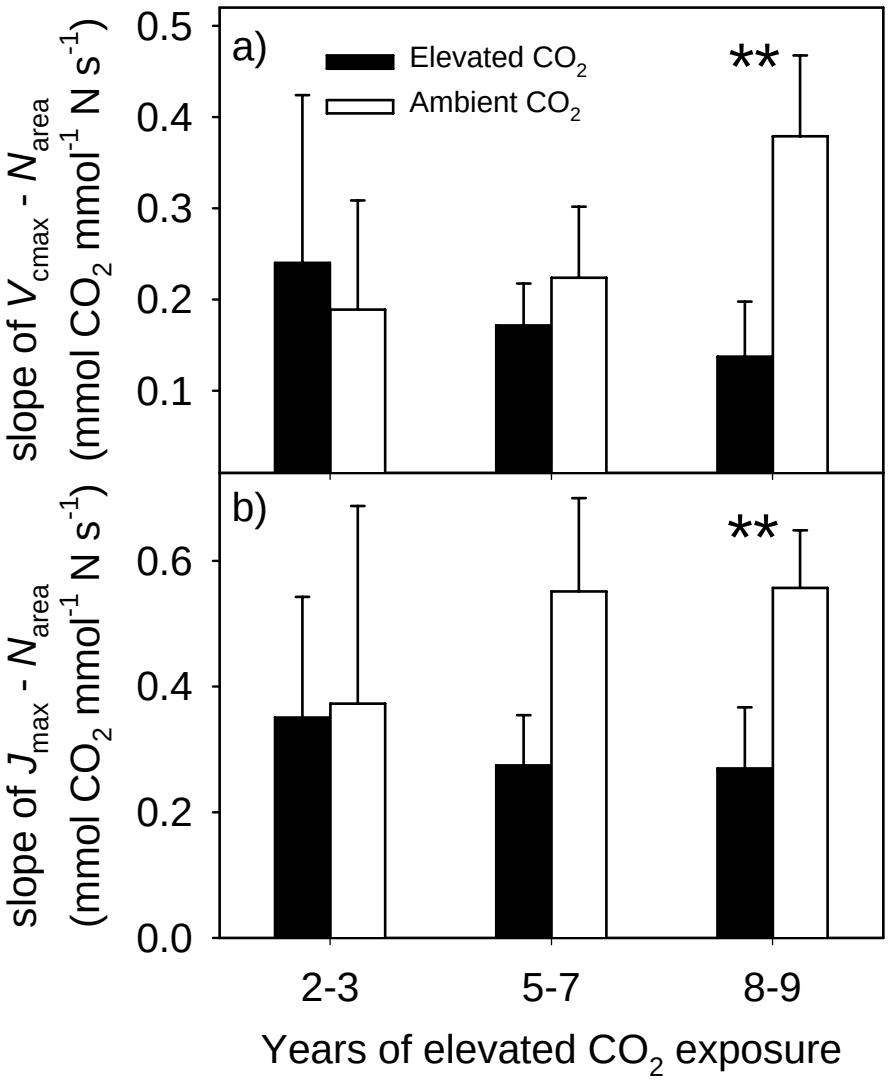

Figure 6: Slope values with estimated standard errors over duration of CO_2 exposure defined as early in the experiment (2^{nd} and 3^{rd} year of elevated [CO_2]), middle (5^{th} to 7^{th} years of elevated [CO_2]) and late (8^{th} and 9^{th} years of elevated [CO_2]). Black bars represent elevated atmospheric [CO_2] and white bars represent ambient [CO_2]. There is a clear decline of slope values over time in elevated [CO_2] but not in ambient [CO_2] in both the V_{cmax}-N_{area} (a) and J_{max}-N_{area} (b) relationships. The exposure [CO_2] difference in slopes is significant ($P \leq 0.05$) in the 8^{th} and 9^{th} years of elevated [CO_2], indicated via the asterices.

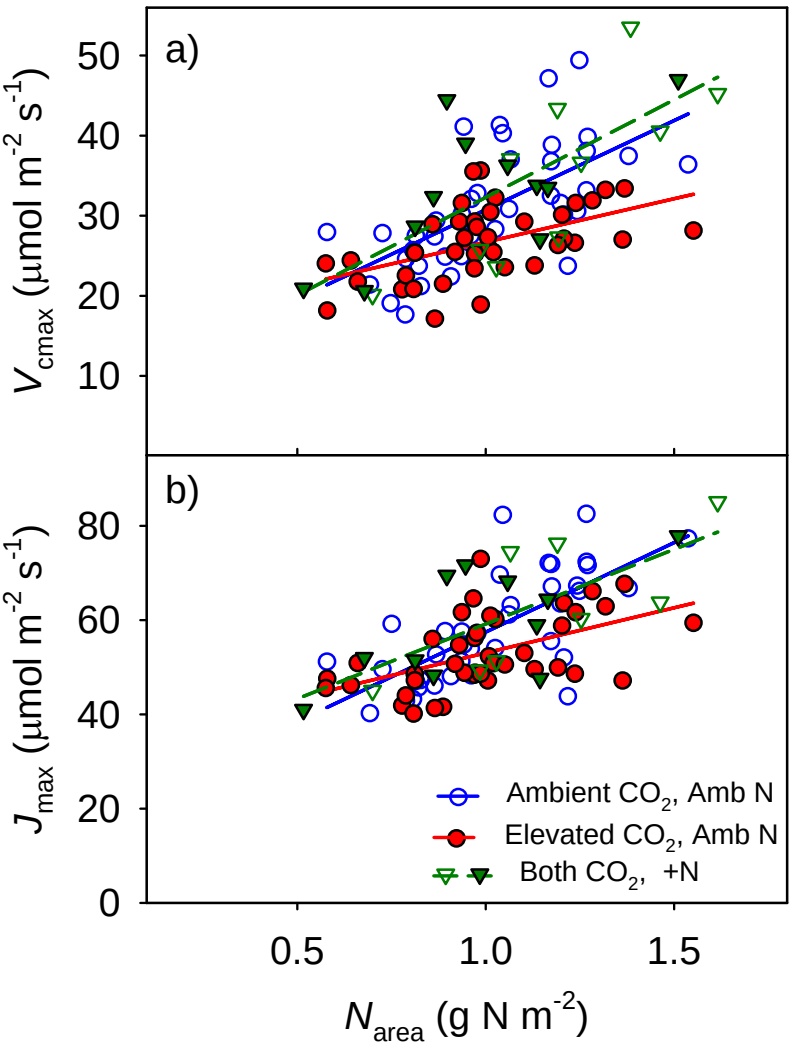

Figure 7: Relationship of maximal carboxylation rate (V_{cmax}, a) and maximal electron transport rate (J_{max}, b) as a function of N_{area} across the fifth to ninth year of elevated CO_2 exposure for unfertilized one-year old foliage (amb N) between CO_2 treatments (circles) and for fertilized one-year old leaves (+N) across CO_2 treatments only (triangles). Fertilization was done in the ninth year of the CO_2 treatment only. Open symbols represent ambient CO_2 conditions, filled symbols represent elevated CO_2 conditions. There was no difference in slope between ambient and elevated [CO_2] for fertilized leaves in the ninth year of elevated CO_2 exposure. Relationship equations and statistics are as follows: For Ambient CO_2, Amb N (solid blue line): $V_{cmax} = 8.45 + 22.29*N_{area}$, $R^2 = 0.40$, $P < 0.0001$; $J_{max} = 17.41 + 39.73*N_{area}$, $R^2 = 0.53$, $P < 0.0001$. For Elevated CO_2, Amb N (solid red line): $V_{cmax} = 15.57 + 10.91*N_{area}$, $R^2 = 0.30$, $P = 0.0002$; $J_{max} = 33.31 + 19.23*N_{area}$, $R^2 = 0.33$, $P = 0.0001$. Relationships in ambient and elevated CO_2 were similar in fertilized leaves and were pooled together. For both [CO_2], +N (dashed green line): $V_{cmax} = 7.86 + 24.40*N_{area}$, $R^2 = 0.52$, $P = 0.0002$; $J_{max} = 27.43 + 31.73*N_{area}$, $R^2 = 0.50$, $P = 0.0007$. The relationships for fertilized trees are not significantly different from that of Ambient CO_2, Amb N trees ($P > 0.10$).

For all three measures of photosynthetic and biochemical capacity in one-year needles (e.g., $A_{net\text{-}Ca}$, V_{cmax}, and J_{max}) the depression in the slopes of their relationships with N_{area} after long term exposure to elevated CO_2 (Table 1) was completely ameliorated by nitrogen fertilization (Fig. 7). Measurements from fertilized needles from both ambient and elevated [CO_2] had similar slopes ($P > 0.1$, Fig. 7 and data not shown). Slopes of both the V_{cmax}-N_{area} and J_{max}-N_{area} relationships in one-year old needles grown in elevated [CO_2] with nitrogen fertilization recovered to values similar to those measured in ambient CO_2 conditions (green dashed line versus solid blue line, Fig. 7).

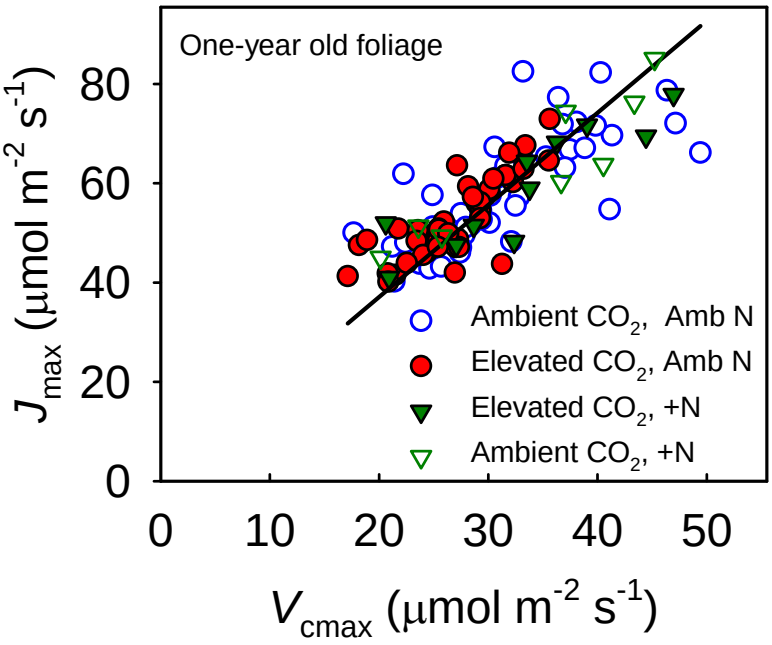

Figure 8: Relationship between J_{max} and V_{cmax} in ambient [CO_2] (open symbols) and elevated [CO_2] (filled symbols) for unfertilized (Amb N) and fertilized (+N) one-year old foliage across 2001-2005. Fertilized leaves (+N, triangles) represent only the ninth growing season of elevated CO_2 exposure. Relationship equations and statistics are as follows: For one-year old needles, Ambient CO_2, Amb N: $J_{max} = 1.82^* V_{cmax}$, $P < 0.0001$; Elevated CO_2, Amb N: $J_{max} = 1.95^* V_{cmax}$, $P < 0.0001$; Ambient CO_2, +N: $J_{max} = 1.83^* V_{cmax}$, $P < 0.0001$; Elevated CO_2, +N: $J_{max} = 1.76^* V_{cmax}$, $P < 0.0001$. All one-year old needles together: $J_{max} = 1.86^* V_{cmax}$, $P < 0.0001$.

The ratio of J_{max} to V_{cmax} was remarkably constant over time and between elevated CO_2 and CO_2 × N fertilization treatments. There was no difference in the slope of J_{max}-V_{cmax} relationship after nine years of elevated CO_2 exposure in one-year old needles (Fig. 8) and in current-year needles (data not shown) across fifth to ninth

growing season of elevated CO_2 exposure. There was also no evidence of an effect of nitrogen fertilization on the J_{max}:V_{cmax} ratio (Fig. 8).

2.5. Discussion

I found evidence for photosynthetic down-regulation in aging pine foliage of *P. taeda* with nine years of elevated CO_2 (Fig. 4). These findings support previous results from the Duke FACE experiment by Rogers and Ellsworth (2002) and Crous and Ellsworth (2004), and are consistent with results from other conifers exposed to elevated CO_2 (Turnbull *et al.*, 1998; Tissue *et al.*, 1999; Jach & Ceulemans, 2000). Contrary to what was previously thought, the observed down-regulation in aging needles was not due to changes in leaf N_{area}, because the overall V_{cmax}-N_{area} and J_{max}-N_{area} relationships were affected via reduced steepness in the slopes (Tables 1, 2), suggesting N allocated awas from photosynthetic components in one-year-old foliage. Moreover, the relationship between $A_{net\text{-}Ca}$ and leaf N_{area}, another measure of photosynthetic capacity, was similarly affected (Fig. 4d). The decreases in V_{cmax}-N_{area} slopes in one-year needles with elevated CO_2 became more pronounced over time (23 to 64% reduction in slope) resulting in a statistically significant CO_2 effect on one-year old needles late in the experiment (Fig. 5, Fig. 6, Table 1). As elevated CO_2 exposure continued, changes in slope steepness suggest significant down-regulation of V_{cmax} over time (Fig. 6), which is mirrored by similar down-regulation in J_{max}. This resulted in a decrease in photosynthetic enhancement of 37% in one-year old needles in elevated CO_2. Down-regulation in one-year-old needles can reduce tree productivity and hence C uptake from the atmosphere because one-year old needles contain the majority of the tree crown.

Given of the magnitude of the positive x-intercepts of the V_{cmax}-N_{area} and J_{max}-N_{area} relationships in one-year old needles (Table 1), the net effect of the relatively large CO_2-induced changes in slope resulted in a smaller proportional decrease in photosynthetic capacity at a common [CO_2] (e.g. about -14%; Fig. 4d). Because elevated CO_2 effects were noticeable on one-year old needles but not current-year needles measured at the same time, it appears that needle age-related declines in photosynthetic capacity are enhanced by long-term elevated CO_2 (Jach & Ceulemans,

2000; Rogers & Ellsworth, 2002; Crous & Ellsworth, 2004) rather than declines in leaf N_{area}.

The CO_2-induced change in form of the V_{cmax}-N_{area} relationship likely represents a reduction of nitrogen allocated to Rubisco (Table 2), and suggests that the nitrogen-use per unit carboxylation capacity is reduced in one-year old foliage. This reduction is compensated by the well-documented stimulation of carboxylation rates in elevated CO_2 with suppression of photorespiration (Long *et al*., 2004, Rogers & Ainsworth 2007). Therefore the overall A_{net} enhancement by elevated CO_2 of 40% in one-year old needles after eight to nine years of elevated CO_2 exposure still results in increased photosynthetic nitrogen use-efficiency in elevated CO_2. Given that new foliage represents a large nitrogen sink for the canopy, the inferred reductions in nitrogen allocated to photosynthetic capacity in one-year old needles may be a determinant of how much foliage can be supported in the *P. taeda* stand in elevated CO_2 on these infertile soils (Finzi *et al.* 2002).

In highly N-limited soils in which where many pine species are important, nitrogen reallocation from one-year old to current-year foliage occurs by mobilization of leaf soluble protein nitrogen (Fife & Nambiar, 1984; Cherbuy *et al.*, 2001). Reallocation of nitrogen from old foliage to current-year foliage can provide a mechanism to supply nitrogen to growing foliage at branch apices and thus maximize whole plant carbon gain (Field, 1983; Hirose & Werger, 1987). I expected that the reduction in photosynthetic capacity in one-year old needles in elevated CO_2 would be alleviated when nitrogen availability was increased (Farage *et al.*, 1998). In my study, there was a large response to the nitrogen fertilization in the first year of application, even in needles that developed prior to fertilization. Nitrogen fertilization ameliorated the CO_2-induced down-regulation effect on photosynthetic capacity in one-year-old foliage. This was shown as a recovery of the slope of both V_{cmax}- and J_{max}-N_{area} relationships in fertilized conditions under elevated CO_2 (Fig. 7). Given that the reduction in photosynthetic capacity in one-year old foliage resulted in a reduction in the apparent fraction of nitrogen in Rubisco (Table 2), the fertilization-induced increase in nitrogen-use efficiency is achieved by an increased nitrogen allocation to the photosynthetic apparatus with increased available nitrogen (Hikosaka & Hirose, 1998; Poorter & Evans, 1998; Westbeek *et al.*, 1999). A strong response to nitrogen fertilization suggests that there may have been N-limitations to growth via changes in

photosynthetic functioning in long-term elevated CO_2 supporting earlier observations of reduced nitrogen mineralization in elevated CO_2 (Finzi *et al.*, 2006).

Taken together, my results suggest changes in nitrogen allocation in response to environmental conditions with nitrogen allocated away from carboxylation and RUBP regeneration in long-term elevated CO_2 (Fig. 6) while, in contrast, nitrogen fertilization induced nitrogen investment towards photosynthetic components (Fig. 7). Therefore, I speculate that nitrogen invested in the carboxylation enzyme may act as an indicator of the physiological nitrogen demand of plants.

The ratio between J_{max} and V_{cmax} was not affected by treatments of long-term elevated CO_2 conditions or short-term fertilization treatments, suggesting strong coordination between photosynthetic components in elevated CO_2. A conservative ratio of J_{max}:V_{cmax} across treatments within one species in this study (Fig. 8) is consistent with patterns across many plant species (Medlyn, 1996; Leuning, 1997; Medlyn *et al.*, 1999; Warren *et al.*, 2003). In contrast, the meta-analysis from (Ainsworth & Rogers, 2007) shows that V_{cmax} was reduced in long-term elevated CO_2 by about twice that of J_{max}. A constant J_{max}:V_{cmax} ratio with environmental conditions that force adjustments in photosynthetic capacity such as shade (Kull & Niinemets, 1998; Hikosaka, 2005), low nutrient availability (Ainsworth *et al.*, 2003), and elevated CO_2 (Medlyn, 1996; Midgley *et al.*, 1999; Onoda *et al.*, 2005) suggests that there may be coordination of the activity of different photosynthetic components (Reynolds *et al.*, 1992; Chen *et al.*, 1993; Medlyn, 1996). A constant J_{max}:V_{cmax} ratio also suggests no nitrogen reallocation between photosynthetic components (Medlyn *et al.* 1999), which is supported in my results by a fairly equal magnitude of down-regulation in carboxylation and electron transport components manifested by a similar reduction in slope in long-term elevated CO_2 (Fig. 5c, f, Fig. 6). Coordination in different components of the photosynthetic apparatus is maintained via the allocation of nitrogen to avoid an imbalance between limitations by the 'light-dependent' and 'light-independent' portions of the photosynthesis process (Chen *et al.*, 1993). Insight into the reallocation of nitrogen and N-partitioning within the photosynthetic apparatus could elucidate the mechanism enabling plants to adjust to changing environmental conditions (Field *et al.*, 1992; Onoda *et al.*, 2004).

2.6. Conclusions

After almost a decade of exposure to elevated [CO_2] in FACE, photosynthesis of different needle age classes of *P. taeda* was still stimulated in elevated [CO_2] compared to ambient [CO_2]. However, reductions in photosynthetic capacity in aging needles in elevated [CO_2] were evident in *P. taeda*. Strong reductions in the slope of the relationship between leaf photosynthetic capacity ($A_{net\text{-}Ca}$) and leaf N_{area} (by 40 ± 3%) in one-year old needles with five to nine years of elevated CO_2 exposure were evident, whereas no significant reduction was observed in current-year needles. I also found evidence for changes in V_{cmax}-N_{area} and J_{max}-N_{area} relationships in one-year old needles after eight to nine years of elevated CO_2, with slopes declining by about 50-60%. Decreasing photosynthetic capacity, evident as reductions in the slope of V_{cmax}- and J_{max} as a function of N_{area}, may suggest limited nitrogen pools for foliage growth at the Duke Face site after nearly a decade of elevated CO_2 exposure. Because nitrogen fertilization caused the recovery of the slopes of these relationships in elevated [CO_2] to those similar to ambient control trees, I attribute the elevated [CO_2]-induced reductions in photosynthetic capacity to reductions in the allocation of nitrogen to Rubisco and proteins involved in the electron transport process. Decreases in the allocation of nitrogen to photosynthetic processes may serve to increase mobile and available nitrogen for new foliage growth. Reallocation of nitrogen pools among foliage cohorts (e.g., from one-year-old to current-year foliage) could provide a mechanism for plant adjustments to environmental perturbations such as rising atmospheric [CO_2] or increasing nitrogen availability.

The relationships between leaf photosynthetic capacity, V_{cmax}, and J_{max} all as a function of leaf N_{area} are widely used in scaling leaf responses to the canopy and for gaining insight into ecosystem-scale responses to elevated atmospheric [CO_2] (McMurtrie & Wang, 1993; Friend *et al.*, 1997; White *et al.*, 2000). Including dynamic photosynthetic nitrogen allocation along with canopy nitrogen dynamics in pines may improve physiological process models in order to estimate future atmospheric CO_2 concentrations and plant feedbacks to these CO_2 concentrations.

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Chapter 3

Maintenance of leaf N controls the CO_2 response of grassland species exposed to nine years of Free-Air CO_2 Enrichment

3.1. Summary

The continued ability of grasslands to serve as sinks for atmospheric CO_2 may depend on species responses to nitrogen (N) availability. To study this, N was added to a C_3 grassland in Minnesota exposed to elevated atmospheric CO_2 (e.g., 560 μmol CO_2 mol^{-1}) in a 9-year experiment. Across seven grassland species, elevated CO_2 reduced leaf photosynthetic capacity measured at a common CO_2 concentration (A_{m365}) and reduced leaf N concentration (N_{mass}) in N-addition plots but not in unamended plots, demonstrating a significant CO_2 x N interaction for A_{m365} ($P < 0.005$) and N_{mass} ($P < 0.05$).

Plant functional groups differed in elevated CO_2 and N treatment responses. With added N, elevated CO_2 reduced in leaf N_{mass} concentrations (-26% in N_{mass}) and photosynthetic capacity (-28% in carboxylation capacity, V_{cmax}) in C_3 forbs but not in C_3 grasses. Hence, leaf photosynthetic rates were enhanced by 68% for C_3 grasses in elevated CO_2, whereas forbs did not significantly increase photosynthesis in elevated CO_2.

Maintenance of leaf N, possibly through increased root foraging, is necessary to sustain stimulation of photosynthesis in this grassland under long-term elevated CO_2. Different effects of elevated CO_2 on leaf N and photosynthesis for forbs versus grasses suggests a high potential for shifts in species composition in this grassland ecosystem.

3.2. Introduction

With increasing CO_2 emissions from human activities driving increases in mean global atmospheric [CO_2], there are concerns over the capacity of natural ecosystems to continue to serve as sinks for atmospheric CO_2 over decades to come (Canadell *et al.*, 2007). During the 20th century, the carbon (C) sink capacity of native grasslands has been variously attributed to changes in climate, atmospheric CO_2, and nitrogen (N) deposition. However, our understanding of the interactions among these factors and the mechanisms of these interactions remains incomplete (Schimel *et al.*, 2001). Long-term field experiments in which multiple factors are manipulated simultaneously provide an important tool for untangling ecological interactions (Hunter, 2001; Mikkelsen *et al.*, 2008). Because ecosystem C and N cycles are strongly coupled, interactive effects of elevated CO_2 and N availability are likely, and may limit the magnitude of photosynthetic enhancement under elevated CO_2 (McMurtrie & Comins, 1996; Rastetter *et al.*, 1997; Luo *et al.*, 2004).

Plant N pools and photosynthesis-leaf N relationships couple ecosystem C and N cycles. Long-term elevated CO_2 can cause a reduction in leaf N and hence plant productivity, particularly when root N uptake is not enhanced to support increased growth demands in elevated CO_2 (Field *et al.*, 1992; Luo *et al.*, 1994). In contrast to the reduction in leaf N under elevated [CO_2] (Yin, 2002), addition of N to soils would be expected to increase leaf N (Field *et al.*, 1992). However, these opposite responses in leaf N to current human activities demand that we understand interactions between N availability and atmospheric CO_2 concentration to predict how ecosystem functioning will change in an increasingly eutrophic biosphere (Vitousek, 1994).

Grasslands cover a wide range of climatic conditions, soil types, and nutrient availabilities across the world, and consist of many interacting species. Thus, in understanding how N availability can affect plant responses to elevated CO_2 in grasslands, it is critical to understand species responses and how these ultimately affect responses of the ecosystem to CO_2 and N, including shifts in species composition and ecosystem services (Vitousek *et al.*, 1997; Scurlock & Hall, 1998; Soussana & Lüscher, 2007). Only a very small number of grassland experiments have assessed long-term interactions between elevated CO_2 and N (Tilman *et al.*, 2006) and most but not all of these have found that increased plant growth under elevated CO_2

can only be sustained with sufficient N supply (Lüscher *et al.*, 2000; Grünzweig & Körner, 2003; Schneider *et al.*, 2004; Reich *et al.*, 2006a). Recent studies have found substantial growth responses in elevated CO_2 because additional N was taken up from deeper soil layers (Iversen *et al.*, 2008). The higher N demands of plants under elevated CO_2 could constrain plant growth if N is limiting, especially when elevated CO_2 has stimulated N immobilization (Henry *et al.*, 2005; Knops *et al.*, 2007).

Plant species can vary in their responses to environmental change, including rising atmospheric CO_2 and N addition (Zanetti *et al.*, 1997; Joel *et al.*, 2001; Lee *et al.*, 2001; Poorter & Perez-Soba, 2001; Reich *et al.*, 2004). Currently, it is unclear how these differential plant responses will affect long-term composition, structure and function of species-rich ecosystems (Potvin *et al.*, 2007). Given the inability to conduct appropriate elevated CO_2 experiments on high numbers of plant species simultaneously, there has instead been a focus on key functional traits shared by species in functional groups that can be represented in models, with varying success (Poorter & Navas, 2003; Reich *et al.*, 2004). It has been hypothesized that a number of intrinsic physiological leaf traits, such as photosynthetic rates, specific leaf area (SLA) and foliar nitrogen, central to how species functional groups are depicted in such models, determine the response of species to elevated CO_2 (Woodward & Cramer, 1996; Lavorel *et al.*, 1997). However, species grouping schemes do not always sufficiently describe physiological responses to elevated CO_2 (Reich *et al.*, 2003; Ellsworth *et al.*, 2004b). Because grasslands are often relatively species-rich, it is important to consider if species groupings provide insight into species responsiveness to elevated CO_2 in grass-dominated systems, which constitute 30% of global land area (Soussana & Lüscher, 2007).

The objectives of this study were to investigate physiological mechanisms underlying species responses to elevated CO_2 and N deposition, and potential interactions among atmospheric [CO_2] and leaf N in species from contrasting functional groups. I studied C_3 grass and forb species across the sixth to ninth years of elevated CO_2 exposure and N addition in a nutrient-poor prairie grassland in Minnesota to address the following hypotheses:

H.1. Long-term reductions in foliar N under elevated CO_2 are reflected in declining photosynthetic capacity such that the instantaneous CO_2 enhancement effect is offset by photosynthetic down-regulation. This results

in little or no change in photosynthetic performance in elevated CO_2 in nutrient-poor grasslands.

H.2. Maintenance of leaf N is necessary to sustain photosynthetic enhancement responses to elevated [CO_2] among C_3 grassland species.

H.3. Nutrient addition can compensate for reduced foliar N under elevated CO_2 such that photosynthetic capacity of C_3 grassland species remains unchanged or increased.

I examined these hypotheses in a long-term grassland free-air CO_2 enrichment (FACE) experiment where atmospheric [CO_2] and soil N were manipulated for multiple C_3 grassland species (Reich *et al.*, 2001a).

3.3. Materials and Methods

3.3.1. Site Description and Experimental Design

The BioCON (Biodiversity, CO_2 and N) FACE experiment is part of the Long-term Ecological Research network and is located in central Minnesota, USA (45° 24' 13.5" N, 93° 11' 08" W). The site is located in a humid continental climate on glacial outwash comprised of loamy sand soils with very low nutrient availability (Grigal *et al.*, 1976). The mean annual precipitation is 660 mm $year^{-1}$ and the mean maximum July temperature is 28.3°C.

The BioCON FACE experiment consists of six circular plots of 20m diameter, three of which control atmospheric [CO_2] to 560 μmol mol^{-1} whereas three plots remain at ambient [CO_2]. Daytime exposure of plots to elevated [CO_2] proceeds continuously from the beginning of the frost-free season in late April until the end of the growing season in September. The plants were established in 1997, with the first season of CO_2 fumigation in 1998.

A subset of the complete FACE experiment (see Reich *et al.* 2001b) was used for the analyses here, specifically 2x2 m subplots within the six FACE rings with monocultures of C_3 grass or non-leguminous forb species. Monocultures were used to assess species responses rather than mixtures since the emphasis was on independent species responses to the treatment factors. Among these subplots, soil N addition treatments had been randomly assigned in two replicates in a split-plot design since the start of the experiment in 1998. N addition consisted of 4 g N m^{-2} yr^{-1} in the form

of solid ammonium nitrate applied each year across May, June and July. There were 8 monoculture subplots of each of the seven species equally divided across the four combinations of CO_2 and N-addition treatments. Above- and belowground biomass of these plots, provided by P.B. Reich, were determined each year in June by harvest of a subsample area of the main plot (Reich *et al.*, 2001b). One m^2 area of vegetation was clipped just above the soil surface for harvest, sorted into live and senesced material, dried and weighed. Roots were isolated from three 5-cm diameter soil samples taken to 20 cm depth from each plot where aboveground biomass had been sampled. The roots were washed and sorted into fine (<1mm diameter), coarse and crown roots, then dried and weighed. The species chosen for this study were four C_3 grasses: *Poa pratensis* L.*, Koeleria cristata* Pers.*, Bromus inermis* Leyss. and *Agropyron repens* L. and three forb species: *Solidago rigida* L. and *Anemone cylindrica* A. Gray and *Achillea millefolium* L. . These species are referred to in figures by a combination of the first three letters of the genus and the first two letters species name.

3.3.2. Gas exchange and leaf nitrogen

Measurements in this study were made during the 6^{th} through 9^{th} growing seasons of the experiment (2003-2006) to assess the long-term effects of elevated CO_2 and nitrogen additions and potential interactions between them. Species composition, biomass and responses to CO_2 and N were relatively stable at this stage of the experiment. Gas exchange measurements were conducted with a portable infrared gas analyzer system (LiCOR 6400, Li-Cor Inc., Lincoln NE, USA) during the main portion of the season when each species was active (May-June of each growing season). To assess instantaneous and long-term (up to nine years) effects of elevated CO_2 on photosynthetic capacity, photosynthetic CO_2 response curves (A-C_i) were measured on leaves of each plant species with a minimum of seven different CO_2 concentrations, using saturating light conditions (photon flux density of 1800 μmol m^{-2} s^{-1}) and controlled temperatures (leaf temperatures of 28-30°C) in the leaf cuvette. Per species, plants in monoculture plots were measured with 2 replicates for each CO_2 and N treatment. All grass measurements were from the top-most fully expanded leaf adjacent to the flag leaf to ensure similar leaf ages. Leaves were collected and placed on ice after each A-C_i response curve to determine projected leaf area in the chamber

(Image J v1.37, National Institutes of Health, Bethesda, MD, USA). In the laboratory, leaves were dried at 70°C, weighed, and finely ground. A subsample was analyzed for total nitrogen and carbon content using an elemental analyzer (Carlo-Erba Strumentazione, Milan, Italy) with appropriate reference standards for herbaceous leaves in each analysis run (National Institute of Standards and Technology, Boulder, CO USA).

Physiological variables were fitted from the A-C_i response curves using the Farquhar photosynthesis model (Farquhar *et al.*, 1980) according to the procedure laid out in Ellsworth *et al.* (2004). To evaluate changes in photosynthetic capacity and assess potential down-regulation of photosynthesis, I analyzed the variables maximum carboxylation rate (V_{cmax}) and the maximum electron transport rate (J_{max}) as well as the measured net photosynthesis in current growth conditions (either ambient or elevated [CO_2]) (A_{net}) and net photosynthesis at a common CO_2 level of 365 μmol mol^{-1}. Net photosynthesis at a common CO_2 level was analyzed both on a mass basis (A_{m365}) and area basis (A_{a365}), concurrent with leaf N expressed on a mass basis (N_{mass}) and on an area basis (N_{area}). A slight increase in LMA (Leaf Mass per Area ratio, g m^{-2}) was observed in elevated CO_2 ($P = 0.07$). Despite this, results were generally similar whether expressed on mass or area bases. I also analyzed net photosynthesis at a common CO_2 level of 560 μmol mol^{-1} corresponding to the CO_2 concentration of the elevated CO_2 treatment, however those results strongly paralleled the results for A_{a365} and hence are not shown. These variables help evaluate the basic physiological mechanisms behind changes in plant growth and productivity in long-term elevated CO_2 and N addition, as well as comparing similar mechanisms in different C_3 species.

3.3.3. Statistical analyses

Because I am interested in the long-term effects of elevated CO_2 and N addition without the influence of climatic annual variation, data from CO_2 response curves were averaged across four years. Averaging across years resulted in similar sample sizes for each species per treatment combination, and represented average responses of each species to long-term elevated CO_2 and N addition. Moreover, there was generally no significant year effect in ANOVAs conducted using this term ($P >$

0.1). All further analyses of variance described below were conducted on variables averaged across years by species, plot, CO_2 and N treatment.

The BioCON experiment was designed as a split-plot with N addition nested within atmospheric CO_2 treatment (Reich *et al.*, 2001b). Treatment effects were tested using the appropriate whole-plot random effect of atmospheric CO_2 or within-plot error variances against the residual error in the F-test. The whole-plot random effect was not significant ($P > 0.1$) in any case. Because my goal was to investigate species-specific responses to the experimental treatments, as well as responses of species within functional groups, I conducted ANOVA using main effects CO_2 level, N level, and Functional Group and Species identity within functional group to test for effects and interactions in the experiment (Table 3). Post-hoc Tukey's tests were used to establish differences among the different species. Because species responded differently to elevated CO_2 (Table 3), I further analyzed differences between ambient and elevated CO_2 for each species separately, including the whole-plot random effect. To evaluate the strength of species effects versus treatment effects, the contribution of each effect (e.g. CO_2, N or species effects and their interactions) to the total variation was calculated using sums of squares of the effect divided by the total sums of squares from the analysis of variance (Hunter *et al.*, 1997). Species and functional group responses to elevated CO_2 are further explored for the N addition treatment because the focus is on plant mechanisms in species and functional groups when both elevated CO_2 and N addition occur. All statistical analyses were conducted in JMP 5.0.1 software, SAS Institute, Cary NC, USA.

Table 3: *P*-values, whole-model error mean squares (MS) and goodness of fit for an ANOVA with CO_2 treatment (CO_2), N addition treatment (N), Functional Group (Funct gr) and species within functional group (Spp(Funct gr)) as main effects, including degrees of freedom (d.f.), for the following variables: maximum carboxylation rate (V_{cmax}), maximum electron transport rate (J_{max}), net photosynthesis in respective growth conditions e.g., either ambient or elevated [CO_2] (A_{net}), net photosynthesis at a common CO_2 level of 365 μmol mol^{-1} on an area basis (A_{a365}) and mass basis (A_{m365}) and foliage N on a mass basis (N_{mass}) and area basis (N_{area}).

		P-Values						
Source	d.f.	V_{cmax}	J_{max}	A_{net}	A_{a365}	A_{m365}	N_{mass}	N_{area}*
CO_2	1	-[1]	-	<0.0001	-	-	<0.0001	-
N	1	0.0157	0.0231	0.0673	-	-	<0.0001	<0.0001
CO_2 x N	1	-	-	-	0.0433	0.0012	0.0209	-
Funct gr	1	-	-	-	-	-	0.0415	-
Spp (Funct gr)	5	<0.0001	0.0002	<0.0001	<0.0001	<0.0001	0.0026	<0.0001
CO_2 x Funct gr	1	0.0113	0.0675	0.0012	0.0056	0.0062	0.0096	-
N x Funct gr	1	-	-	-	-	0.0147	-	-
CO_2 x Spp(Funct gr)	5	0.0392	0.0601	-	-	0.0213	0.0047	-
N x Spp(Funct gr)	5	0.0162	0.0082	-	-	-	0.0078	-
CO_2 x N x Funct gr	1	0.0219	0.0158	0.0806	0.0587	0.0011	-	-
CO_2 x N x Spp(Funct gr)	5	-	-	-	-	-	-	-
Error MS	35-38	128.0	398.7	8.694	7.435	1465.0	3.02	0.034
Whole model R^2		0.76	0.73	0.81	0.69	0.81	0.85	0.84

[1]dash denotes that results were not significant (P>0.1)

* transformation used to meet normality assumption: Log(N_{area}-0.2)

3.4. Results

Because the BioCON FACE experiment was designed with [CO_2] and N as the two central experimentally-manipulated factors, I first focus my results on the main and interactive effects of these factors. I then present species and functional group effects as well as higher-order interactions of elevated CO_2 and N with these factors.

3.4.1. Effects of elevated CO_2 and N treatments on leaf nitrogen and photosynthesis across species

A number of photosynthetic and nitrogen-related traits varied significantly with CO_2 treatment, N addition treatment and their interaction across all seven grassland species (Table 3). Long-term elevated CO_2 exposure significantly decreased foliar N on a mass basis (-11%, $P < 0.0001$, Table 3) more than on an area basis (N.S. in Table 3). As expected, foliar N concentration was increased by 23% by N addition across all species (both area- and mass-based N, $P < 0.0001$, Table 3). However, a significant CO_2 x N interaction in N_{mass} ($P = 0.0209$, Table 3) showed no differences in N_{mass} between CO_2 treatments at low N levels whereas with N addition, N_{mass} responded significantly more to N addition in ambient CO_2 (+ 27%, $P = 0.0031$, Fig. 9a) compared to elevated CO_2 plots. There were similar trends for N_{area} to those for N_{mass}, but CO_2 x N was not statistically significant for this parameter ($P > 0.10$, Fig. 9b).

With a +200 μmol CO_2 mol^{-1} enrichment in CO_2, there was a significant enhancement in photosynthesis under growth conditions (A_{net} = +41%, $P < 0.0001$, Table 3) across all species and functional groups. In contrast, A_{net} responded weakly to N addition (+8%, $P = 0.0673$, Table 3). There was no significant CO_2 x N interaction for A_{net} across species. However, insights into the long-term effects of elevated [CO_2] and N on intrinsic photosynthetic capacity may be better considered by comparing photosynthesis per unit area at a common CO_2 level across treatments rather than A_{net} (Ellsworth *et al.*, 2004; Ainsworth & Rogers, 2007). Here, among all species, photosynthesis at a common CO_2 level on both mass and area bases showed no significant main effect of CO_2 treatment, but showed a significant CO_2 x N interaction

(Table 3; Fig. 9c,d). Both area-based (A_{a365}) and mass-based (A_{m365}) photosynthesis at a common measurement [CO_2] showed CO_2 treatment-induced downregulation under added N but not under ambient N.

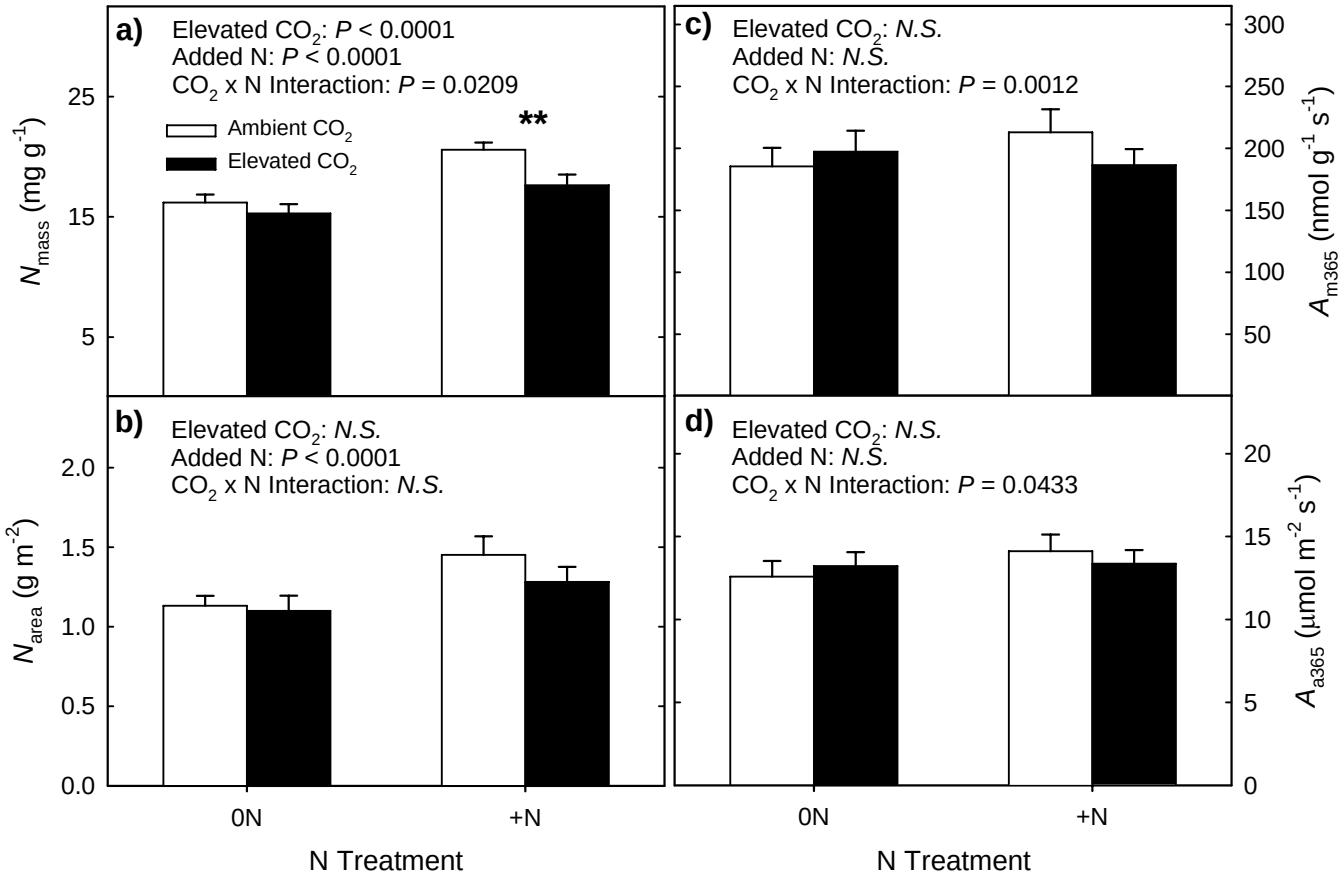

Figure 9: Effects of elevated CO_2 and N addition treatments on (a) foliar N on a mass basis (N_{mass}), (b) foliar N on an area basis (N_{area}), c) photosynthesis at a common CO_2 level on a mass basis, A_{m365} and (d), photosynthesis at a common CO_2 level on an area basis, A_{a365} across seven grassland species. Species means in ambient CO_2 (open bars) and elevated CO_2 treatment (black bars) are shown. 0N denotes unamended plots and +N denotes N-addition plots. Sample size associated with the means in this figure varied between 6 and 8 (i.e. number of species in two replicates). ** indicates a significant difference between elevated and ambient CO_2 treatment within a N treatment ($P < 0.01$).

V_{cmax} and J_{max}, did not significantly differ between CO_2 treatments across the different species (Table 3), because species or functional groups differed in their response to elevated CO_2 or elevated CO_2 and N (see below). Both V_{cmax} and J_{max} increased

significantly with N addition (+11% and +10% respectively, Table 3) across species. More insight into the observed CO_2 x N interactions may be drawn from examining species and functional group differences in response to elevated CO_2 and N addition.

3.4.2. Species effects and higher-order interactions

Species differed significantly in all measures of photosynthetic capacity and leaf N ($P \leq 0.003$, Table 3). All grass species had higher N_{mass} values than forb species, resulting in a significant functional group difference ($P = 0.0415$). Across CO_2 and N treatments, species ranked similarly in V_{cmax}, J_{max}, A_{a365} and A_{net}. *Solidago* had consistently the highest photosynthetic capacity, and *Achillea, Bromus* and *Poa* always represented the lowest three values (in descending order, Fig. 10 and data not shown).

For some variables, there were significant CO_2 x species interactions (Table 3). Only *Solidago* and *Bromus* had significantly reduced V_{cmax}, J_{max} or N_{mass} in elevated CO_2 across N treatments. The same physiological variables also showed significant N x species interactions (Table 3). Species consistently responded to N addition with a significant increase in N_{mass} (11-45% increase, $P < 0.04$), except *Anemone*. For V_{cmax} and J_{max}, only *Poa* and *Anemone* showed a significant increase with N-addition. These species differences were often consistent with functional group differences in response to elevated CO_2. Across N treatments, forbs reduced photosynthetic capacity and leaf N in elevated CO_2 by at least 15%, whereas grasses did not show significant reductions in elevated CO_2. Moreover, there were significant 3-way interactions of CO_2 x N x functional group for variables reflecting photosynthetic capacity: V_{cmax} ($P = 0.0219$), J_{max} ($P = 0.0158$), A_{m365} ($P = 0.0011$) and A_{a365} ($P = 0.0587$) (Table 3). These measures of photosynthetic capacity were generally reduced by elevated CO_2 more for forbs than grasses. These reductions were more pronounced in N addition treatments (Table 3), and hence were examined in more detail (see below). Conditions of elevated CO_2 and increased N availability are likely to co-occur due to global change. Also, understanding the CO_2 responses of different functional groups in the N-added plots provides insight into CO_2 x functional group interactions that are difficult to visualize as three-way interactions with N.

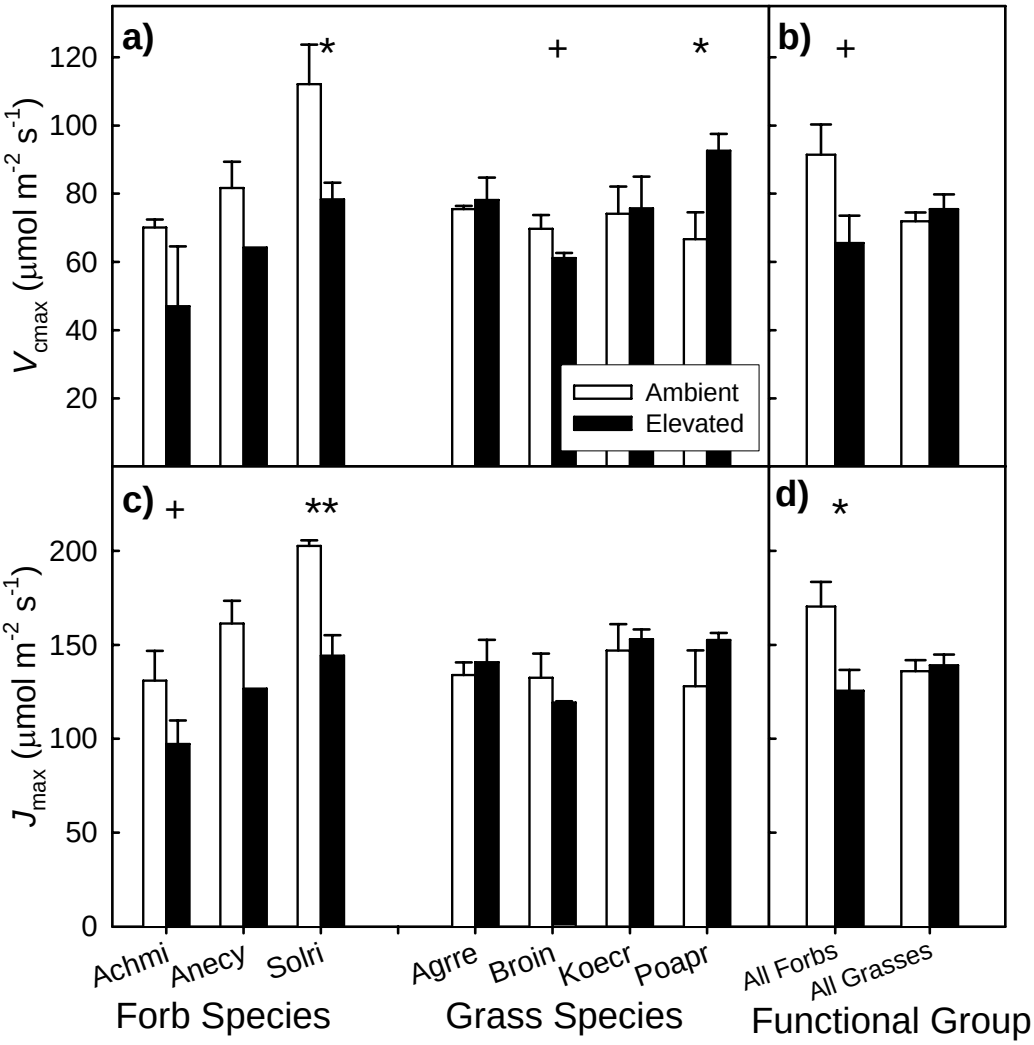

Figure 10: Species-specific responses (left panels) to elevated CO_2 in N-addition plots for maximum carboxylation rate, V_{cmax} (a, b) and maximum electron transport rate, J_{max} (c,d). The aggregate functional groups responses of V_{cmax} and J_{max} to elevated CO_2 are shown at right in panels b and d. Open bars represent the ambient CO_2 treatment and black bars are the elevated CO_2 treatment. Significant differences between CO_2 treatments within either each species or each functional group are represented by $^{+}$ for $P < 0.1$, * for $P < 0.05$, ** for $P < 0.01$ and *** for $P < 0.001$. Samples sizes ranged from 1-3 for species effects (a,c) and 6-8 for functional group effects (b,d). Species abbreviations are as follows: Achmi = *Achillea millefolium*, Anecy = *Anemone cylindrica*, Solri = *Solidago rigida*, Agrre = *Agropyron repens*, Broin = *Bromus inermis*, Koecr = *Koeleria cristata*, Poapr = *Poa pratensis*.

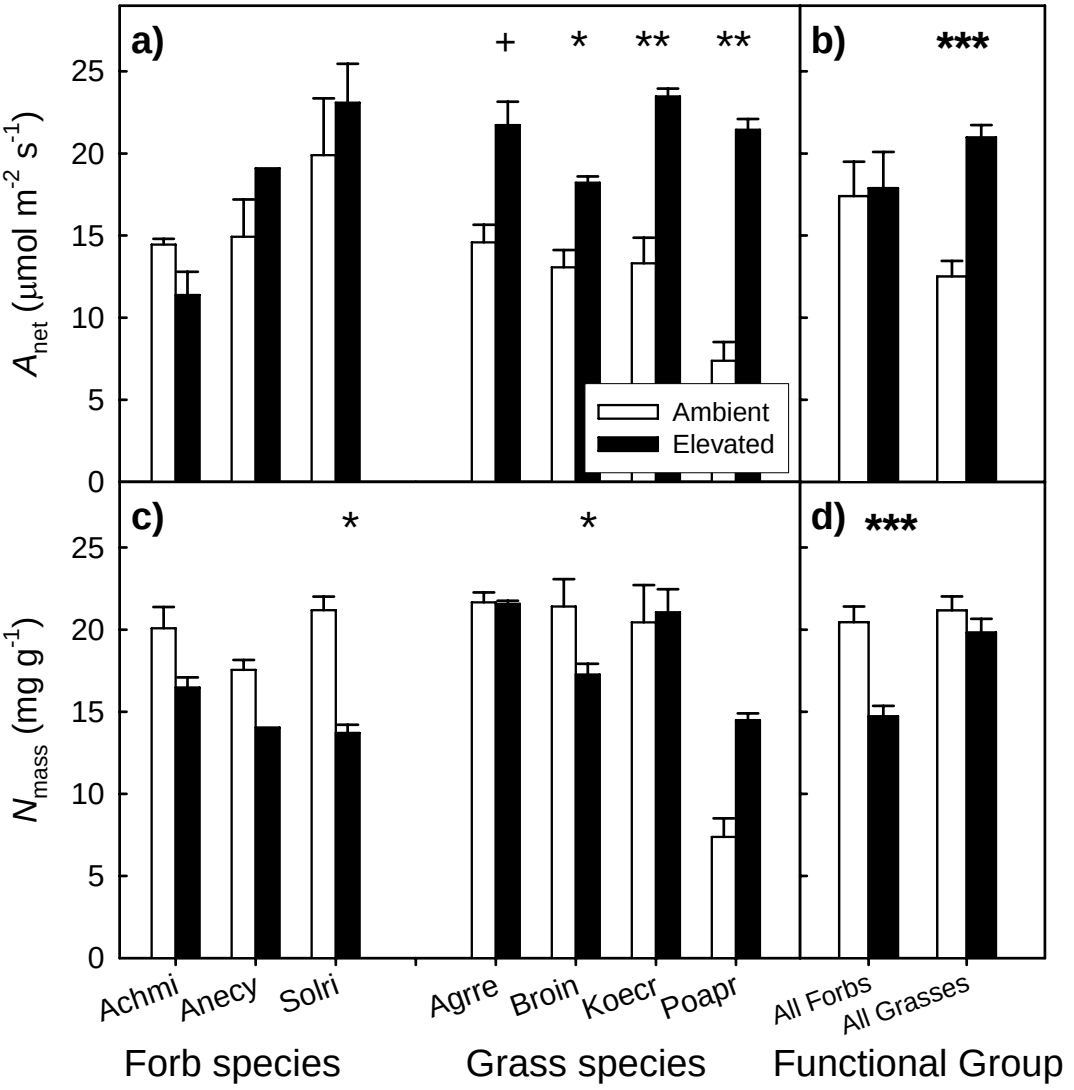

Figure 11: Species-specific responses (left panels) to elevated CO_2 in N-addition plots for net photosynthesis in respective growth conditions, A_{net} (a,b) and mass-based foliage nitrogen concentration, N_{mass} (c,d). The aggregate functional groups responses of A_{net} and N_{mass} to elevated CO_2 are shown at right in panels b and d. Open bars represent the ambient CO_2 treatment and black bars are the elevated CO_2 treatment. Significant differences between CO_2 treatments within either each species or each functional group are represented by $^{+}$ for $P < 0.1$, * for $P < 0.05$, ** for $P < 0.01$ and *** for $P < 0.001$. Samples sizes ranged from 1-3 for species effects (a,c) and 6-8 for functional group effects (b,d). Species abbreviations as in Figure 10.

3.4.3. Elevated CO_2 responses of functional groups under N addition

With added N, the response of photosynthetic capacity to elevated CO_2 varied in each functional group with significant CO_2 x functional group interactions. Photosynthetic capacity variables (V_{cmax}, J_{max} and A_{m365}) were all reduced in all forb species in response to elevated CO_2 (by >25%), whereas grasses showed no change in these variables in elevated CO_2 (Fig. 10). These effects were generally consistent among species within each group (Fig. 10) and hence represent functional group differences rather than species to species variation. *Poa*, *Koeleria* and *Agropyron* did not show down-regulation of photosynthetic capacity in elevated CO_2 (V_{cmax} = +39%, +2% and +4% enhancement respectively) in combination with N addition (Fig. 11a, 12a) while *Bromus* showed a -10% change in V_{cmax} (Fig. 10a). In contrast, *Achillea*, *Anemone* and *Solidago* all had lower V_{cmax} in elevated CO_2 ($P < 0.1$ across all forb species; -33, -21%, -30% and respectively) in N addition plots (Fig. 10a). The larger magnitude of down-regulation in forbs versus grasses resulted in no significant enhancement of net photosynthesis in elevated CO_2 for forbs treated with N addition (Fig 11b).

I found similar trends in N_{mass} to those for photosynthetic capacity. Though leaf N was intrinsically lower in forbs compared to grasses ($P = 0.0415$), the significant CO_2 x functional group interaction on leaf N_{mass} ($P = 0.0096$) showed that elevated CO_2 negatively affected the leaf N concentration in forbs but not in grasses (Fig. 11d). N_{mass} in forb leaves with added N was 26% lower in elevated CO_2 ($P = 0.0004$) compared to ambient CO_2 (range among species within this group of –18% to –35%; Fig. 11c). In contrast, there was no consistent CO_2 effect on N_{mass} among grass species (Fig. 11d), though *Bromus* did in fact show a slight decrease of 19% ($P = 0.056$; Fig. 11c). Thus, leaf N concentrations were reduced strongly in forbs when exposed to elevated CO_2, whereas grasses on average were able to maintain leaf N concentrations in elevated CO_2.

Do leaf N responses to long-term elevated CO_2 drive down-regulation of photosynthetic capacity? Because most nitrogen is invested in photosynthetic components, a CO_2-induced reduction in N_{mass} resulted in no significant CO_2-induced enhancement of photosynthesis in forb species receiving N addition, so that reduced photosynthetic capacity offset increased CO_2 supply. In contrast, C_3 grasses were able to

maintain leaf N concentrations and hence sustained a strong CO_2 enhancement response (+68%) at high N supply ($P < 0.0001$, Fig. 11b).

3.4.4. Root biomass allocation patterns in forb and grass species

Table 4: *P*-values, whole-model error mean squares (MS) and goodness of fit for an ANOVA with CO_2 treatment (CO_2), N addition treatment (N), Funtional Group (Funct gr) and species within functional group (Spp(Funct gr)) as main effects, including degrees of freedom (d.f.) for total root biomass and root-to-shoot ratio. Total sample size across all species was n=56. All variables were transformed to meet normality assumptions for ANOVA; Root:Shoot ratio was log transformed, a square root transformation was used for all other biomass variables.

		P - Values			
Source	d.f.	Root:Shoot ratio	Total root biomass	Total leaf biomass	Total biomass
CO_2	1	-[1]	-	-	-
N	1	-	0.0009	0.0003	0.0001
CO_2 x N	1	-	-	-	-
Funct gr	1	-	0.0277	-	0.0549
Spp (Funct gr)	5	<0.0001	<0.0001	<0.0001	<0.0001
CO_2 x Funct gr	1	-	-	-	-
N x Funct gr	1	0.0177	0.0324	-	0.0756
CO_2 x Spp(Funct gr)	5	-	-	-	-
N x Spp(Funct gr)	5	-	-	0.0097	-
CO_2 x N x Funct gr	1	-	-	-	-
CO_2 x N x Spp(Funct gr)	5	-	-	-	-
Error MS	23	0.233	12.707	2.2706	11.876
Whole Model R^2		0.83	0.93	0.95	0.94

Though there were no significant CO_2 treatment differences in total root biomass and root:shoot ratio (Table 4), total root biomass was larger in N-added plots than in unamended plots across species ($P = 0.0009$). In addition, C_3 grasses showed higher root biomass compared to the forb species ($P = 0.0277$, Table 4, Fig.12a). However, root biomass also showed a significant interaction between N-addition and functional group ($P = 0.0324$, Table 4). Whereas root biomass was no different in forbs between N-amended and unamended plots, grasses increased root biomass significantly more (+ 71%) in response to N-addition compared to forb species. This resulted in a significantly

higher root:shoot ratio in C_3 grasses compared to non-leguminous forbs in N-added plots ($P = 0.0177$, Fig. 12b).

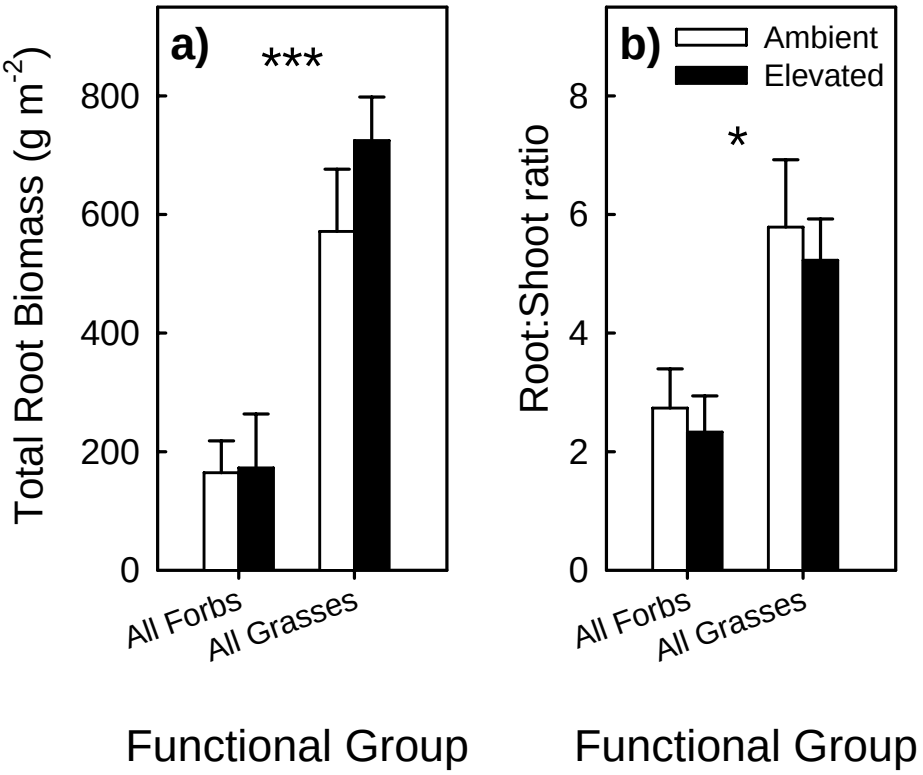

Figure 12: Means with standard error of total root biomass (a) and root:shoot ratio (b) for each functional group (e.g. all C_3 grasses and all non-leguminous forbs) in N-added plots in ambient [CO_2] (open bars) and elevated [CO_2] (black bars). Stars indicate significant differences between functional groups: * $P < 0.05$ and *** for $P < 0.001$. Sample size for forbs equaled n=6, for grasses n=8.

3.5. Discussion

I sought to investigate mechanisms of long-term responses to elevated atmospheric CO_2 and N addition for C_3 species from two functional groups to understand how physiological responses might influence physiology in a way that could alter species dynamics. C_3 forb responses to elevated CO_2 have been previously compared with those of grasses in a number of experiments (Knapp *et al.*, 1996; Anderson *et al.*, 2001; Morgan *et al.*, 2001). However, few of these experiments have examined such responses

under combinations of elevated CO_2 and enhanced N supply, or for long time periods. After six to nine years of elevated CO_2 exposure, significant CO_2 x N interactions were observed across species for photosynthetic capacity (A_{m365}, Fig. 9c) and leaf nitrogen (N_{mass}, Fig. 9a). These interactions indicated that elevated CO_2 had a stronger effect on physiological variables in the N addition treatment than in ambient N (Fig. 9). Moreover, these effects were marked for forbs, showing strong and consistent photosynthetic downregulation, in contrast to C_3 grasses. Based on these results, I conclude that my first two hypotheses were supported. In species or functional groups in which reductions in N were observed under elevated CO_2, photosynthetic down-regulation followed. However, in contrast with the third hypothesis, down-regulation responses were stronger with N addition than with ambient N,.

As a result of the interactive effects of elevated CO_2 and N interactions on photosynthetic capacity (A_{m365} and A_{a365} in Table 3), I observed less photosynthetic enhancement from elevated CO_2 under N enrichment. Despite this, recent findings across a larger set of grassland species and species mixtures have shown sustained plant growth responses under elevated CO_2 in soils with enriched N (Reich *et al.*, 2006a). Hence, biomass enhancement is greater under the combination of elevated CO_2 and N-addition across a wide set of sixteen grassland species, despite the occurrence of photosynthetic down-regulation at the leaf level in at least three of these species (all forbs I studied here, Fig. 10). My findings can be reconciled with those in Reich et al. (2006) because two of these three forb species (*Achillea* and *Anemone*) showed greater biomass enhancement due to elevated CO_2 under ambient N than under N-addition plots.

Although stimulation of photosynthesis in elevated CO_2 is still possible with reduced leaf N concentrations because of increasing plant nitrogen-use efficiency, N-redistributing mechanisms likely do not provide all plant growth demands for N in elevated CO_2 (BassiriRad *et al.*, 2001; Hungate *et al.*, 2006). If plant N demand exceeds N supply, then the stimulated growth response in elevated CO_2 is likely not sustainable though plants may initiate several mechanisms to cope with possible reduced N availability (Gill *et al.*, 2006; Millard *et al.*, 2007). One major mechanism for doing so is via higher root biomass fractions (Luo *et al.*, 1994), which is consistent with the observation for the four C_3 grass species in my study (Fig. 12).

Increased fine root growth is a potential mechanism to cope with increasing nutrient demands in elevated CO_2 (BassiriRad *et al.*, 2001). There is a clear link between N availability and leaf N (Reich *et al.*, 2003) and it is expected that extending fine root systems for N capture could be central to sustaining long-term growth enhancement in elevated CO_2 (Norby *et al.*, 2002; Luo *et al.*, 2004). Though there were no significant CO_2 treatment differences in total root biomass and root-to-shoot ratio (Table 4), C_3 grasses showed higher root biomass and root-to-shoot ratio compared to the forb species ($P = 0.0113$, Table 4, Fig. 12a). I suggest that these differences could play a key role in N acquisition and hence result in the maintenance of leaf N in C_3 grasses in elevated CO_2 combined with added N (Luo *et al.*, 1994; BassiriRad *et al.*, 2001).

My study showed a strong effect of species identity on physiology, explaining >30% of the total variation in each variable. Experimental factors such as elevated CO_2 and N-addition also had a substantial influence on the physiological traits that they affect directly (e.g. >20% of variation in photosynthetic rates and leaf N concentration). However, I also observed strong differences in photosynthetic responses to elevated CO_2 between functional groups. Forbs were negatively affected in elevated CO_2, which was exacerbated in high N conditions. Grasses maintained a substantial photosynthetic stimulation even after 9 years of elevated CO_2 exposure.

In my study, the ability to maintain leaf N appears to correspond to increased biomass allocation to roots (Fig. 12a), enabling C_3 grasses to forage for additional soil N and hence prevent reduction of N_{mass} in elevated CO_2. Forbs showed significantly lower total root biomass ($P = 0.0131$, Fig. 12a) than C_3 grasses and also showed strong reductions in leaf N in elevated CO_2 with down-regulation of photosynthetic capacity (Fig. 11). The negative response of forbs to elevated CO_2 (Morgan *et al.*, 2001; Grünzweig & Körner, 2003) and to N addition (Reich *et al.*, 2003) may indicate that shifts in competitive balance among species are possible (Joel *et al.*, 2001; Zavaleta *et al.*, 2003). If shifts in the competitive balance of grasses and forbs occur over time, it may lead to less diverse grasslands dominated by graminoids in elevated CO_2. Hence, increased biomass allocation to roots and maintenance of leaf N can be a major mode for sustaining photosynthetic stimulation in elevated CO_2.

3.6. References

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Chapter 4

A comparison of leaf- versus canopy-level responses to elevated CO_2 in grassland species using a simulation analysis

4.1. Summary

I used a simple sun-shade canopy model to investigate how leaf-level photosynthesis and canopy leaf area index (LAI) interact in affecting the gross canopy photosynthesis (GPP_{day}) responses to elevated CO_2 in seven different grassland species at the BioCON FACE site. Consistent with leaf-level results, grasses increased GPP_{day} by 47% in elevated CO_2 whereas forb species only showed a 9% GPP_{day} CO_2-enhancement across N treatments. The direct effect of elevated CO_2 contributed 27% on average to the total GPP increase in elevated CO_2, while LAI and photosynthetic acclimation contributed respectively +15% and -5% on average to the total change in gross canopy photosynthesis (GPP_{day}) in elevated CO_2, though species-specific responses varied. In elevated CO_2, grasses showed large increases in LAI and no differences in photosynthesis, while forbs showed little enhancement in LAI and down-regulated photosynthesis. The small LAI enhancement in forbs in elevated CO_2 was offset by down-regulation of photosynthesis. As a result, canopy photosynthetic enhancements in forbs were only due to the direct effect of elevated CO_2 itself whereas grasses showed larger enhancements of daily GPP in canopies exposed to elevated CO_2. N addition had a positive effect on LAI resulting in higher GPP_{day} when N was added. Daily modeled GPP correlated well with total biomass though only when different biomass allocation patterns via root-to-shoot ratios were taken into account.

4.2. Introduction

Gross primary production (GPP), the balance between photosynthetic CO_2 assimilation and respiratory CO_2 release represents the total amount of autotrophic carbon available for growth, storage and energy demands at the ecosystem level (Wittig *et al.*, 2005). Given that photosynthesis and respiration are highly sensitive to variations in atmospheric CO_2 concentration, temperature, light, and other climate factors, GPP exhibits daily, seasonal, annual and inter-annual variations in response to climatic conditions (Williams *et al.*, 1997). Therefore, the annual carbon (C) fixation rate is sensitive to long-term climate change. Predicting responses to climate change and how physiological processes and canopy structure (e.g., LAI) affect GPP in ambient and elevated CO_2 is key to estimate how C balance of terrestrial ecosystems may change due to elevated atmospheric CO_2.

The physiological controls on carbon fixation at the leaf-level in response to elevated CO_2 have been relatively well understood for more than two decades (Farquhar *et al.*, 1982; Long & Drake, 1991; Sellers *et al.*, 1997). Thus the direct stimulatory effect of elevated CO_2 on photosynthesis and its modification by temperature and light can be represented well in current canopy photosynthesis models (McMurtrie & Comins, 1996; Friend *et al.*, 1997). However, experiments using elevated CO_2 have also documented other, more indirect effects associated with physiological acclimation to long-term CO_2 enrichment, such as photosynthetic down-regulation (Crous & Ellsworth, 2004; Long *et al.*, 2004; Nowak *et al.*, 2004; Ainsworth & Long, 2005). Such photosynthetic adjustments can be expected to feed-forward to plant growth (Hikosaka, 2005). However, understanding the implications of these responses for canopy function has lagged, largely because direct measurements of canopy-scale photosynthesis are rare (Stocker *et al.*, 1997; Aeschlimann *et al.*, 2005).

An understanding of canopy leaf area responses to long-term CO_2 enrichment is central to scaling physiological results to the entire canopy (Norby *et al.*, 2005), yet such data are often lacking (Norby *et al.*, 1999). One tool for linking CO_2-induced changes in photosynthetic capacity and changes in canopy structure to understand whole-canopy

level photosynthesis under enriched CO_2 concentration is mathematical modeling (Luo *et al.*, 2000). When parameterized with both leaf-level and whole-canopy data, such as in this study, photosynthesis models can help to distinguish important species differences driving ecosystem-scale processes such as C cycling.

Given the presence of many different species and plant functional types in grasslands, the BioCON FACE experiment provided a unique opportunity to understand how the physiology and canopy structure of different species affect GPP response under long-term elevated CO_2 and N addition. My goal was to 1) quantify the response of modeled daily GPP (GPP_{day}) to elevated CO_2 with inputs for different grassland species monocultures exposed to elevated CO_2 and/or N availability using a canopy photosynthesis model, 2) analyze possible sensitivities of modeled GPP_{day} to elevated [CO_2] and 3) evaluate the proportion of the modeled GPP_{day} response that can be ascribed to direct effects of CO_2 and indirect effects of physiological acclimation, and changes in canopy structure (e.g., total leaf area) for each species. In this way, I determined the relative contributions of physiology and canopy structure as major components regulating overall modeled GPP_{day} to help refine future models and determine underpinnings of the biomass response to elevated CO_2.

4.3. Material and Methods

4.3.1. Site description and species

The BioCON (Biodiversity, CO_2 and N) FACE experiment is located in a humid continental climate on glacial outwash comprised of loamy sand soils with very low nutrient availability in central Minnesota, USA (45° 25' 59 N, 93° 12'02 W). The mean annual precipitation is 660 mm $year^{-1}$ and the mean July temperature is 22°C, with an average growing season of approximately 145 days. Exposure to elevated CO_2 starts at the beginning of the frost-free season in late April and continues until the end of the growing season in September. The elevated CO_2 treatment represents ambient [CO_2] + 200 μmol mol^{-1}. The N addition treatment consisted of 4 g N m^{-2} yr^{-1} applied each year in May, June and July. The experimental design is a split-plot where six circular plots of 20

m diameter from the elevated CO_2 treatment and within which 2m x 2m subplots represent the N addition treatments for combinations of species plots. Here, two replicates for each combination of elevated CO_2 and N treatment in monoculture plots are used, each of which has been grown in either ambient or elevated CO_2 for six year prior to the start of this study. The species chosen for this study were four C_3 grasses: *Poa pratensis* L., *Koeleria cristata* Pers., *Bromus inermis* Leyss. and *Agropyron repens* L. and three non-leguminous forbs: *Solidago rigida* L., *Anemone cylindrica* A. Gray and *Achillea millefolium* L. Plant species are hereafter and in the figures referred to with the first three letters of the genus name and the two first letters of the species name. More details about the site are found in Lee *et al.* (2001) and Reich *et al.* (2001).

4.3.2. Model description and inputs

I used the two-layered sun-shade canopy model BEWDY, which was described by Medlyn *et al.* (2000) with parameters estimated from site measurements. The model estimates plant canopy CO_2 assimilation from the Farquhar model, given the biophysical and biochemical mechanisms of photosynthesis and their responses to atmospheric CO_2 concentration and temperature and light (Farquhar *et al.*, 1980; Long & Drake, 1991; Medlyn *et al.*, 2000). The Farquhar model (Farquhar *et al.*, 1980) determines that leaf photosynthesis is given by the minimum of the rate of carboxylation when rubsico activity is limiting (A_c) and the rate of carboxylation when RuBP regeneration is limiting (A_j) :

$$A_n = \min (A_c, A_j)$$

A_c is the rate of photosynthesis when Rubisco activity is limiting and A_j the rate when RuBP-regeneration is limiting. Rubisco-limited photosynthesis is given by

$$A_c = V_{c\max}\left(\frac{C_i - \Gamma^*}{C_i + K_m}\right)$$

where V_{cmax} is the maximum rate of Rubisco activity, C_i is the intercellular concentrations of CO_2 and, Γ^* is the photosynthetic CO_2 compensation point without mitochondrial respiration contributions, and K_m is the Michaelis-Menten coefficient of Rubisco activity for CO_2 (Medlyn *et al*., 2000). In BEWDY, $K_m = 39.05e^{0.086T} + 9.58\Gamma^*$ (see equation B8b in Medlyn *et al.*, 2000). The rate of photosynthesis when RuBP regeneration is limiting is given by

$$A_j = \frac{J}{4}\frac{C_i - \Gamma^*}{C_i + 2\Gamma^*}$$

where J is the rate of electron transport which is a saturating function of absorbed light with a maximum value of J_{max}. Both V_{cmax} and J_{max} were assumed to be linear with leaf N (N_f) content according to:

$$V_{cmax} = V_N (N_f - N_{min}) \text{ and } J_{max} = J_N (N_f - N_{min})$$

where N_{min} represents the minimum amount of N required for photosynthesis (e.g. the intercept of the $V_{cmax} - N_a$ relationship). V_N and J_N are temperature dependent coefficients according to Medlyn *et al.* (2000) (equations B8c and B8d).

The response of instantaneous gross photosynthesis (A_g) to light (i.e. absorbed radiation) is calculated by the model based on a rectangular hyperbola function given by:

$$A_g = \frac{a\alpha_A I A_{max}}{a\alpha_A I + A_{max}}$$

where a is the leaf absorptance, α_A is the quantum yield of absorbed radiation, I is the quantum flux density on the leaf (i.e., incident light). A_{max} is the light-saturated rate of photosynthesis, assumed to be linearly related to leaf N (see Medlyn *et al*., 2000, equation A3). The sun-shade model computes the average light levels for the sunlit and shaded fractions of the canopy using absorbed radiation derived from the total leaf area and the Beer-Lambert law. Direct radiation of beam radiation (I_b) does not decrease throughout the canopy whereas diffuse radiation (I_d) is assumed to extinguish through the

canopy according to the Beer-Lambert law with k being the light-extinction coefficient and L representing the canopy depth (i.e., LAI). Therefore,

$$I_{sun}(L) = k\,(I_b + I_d\,e^{-kL}) \text{ and } I_{shade}(L) = k\,I_d\,e^{-kL}$$

Gross daily canopy photosynthesis (GPP_{day}) then is predicted by addition of the calculated gross photosynthesis from sunlit and shaded foliage at hourly time steps over the course of one day and integrated across the canopy depth (see equation A6 in (Medlyn *et al.*, 2000).

More detailed models of canopy photosynthesis exist (Baldocchi & Meyers, 1998; Wang & Leuning, 1998; Schäfer *et al.*, 2003) but BEWDY incorporates the major elements regulating canopy photosynthesis by considering sunlit and shaded leaf area separately (dePury & Farquhar, 1997; Medlyn *et al.*, 2000; Friend, 2001). The hourly time-step integrated over the day allows for accurate scaling of the many non-linear processes associated with photosynthesis such as the diurnal variation in light-attenuation through the canopy, while requiring minimal data input (Williams *et al.*, 1997; Medlyn *et al.*, 2003). Key assumptions of the model are: 1) that both light and foliar nitrogen concentrations decrease proportionally with canopy depth, 2) that Rubisco capacity is linearly related to leaf N concentration, 3) the light extinction coefficient is assumed to be the same for both direct and diffuse radiation, 4) stomatal closure is assumed to be homogeneous, and 5) the grassland and forb canopies are considered to be spatially homogenous (i.e. no clumped leaves).

My goal was to estimate daily gross canopy photosynthesis (GPP_{day}) in different grassland species using data across 2003 to 2006, the 6^{th} to 9^{th} year of CO_2 exposure in the BIOCON experiment. These years were simulated in order to assess long-term effects of elevated CO_2 and N addition on daily GPP. Inputs for the canopy photosynthesis model were meteorological data, physiological data, functional relationships, and canopy structure. Meteorological data, comprising hourly means for radiation (PAR), precipitation, and temperature, were taken from a nearby weather station (40 km south of the site in St. Paul, MN USA). Physiological data such as leaf N content, parameters for stomatal responses to the environment (Collatz *et al.*, 1991), and slopes and intercepts of

the V_{cmax}- N_a and J_{max}- N_a relationships were derived for each species separately based on data from the site in each treatment. Temperature dependence of physiological parameters reflecting the carboxylation (V_{cmax}) and electron transport (J_{max}) capacity of leaves were taken from (Medlyn *et al.*, 2002). I used a single averaged value of leaf N_a per species and treatment representing N_a at the top of the canopy (N_0). The model then calculates the N gradient throughout the canopy according to the attenuation of light following (Hirose & Werger, 1987) (see Equation A3 in Appendix A of Medlyn *et al.*, 2000). Assuming that the N-gradient follows the light gradient is a robust assumption in canopies with low LAI because the difference in canopy photosynthesis with a uniform N distribution versus a calculated gradient (e.g., optimal N distribution) is very small (<1%) (Field, 1983; Hirose & Werger, 1987).

Canopy structure was estimated using light penetration measurements from each replicate plot of each species monoculture (Pierce *et al.*, 1994) and assuming an ellipsoidal distribution for the vertical profile of leaf area (Wang *et al.* 2007). Leaf Area Index (LAI) of each monoculture canopy was determined using an inversion of photosynthetically-active radiation (PAR) transmitted to the base of the canopy (Decagon Instruments, WA USA) following the Beer-Lambert law (Campbell & Norman, 1998). LAI data of each plot were collected monthly by summer interns at BIOCON and provided to me by P.B. Reich for modeling purposes. LAI was averaged across the four years for each monoculture plot. The fraction of sunlit and shaded leaf area in the model is calculated based on the total LAI and sun zenith angle according to equations from Campbell & Norman (1998), where the light extinction coefficient is assumed to be the same for both direct and diffuse radiation.

Gas exchange measurements that were used as input, such as V_{cmax} and J_{max}, are described in detail in Crous *et al.* (submitted, chapter 3). Total leaf *N* concentration was determined using an elemental analyzer (Carlo-Erba Instrumentations, Milan, Italy), and converted to area-based N (N_a) using specific leaf area determined for each species plot. Slopes and intercepts of relationships between V_{cmax} - N_a and J_{max} - N_a were determined by fitting a linear regression for each species x CO_2 treatment combination. These served as inputs to the model to drive photosynthesis, and are summarized in Table 5.

Table 5: Summary of significant V_{cmax}-N_a and J_{max}-N_a relationships at 25°C across three forbs species and four C_3 grass species grouped in relationships per functional group at BioCON in ambient and elevated CO_2 treatments across N treatments. The relationships were used along with leaf N_a from Crous *et al.* (Chapter 3) to calculate V_{cmax} in the daily GPP model. The number of observations per relationship is represented by N_{obs}.

Functional group	**CO_2 Treatment**	**Equation**	**R^2**	***P*-value**	**N_{obs}**
Forb species	Ambient	$V_{cmax} = 29.29 + 43.21\ N_a$	0.29	0.018	19
	Elevated	$V_{cmax} = 24.80 + 34.69\ N_a$	0.35	0.015	16
	Ambient	$J_{max} = 86.52 + 74.54\ N_a$	0.39	0.0058	18
	Elevated	$J_{max} = 82.18 + 56.35\ N_a$	0.21	0.086	15
C_3 grasses	Across CO_2 treatment	$V_{cmax} = 48.65 + 14.80\ N_a$	0.12	0.0003	110
	Across CO_2 treatment	$J_{max} = 84.43 + 55.20\ N_a$	0.19	<0.0001	104

The model simulated GPP over the month of June, calculating an averaged daily GPP (GPP_{day}). I chose to examine the relationships between canopy-level and leaf-level variables during that month because that time has the most favorable climatic conditions for C_3 species photosynthesis and growth for this region. Also, intensive leaf-level measurements were conducted in June when the monocultures were at peak LAI, and harvests were conducted at the end of the month. Given that V_{cmax} decreases towards the end of the growing season and when leaves age (Wilson *et al.*, 2000), I chose to shorten the model period rather than assuming the same carboxylation rate throughout the growing season. Averaged across years, the model should reflect GPP_{day} values affected by the experimental treatments but minimally affected by inter-annual climatic variability.

Sensitivity analysis was used to assess the contribution of physiological and structural canopy properties to C uptake in the model. GPP_{day} was simulated when the slope of the V_{cmax}-N_a relationship, N_a or LAI were independently increased or decreased by 10, 20 or 30%. This range corresponded with observed changes in physiological

variables across species in the BioCON experiment (Crous *et al.*, submitted). The importance of each class of inputs driving the model ouput for GPP_{day} was assessed over the month prior to biomass harvest.

4.3.3. Total Biomass

I analyzed biomass accumulation as a measure of productivity of the grassland species at BioCON (Reich *et al.*, 2001b). Biomass data from P.B. Reich were available aboveground biomass, total root biomass and total biomass for monocultures of C_3 grasses and C_3 forbs averaged across the sixth to ninth growing season of the experiment. Monocultures of each species had two replicates of each combination of CO_2 and N treatments. Above- and belowground biomass was assessed in June of each growing season as described in Reich *et al.* (2001). In brief, a $1m^2$ area of vegetation was clipped just above the soil surface for harvest, sorted into live and senesced material, dried and weighed. Roots were isolated from three soil samples taken to 20 cm depth from each plot where aboveground biomass had been sampled. The roots were washed and sorted into fine (<1mm diameter), coarse and crown roots, then dried and weighed. Biomass data were used to correlate model results and gain more insight into species-specific outcomes of GPP in elevated CO_2 and N addition.

4.4. Results and Discussion

First, I examined the sensitivity of different inputs in the model on daily GPP (GPP_{day}). The sensitivity analyses were designed to evaluate three effects of elevated CO_2 on GPP_{day}: the direct response of increased concentrations of CO_2 ($[CO_2]$), the indirect effect of leaf physiology and the indirect effect of leaf area index (LAI). Evaluating these effects help to explain species-specific responses of modeled GPP_{day} in elevated CO_2 and N addition. I then used the model to examine actual outcomes at BIOCON and compared these with results of total biomass.

4.4.1. Sensitivity of GPP_{day} to changes in V_{cmax}

To examine the sensitivity of modeled GPP_{day} to leaf-level physiology (i.e. changes in V_{cmax} and N_a in elevated CO_2), I tested how GPP_{day} changed in response to leaf-level changes in V_{cmax} and leaf nitrogen at ambient and elevated CO_2 (Figures 13 and 14).

Increasing foliar N by 10-30% increased V_{cmax} whereas decreasing foliar N decreased V_{cmax} in all species (Fig.13). The different slopes of the V_{cmax} - N_a sensitivity isolines in figure 13 indicated that some species were more sensitive to changes in leaf N_a than others. Stoloniferous grasses, e.g. *Bromus* and *Agropyron*, were most sensitive to a change in N_a, where a 20% increase in N_a increased V_{cmax} values by 18%. The V_{cmax} in bunchgrass *Poa pratensis* was least sensitive to leaf N because a 20% increase in N_a in *Poa* had little effect on V_{cmax} (e.g., +2% change in V_{cmax}). However, the different slopes in Fig. 13 are correlated with the magnitude of the intercepts and the strength of the V_{cmax}-N_a relationship. When intercepts were high, slopes were low (Table 5) and V_{cmax} was less sensitive to changes in N_a. Fast-growing, productive species (e.g. *Solidago*, *Bromus* and *Agropyron*) had higher carboxylation rates associated with high leaf N. Because V_{cmax} accounts for both leaf N content and the N allocated to Rubisco via the V_{cmax} - N_a relationship slope, V_{cmax} is a key variable to compare in terms of species' modeled GPP_{day} sensitivity to physiology (Fig. 14).

The sensitivity of modeled GPP_{day} to changes in V_{cmax} was similar in each species resulting in one single relationship for all grass and forb species in ambient CO_2

conditions (Fig. 14). Among all species, a change of 10% in V_{cmax} resulted in a 7% change in daily GPP in ambient CO_2 conditions, though species differed in the magnitude of change in N_a that brought about a 10% change in V_{cmax} (Fig. 13). This response of photosynthetic capacity was similar for all species because of the same basic mechanism represented in the model. Sensitive species (*Bromus, Agropyron* and *Solidago*) with larger slopes in Fig. 13, also had larger changes in modeled GPP_{day} with physiological adjustments in V_{cmax} because those species were positioned at the outer ends of the GPP_{day}-V_{cmax} relationship in Fig. 14. As a result, modeled GPP_{day} can be considered sensitive to down-regulatory changes in V_{cmax} as a driver of canopy-scale CO_2 assimilation. This would include down-regulation of V_{cmax} due to low N as well as other environmental conditions.

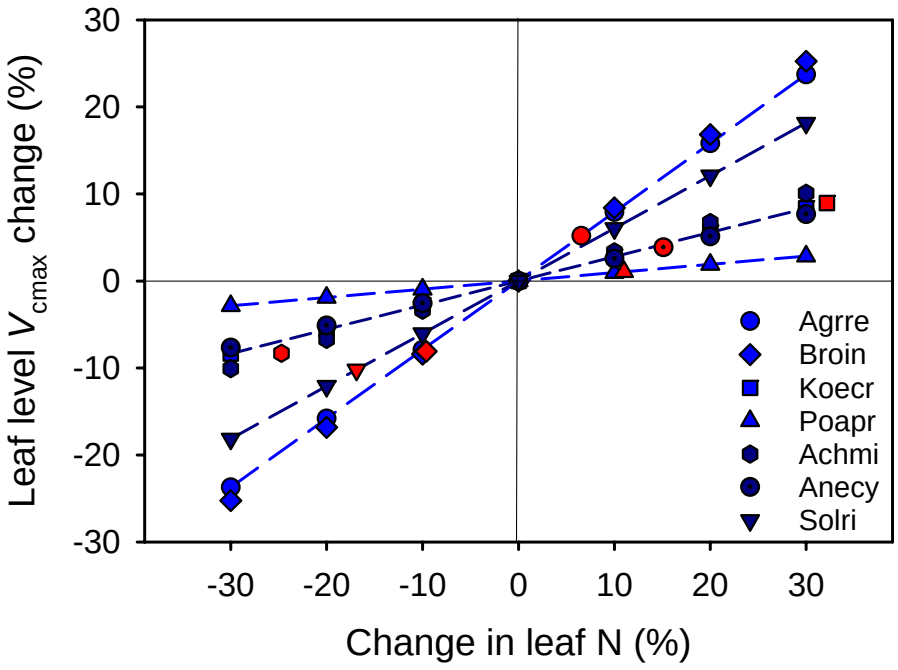


Figure 13: Relative changes in V_{cmax} (%) for different grassland species as a function of the change in foliar N (%) in a sensitivity analysis. The lines indicate species-specific V_{cmax} sensitivity to leaf N. The red points indicate the observed mean elevated CO_2 effect on foliar N values for each species, and the CO_2 effect on V_{cmax} based on averaged data from years 6-9 in BIOCON.

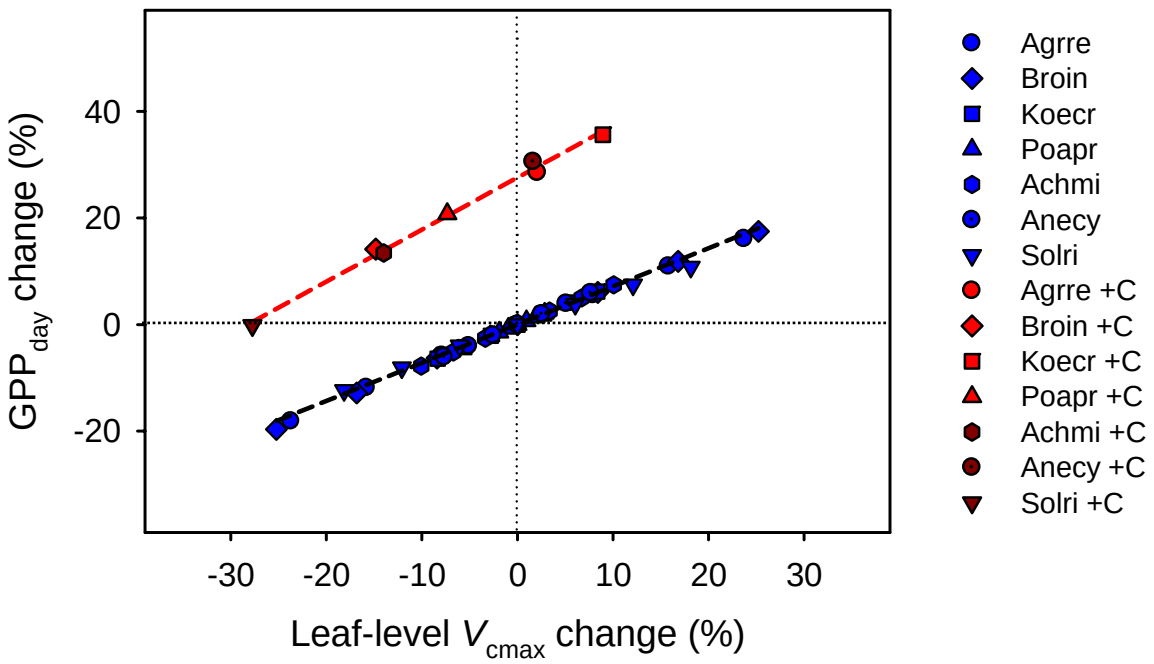


Figure 14: Relative changes in daily GPP (GPP_{day}) (%) for different grassland species as a function of changes in V_{cmax} (%) in ambient (blue points) and elevated CO_2 (red points). Points represent values for all 7 species at 6 sensitivity levels in a sensitivity analysis. The observed elevated CO_2 effect based on field measurement for each species on V_{cmax} is represented in red with the corresponding change in GPP_{day}. Linear regressions showed the following relationships: Y = 0.717x in ambient CO_2 (blue points) and Y = 0.976x + 27.6 in elevated CO_2 (red points).

The overall sensitivity of modeled GPP_{day} to elevated CO_2 must incorporate both short- and long-term effects of elevated CO_2. Short-term effects in the model reflect the direct effect of elevated CO_2, whereas long-term effects include other adjustments such as changes in V_{cmax}. For modeled GPP_{day} at an elevated [CO_2] of 560 μmol CO_2 mol^{-1}, variation in V_{cmax} responses among species represented a relationship with higher slope than in ambient CO_2 conditions and an intercept of 27.6%. Therefore, GPP_{day} at elevated CO_2 is more sensitive to V_{cmax} than GPP_{day} in ambient CO_2 conditions (Fig. 14). According to the slopes of the linear regressions in each respective CO_2 treatment (Fig. 14), every 10% change in V_{cmax} results in a 9.8% change in GPP in elevated CO_2 conditions compared with a 7.2% change in GPP_{day} in ambient CO_2. The Y-intercept of the relationship was 27% higher in elevated CO_2 than ambient CO_2 representing the modeled magnitude of the direct effect of elevated CO_2 (53% increase in [CO_2]) on

GPP_{day}. Without any changes in V_{cmax}, the GPP_{day} is still 27% increased in elevated CO_2. This increase in canopy C uptake is lower than the expected short-term leaf-level photosynthetic CO_2 enhancement at BioCON (~50%, see chapter 3, (Lee *et al.*, 2001) because diurnal effects and effects of sunlit and shaded leaves diminish the potential for photosynthetic enhancement from that expected for single leaves in full sun.

4.4.2. Sensitivity of GPP_{day} to changes in LAI

Total leaf area is a core ecosystem attribute that controls CO_2 fluxes between ecosystems and the atmosphere (Baldocchi *et al.*, 2000). LAI ranged from 0.2 to 2.0 in the herbaceous monoculture plots at all CO_2 and N levels in this study. All species showed a very high sensitivity of modeled GPP_{day} to LAI (Fig. 15a), with a correspondence close to 1:1 between the relative variation in LAI and relative change in modeled GPP_{day} (slope = 0.95). Due to self-shading, species monocultures with higher LAI such as *Solidago* showed a lower instantaneous enhancement response of modeled GPP_{day} to elevated CO_2 than other species (e.g., 21% versus a mean of 26% for the remainder of the species). However, the observed increase in LAI in elevated CO_2 can be a driver of increased GPP_{day}, as has been shown for some forests (Baldocchi *et al.*, 2000). However, the long-term cumulative effects of increased GPP_{day} can also drive leaf area growth, especially in plant species that prefer to allocate photosynthetic resources to aboveground biomass. The model also predicted that LAI would need to be reduced by 22% in order to completely compensate the direct effect of elevated CO_2 (crossing point of 0% change in GPP_{day} and change in LAI, Fig. 15a).

I found a steeper relative slope in modeled GPP_{day} with variation in LAI than for variation in V_{cmax} in the sensitivity analysis at elevated CO_2 (Fig. 15b). Whereas a 10% increase in V_{cmax} resulted in a 9.8% increase in modeled GPP_{day} (Fig. 14), here a 10% increase in LAI resulted in an 11.9% increase in GPP_{day} in elevated CO_2. As a result, across all species and N levels there was generally a greater sensitivity of modeled GPP to LAI than V_{cmax} (Table 6).

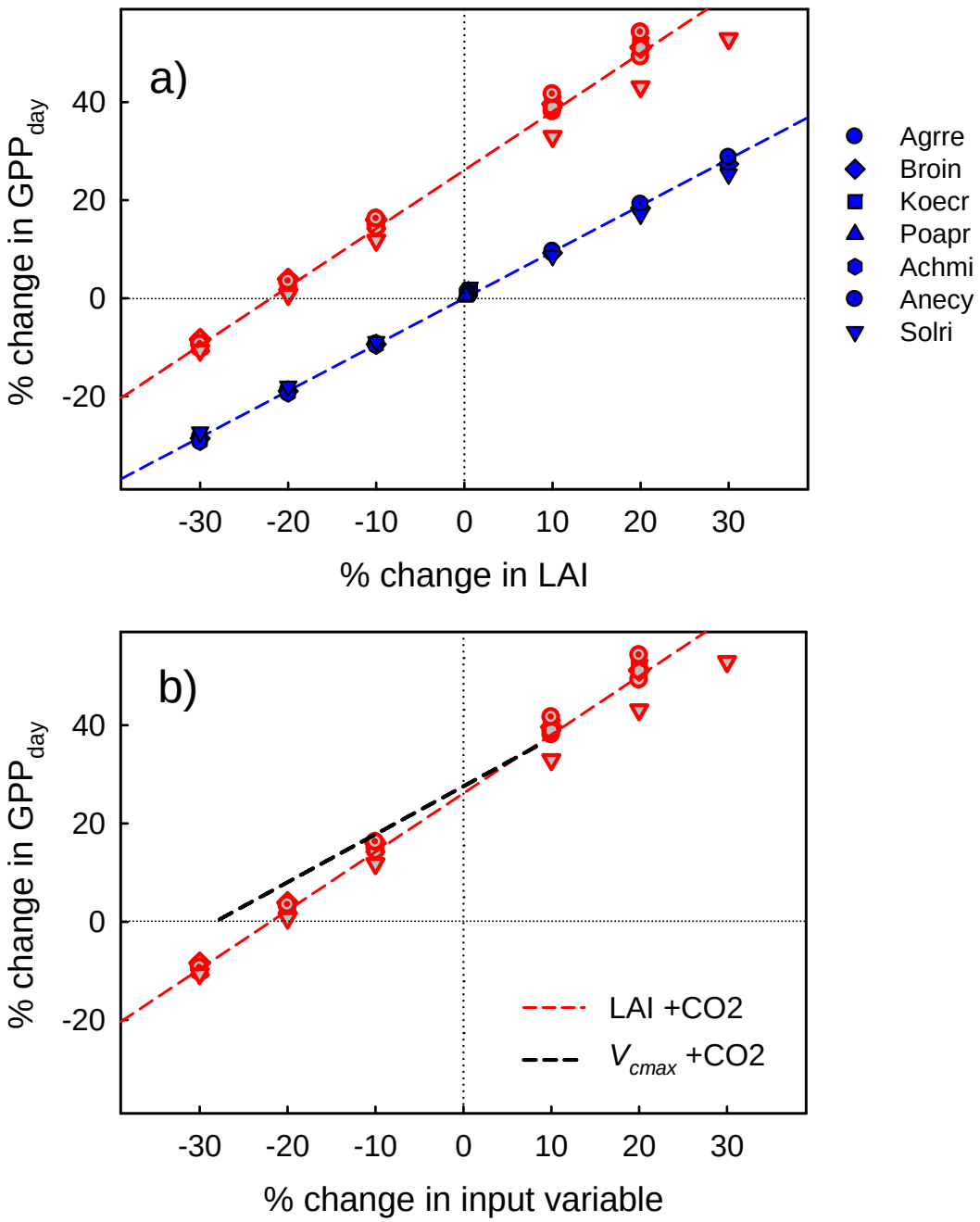


Figure 15: (a) Relationship between the change in LAI and the change in GPP_{day} across N treatments in ambient CO_2 (Y = 0.95x, blue symbols) and in elevated CO_2 (Y = 1.19x + 26.14, red symbols) from a sensitivity analysis for each species. (b) Comparison of relationships resulting from relative changes in GPP_{day} as a function of changes in V_{cmax} (thick black dashed line, from Fig. 14) and as a function of LAI (red dashed line, from Fig. 15a) in elevated CO_2 (+CO2) to assess the sensitivity of each variable on modeled GPP_{day}.

4.4.3. Contributions of V_{cmax} and LAI to the total change in modeled daily GPP

The sensitivity analyses indicated that both LAI and physiological adjustments are critical to estimates of GPP_{day}. Therefore I examined the relative contributions of these variables to the overall CO_2 enhancement for each species, in addition to the direct effect of CO_2 itself (Table 6). The relative contribution of each component to a change in modeled GPP_{day} was calculated as a ratio of elevated/ambient CO_2 conditions. Decomposing the total CO_2-induced change in modeled GPP_{day} into component contributions of direct [CO_2] plus carboxylation (V_{cmax}) and LAI gave insight into the relative proportions of these components as well as species-specific variation of these responses.

Modeled GPP_{day} was most sensitive to the direct effects of an increase in atmospheric CO_2 from 360 μmol CO_2 mol^{-1} to 560 μmol CO_2 mol^{-1}, via a consistent contribution of +21 to +28% to the overall GPP_{day} response (Table 6). While there was considerable variation in modeled GPP_{day} responses to elevated CO_2 (Table 6) the greatest enhancements were observed for species and N availability conditions that showed the largest LAI increases. The change in LAI contributed between -47 to +186% to the total change of GPP in elevated CO_2, depending on species and N treatment. LAI increased more in the N-added treatments than in ambient N. Despite some degree of photosynthetic down-regulation in most species with added N (+V_{cmax} in Table 6), increased LAI in the N-addition treatment ultimately resulted in a strong enhancement of canopy photosynthesis in elevated CO_2 (Table 6). Therefore, the total change in GPP_{day} in elevated CO_2 was larger in N-added treatment than in unamended N for all species except *Agropyron, Poa* and *Anemone*. A higher modeled GPP_{day} at high N conditions can be attributed to increased N availability, enabling plants to sustain higher growth rates in elevated CO_2 and accumulate more biomass (Schneider *et al.*, 2004b; Aeschlimann *et al.*, 2005; Reich *et al.*, 2006a). Both *Poa* and *Agropyron* had smaller CO_2 enhancement in GPP in high N compared to low N was due to reductions in LAI in high N (Table 6). These species may allocate more resources to roots in order to forage for nutrients because both species had high root:shoot ratios (Chapter 3). Strong reductions in LAI in

combination with down-regulation reduced the positive CO_2 fertilization effect in *Bromus, Achillea* and *Anemone* (Table 6) in ambient N.

Species with more down-regulation in elevated CO_2, such as forbs, had less overall GPP enhancement especially when this effect was not compensated with increased LAI (Table 6). Even in the N-addition treatment, LAI responses to elevated CO_2 were offset by photosynthetic down-regulation in forbs such that the net effect of these two components was close to zero. Instead, any modeled GPP_{day} enhancements in forbs were due to direct effects of elevated CO_2 on photosynthesis, generally resulting in smaller GPP_{day} enhancements in elevated CO_2 than those exhibited by C_3 grasses. While C_3 grasses responded to elevated CO_2 with increased LAI, forbs showed little or no LAI enhancement in elevated CO_2 and hence small positive to large negative effects of changes in LAI on modeled GPP_{day} (Table 6).

Table 6: Relative contribution of increased atmospheric CO_2 ($+CO_2$), canopy structure (+LAI), and leaf photosynthetic acclimation ($+V_{cmax}$) to the total response of GPP_{day} to elevated CO_2 for each species in high and low nitrogen. Leaf photosynthetic acclimation is considered to be adjustments in $V_{cmax,}$ as the major driver for photosynthesis. The total shown is the sum of all the components.

	Ambient N				**Added N**			
Species	+ CO_2	+LAI	+V_{cmax}	Total % change	+ CO_2	+LAI	+V_{cmax}	Total % change
Agrre	+26	+7	+1	+34	+26	-6	+2	+22
Broin	+28	-17	-2	+9	+27	+32	0	+59
Koecr	+26	+1	+7	+36	+26	+186	-3	+209
Poapr	+27	+32	+2	+61	+26	-9	+3	+20
Achmi	+25	- 47	-24	-46	+25	+16	-24	+17
Anecy	+27	-12	+6	+21	+26	-1	-7	+18
Solri	+23	+8	-19	+12	+21	+20	-17	+24

Forb species had higher down-regulation of photosynthetic capacity in elevated CO_2 in both high and ambient N treatments than grasses. All forbs down-regulated in elevated CO_2 with ambient N, whereas only *Bromus* showed down-regulation in grasses in elevated CO_2. In high N availability, the down-regulation of forbs in elevated CO_2 remained of similar magnitude whereas grasses did not show as much down-regulation as

in ambient N conditions. This supports my previous observation of photosynthetic down-regulation in forbs vs. grasses in elevated CO_2 (Crous *et al*., submitted) and places these results in context of the overall ecosystem GPP_{day} response. Overall, grasses increased GPP_{day} in elevated CO_2 by 47% on average whereas forbs only increased GPP_{day} by 9%. A negative effect of N addition or elevated CO_2 has also been found in forbs species at other sites, suggesting that forbs species may be more prone to global change effects than graminoids (Zavaleta *et al.*, 2003; Stevens *et al.*, 2006). This was attributed to increased competition with nitrophilic grasses (Stevens *et al.*, 2006) potentially reducing species diversity of grasslands (Suding *et al.*, 2005; Clark & Tilman, 2008)

4.4.4. Comparison of modeled GPP_{day} with measured total biomass

The canopy photosynthesis estimated by the BEWDY model for each species and treatment correlated well with total biomass data for each species across treatments (Fig. 16). There were three distinct species relationships, however, which were significantly different from one another ($P \leq 0.01$). Two of these groups were represented by a single species: *Poa pratensis* (up triangles, Fig. 16) and *Solidago rigida* (down triangles, Fig. 16). The third group included the other five species, because the slopes of each individual species fit were not significantly different from each other ($P > 0.1$). All models accounted for 60-98% of the variation in measured biomass (Fig. 16), indicating that this model based on few physiological inputs and LAI, does reasonably well estimating daily gross canopy photosynthesis (GPP_{day}) especially because biomass accrual reflects additional processes such as turnover and allocation that are not captured by the model. Therefore, predicted changes in modeled GPP_{day} between treatments do not necessarily correlate well with observed changes in biomass.

Daily GPP and total biomass are different, because carbon losses to respiration, turnover and allocation to foliage are represented in biomass data whereas modeled GPP_{day} only reflects the gross canopy photosynthesis. These different entities prevent a true validation of the model, though we can evaluate how well modeled GPP_{day} correlated with total biomass (Fig. 16 and Appendix). *Poa pratensis* differed from other species due

to its large total biomass with low GPP_{day}, whereas *Solidago rigida* had the highest modeled GPP_{day} of all species for a relatively small amount of total biomass (Fig. 16).

The reason for these different slopes in *Solidago* and *Poa* compared to other species can be explained by differences in biomass allocation patterns, more specifically by examining the root:shoot ratios (Fig. 17). *Poa* had the largest amount of root biomass (792 g C m^{-2} across CO_2 treatments), resulting in the highest root:shoot ratio of all species (Fig. 17). In contrast, *Solidago* had the lowest root:shoot ratio of all species, with proportionally more aboveground biomass compared to other species. This resulted in higher LAI and higher GPP_{day} in *Solidago* than any other species examined (Fig. 16). Along with high LAI, *Solidago* had the highest maximum carboxylation rate whereas *Poa* had the lowest rates. This suggests that productive, fast-growing species may preferentially invest resources in photosynthetic capacity whereas slower growing species such as *Poa* first allocated resources to the root system.

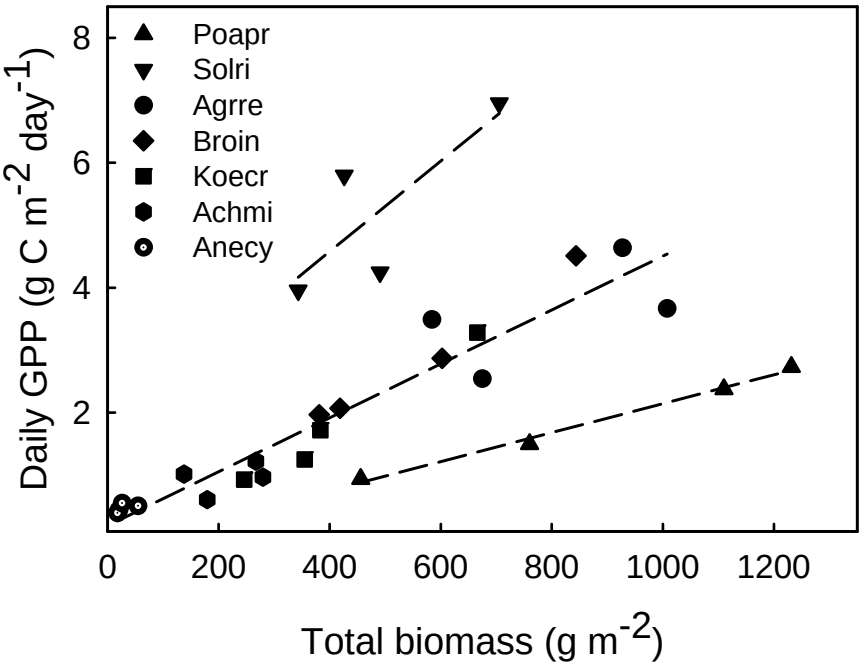


Figure 16: Relationship between total biomass (g m^{-2}) and modeled daily GPP (g C m^{-2} day^{-1}) for seven grassland species, each indicated with different symbols. Each point represents one of four possible treatment combinations of elevated CO_2 and N addition for a given species. Relationships for *Solidago* (Solri, down triangles): $Y = 7.24x + 1.7$, $R^2 = 0.64$; *Poa* (Poapr, up triangles) $Y = 3.32x - 0.18$, $R^2 = 0.98$; all remaining species: $Y = 4.32x + 0.19$, $R^2 = 0.91$.

In theory, patterns of allocation and turnover represented in biomass can be correlated to modeled GPP_{day}. The foliage biomass (B_f) or root biomass (B_r) over time is represented by the total biomass increment (net growth or NPP) multiplied by the fraction allocated to foliage (a_f) or root (a_r) and divided by the amount of foliage or root turnover (t_f or t_r respectively). Hence,

$$B_f = a_f/t_f * \text{NPP} \qquad \text{and} \qquad B_r = a_r/t_r * \text{NPP}$$

where at BIOCON the foliage part completely turns over due to winter die off ($t_f = 1$) and the root turnover is assumed to be constant over the course of one month, for which the model ran. This assumption would need to be adjusted if the model was extended over one growing season or several years due to temporal patterns of root mortality. Given that $a_f + a_r = 1$ then $a_r = 1 - a_f$. So the total biomass (B_{TOT}) is the sum of biomass components of roots (B_r) and shoots (B_f):

$$B_{TOT} = B_f + B_r = a_f*\text{NPP} + (1- a_f)*\text{NPP}*1/t_r$$

Given that carbon use-efficiency (CUE) is defined as the ratio of net primary production to gross primary production (Cannell & Thornley, 2000), GPP can be related to NPP by NPP = GPP * CUE.

Therefore,

$$B_{TOT} = B_f + B_r = GPP_{day}*\text{CUE} *[a_f+(1-a_f)\ 1/t_r]$$

Hence, root:shoot ratios, defined as the ratio of total root biomass to total foliage biomass (B_r/B_f) can be calculated as

$$\frac{Br}{Bf} = \frac{(1-a_f)*CUE*GPP_{day}*1/t_r}{a_f*CUE*GPP_{day}} = \frac{1-a_f}{t_r*a_f}$$

Assuming a constant root turnover (t_r) and ignoring other C losses such as herbivory and root exudation, the equation above indicates that different root:shoot ratios (B_r/B_f) are related to different allocation patterns (a_f) among the different prairie grassland species, resulting in different slopes when correlating GPP_{day} with total biomass (Fig. 16).

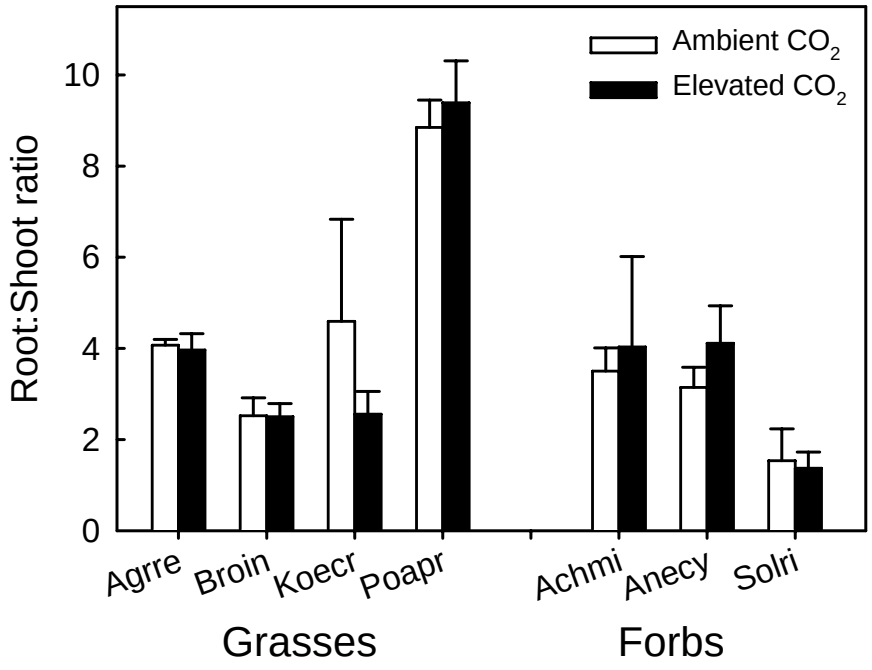


Figure 17: Means and standard error of measured root:shoot ratio of 7 grassland species at BioCON in ambient (open bars) and elevated CO_2 (black bars) across both N treatments.

Estimates of LAI and biomass, although obtained through independent measurements, are likely to be correlated among plots. This means that a statistical relationship (i.e. *P*-value) between total biomass and daily GPP might be inflated because LAI is related to above ground biomass. However, my interest here is not in the statistical strength of the relationship, but rather to gain the best possible predication of daily GPP for each species under climate change.

Improvements to the model can be made by including inputs for LAI, allocation and turnover to estimate total biomass in a growth model across the growing season. The model would calculate biomass increments each day based on inputs of LAI, allocation and turnover and integrate these daily increments across a growing season to estimate total biomass. Obtaining a model estimate of total biomass would allow validating the model results against the harvested total biomass. However, different root:shoot ratios between forbs and grasses (Fig. 17) may indicate different N-uptake mechanisms, which also may need to be incorporated in the growth model. Though I clearly showed good

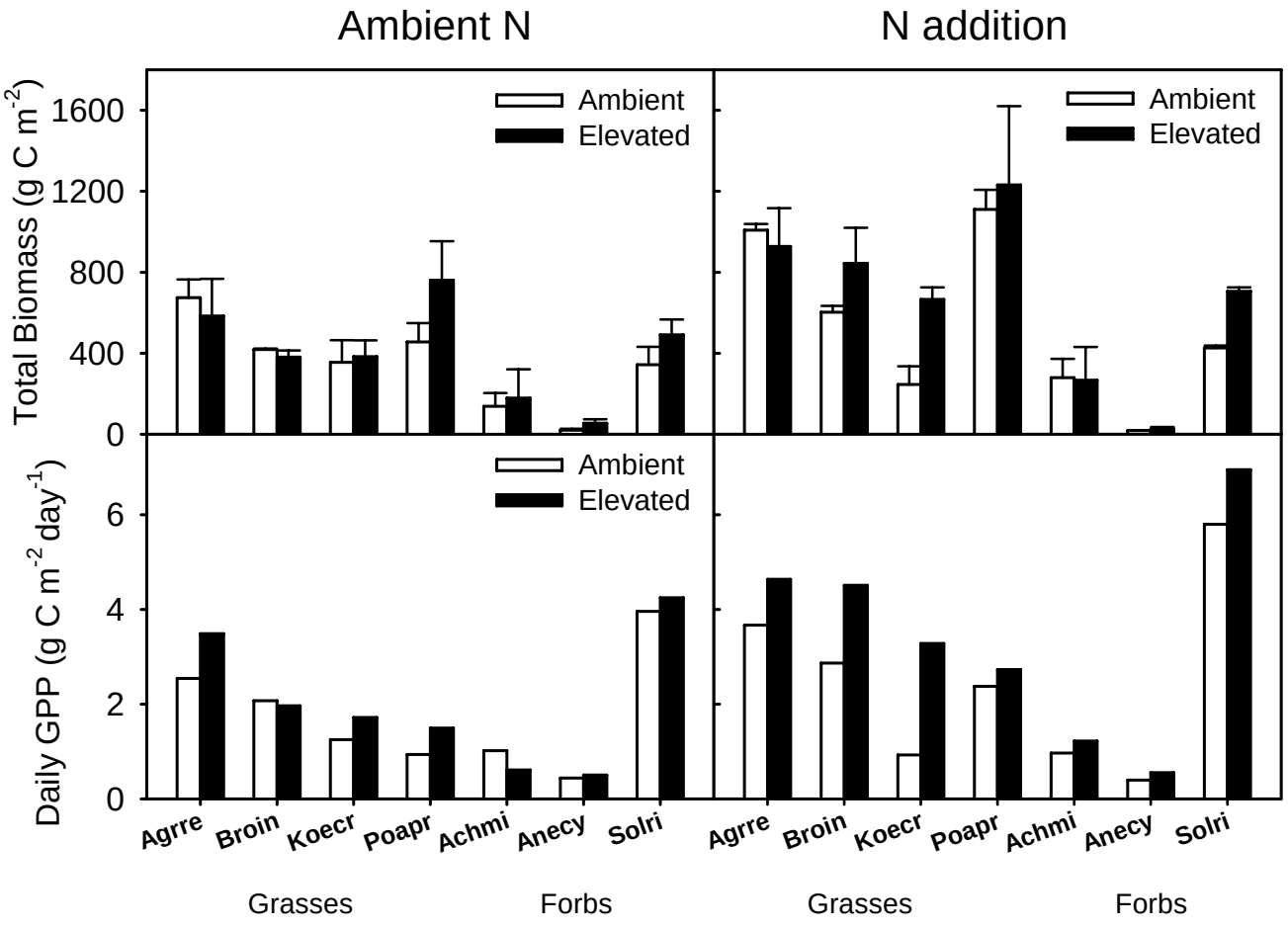
correlations between daily GPP and total biomass and how GPP_{day} is related to root:shoot ratios, a growth model would decrease the direct influence of LAI in addition to improving insights into biomass responses of different grassland species to elevated CO_2 and N addition and how these responses may affect biomass proportions and species composition in diverse species plots.

4.5. Conclusions

This study assessed differences in GPP_{day} between different CO_2 and N treatments for different grassland species to understand how this response is influenced by physiology and canopy LAI in a simple sun-shade model. Evaluating the relative contributions of each component in the model, the increase in elevated CO_2 itself contributed ~27% of the total GPP increase in elevated CO_2. In addition to this direct effect were the contributions of change in LAI and photosynthetic acclimation to differences in GPP_{day} between elevated and ambient CO_2. Though few analyses have discussed the importance of LAI responses to elevated CO_2 (Norby *et al.*, 2003), the model showed that both responses of LAI and physiological acclimation varied among species and functional groups. Grasses showed large increases in LAI in addition to the direct effect of CO_2, while forbs showed little LAI enhancement to elevated CO_2. Therefore, the small LAI response in forbs was offset by the amount of photosynthetic down-regulation resulting in a net effect in GPP_{day} due only to the direct effect of elevated CO_2. Different allocation patterns in different species were important with respect to their responses to elevated CO_2. These processes were not considered in this model simulating canopy photosynthesis but could be incorporated into a growth model.

4.6. Appendix

Model simulation results of modeled daily GPP for C_3 grass and forb species at BioCON, Minnesota, USA and measured total biomass (g C m^{-2}). All data were averaged over years 6-9 of the experiment. Ambient CO_2 data are indicated by open bars. Elevated CO_2 data in FACE are indicated by dark bars.



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Chapter 5

Photosynthetic adjustments to elevated CO_2 within and across functional groups of plants: a synthesis.

5.1. Introduction

The overarching goal of this dissertation was to determine potential changes in the fundamental photosynthesis-nitrogen relationship under elevated CO_2 concentrations and to quantify plant responses to elevated CO_2 and N-addition including potential interactions. If the strong positive relationship between photosynthesis and foliar nitrogen were to change under elevated CO_2, then this could affect C uptake estimates at higher scales because many models employ this relationship at the leaf-level to scale up to larger scales. Because most leaf N is invested in photosynthetic proteins (Evans, 1989), V_{cmax} is also strongly correlated with leaf N (Walcroft *et al.*, 1997; Medlyn *et al.*, 1999).

The relationship between photosynthetic capacity (e.g. maximum leaf CO_2 assimilation) and leaf N is a tool to evaluate variation in carbon processing among diverse plant species. Changes in this relationship in response to elevated CO_2, such as a declining slope in the relationship between carboxylation rate (V_{cmax}) and leaf N_{area}, could indicate a decoupling between photosynthesis and leaf nitrogen and may reveal a mechanism by which plants adjust physiologically to long-term elevated CO_2. This last chapter will aim at summarizing my results while determining general patterns in my data.

Only a few experimental studies have exposed vegetation to long-term elevated CO_2, currently limiting our knowledge about multi-factor interactions and insight into ecosystem functions with respect to global change. My dissertation work aimed at finding

a general mechanism by which plants adjust to changing environmental conditions such as elevated CO_2 and N fertilization. In the first chapter I reported down-regulation of photosynthesis in one-year old loblolly pine leaves but not in current-year leaves. This down-regulation was caused by a reduced allocation of N to Rubisco in leaves grown in elevated CO_2. The reduced allocation of N to Rubisco resulted in declining slopes of the V_{cmax}-N relationship after eight to nine years of elevated CO_2 exposure. The slope of this relationship was restored by N fertilization, implying that N availabiliy modulated the CO_2 response in pines.

A modulated CO_2 response with N addition was also found in herbaceous species at BioCON, resulting in significant CO_2 x N interactions. Both mass-based foliar N and photosynthesis at a common CO_2 level increased more when N was added in ambient CO_2 than in elevated CO_2. In addition, significant CO_2 x N x functional group interactions indicated that forb species were negatively affected long-term by elevated CO_2 which was further exacerbated with N addition. Non-leguminous forb species did not show any CO_2 enhancement in long-term elevated CO_2 in N addition plots. This was attributed to significant reductions (- 28%) in leaf N_{mass} concentration in forbs. In contrast, grasses were able to maintain leaf N concentrations along with a photosynthetic CO_2 enhancement of 68%. Differences in leaf N reductions in response to elevated CO_2 were linked to differences in allocation. Grasses grew significantly more root biomass than did forbs in elevated CO_2 than forbs, presumably allowing them to forage for more nutrients and meet enhanced growth demands in elevated CO_2.

These leaf-level differences between plant functional groups persisted at the canopy level because increased photosynthesis rates in elevated CO_2 resulted in higher LAI and higher daily GPP. In ambient CO_2 conditions, GPP_{day} values were similar in grasses and forbs but grasses had larger GPP_{day} enhancements (+43%) in elevated CO_2 compared to forb species (+12%). Both LAI and physiological traits contributed to the overall GPP_{day} enhancement in elevated CO_2, though these contributions were variable for each species. The relationship between total biomass and modeled GPP_{day} confirmed the importance of different allocation patterns between the species, which seems to be important in their responses to elevated CO_2 and N addition. In this chapter I will tie my

data together with the literature focusing on general mechanisms at the leaf scale in different functional groups.

5.2. Climatic differences and site location

Because elevated CO_2 experiments are sufficiently intensive that they are not replicated across many sites or landscapes, some ecological information from them is likely to be site-specific. I asked, were the differences in results between the Duke Forest and BioCON experiments due to differences between the sites, or more generally due to biological differences? Both of these experiments were established in artificial vegetation that represents the major dominant natural vegetation type in their respective regions. The *Pinus taeda* L. stand in Duke Forest can be considered representative of 23 million ha of southern pines in the U.S., while C_3 grass-dominated prairies once covered tens of millions of ha across the northern plains region of the U.S. and Canada. Both sites are nutrient-poor and limited by N availability as indicated by low rates of N mineralization, but they represent different soil Orders and different textural classes (sand vs. loam; Table 7).

The FACE sites that were intensively studied differed in a number of important regards (see Table 7). There were strong climate differences as expected from the latitudinal differences between the sites, including a shorter growing season, colder average temperatures, and lower annual precipitation at BioCON than Duke Forest (Table 7). While growing season duration has important effects on plant phenology and may constrain leaf longevity, apart from this there is strong convergence in leaf traits across large climatic gradients worldwide (Wright *et al.*, 2005; Enquist *et al.*, 2007a). Despite climate differences between the sites, summertime temperatures are comparable between them though slightly cooler on average for the more northern BioCON site than Duke Forest. Recent evidence suggests that long-term climate and temperature in particular has a small to negligible role in explaining latitudinal differences in photosynthesis and plant metabolism across many sites (Enquist *et al.*, 2007b; Helliker & Richter, 2008). Moreover, the physiological variables presented for Duke Forest and BioCON in previous chapters and in here Fig. 18 and Fig. 21 such as V_{cmax} and J_{max} were measured at

temperatures similar to the mean July maxima in Table 7 and hence these differences are relatively small and cannot account for the responses that were observed. Given the decadal time scale of these experiments and in the absence of strong evidence that climate or site factors co-vary with the leaf traits or elevated CO_2 responses observed previously in Chapters 2 and 3, I consider these differences to be driven by intrinsic biological differences between species. One major aspect of these biological differences can be ascribed to plant functional group, and hereafter I analyze my data by functional group.

Table 7: Site characteristics of two major Free-Air CO_2 Enrichment experiments where data for this dissertation were collected.

Site Characteristic	**Duke Forest, N. Carolina**	**BioCON, Minnesota**
Latitude, Longitude	35.9°N, 79.1°W	45.4°N, 93.2°W
Mean annual temperature[1]	16.5° C	7.2° C
Mean daily maximum July temperature[1]	31.5° C	28.4° C
Growing season[1]	March – October	Late April – early September
Growing season duration (frost-free days)[1]	~ 200 days	~ 145 days
Mean annual precipitation[1]	1150 mm	700 mm
Soil type	Hapludalf	Psamment
Dominating soil texture	Clay	Sand
N Mineralization rate[2]	3 g N m^{-2} yr^{-1}	2-3 g N m^{-2} yr^{-1}
N fertilization treatment	11 g N m^{-2} yr^{-1}	4 g N m^{-2} yr^{-1}
CO_2 treatment in FACE	+200μmol CO_2 mol^{-1}, for 570 μmol CO_2 mol^{-1}	560 μmol CO_2 mol^{-1}
FACE ring diameter	30 m	26 m
Subplots	Two ring halves (265 m^2)	61 4m^2 plots per ring

[1] Data from: http://cdo.ncdc.noaa.gov/cgi-bin/climatenormals/climatenormals.pl from http://www.ncdc.noaa.gov/oa/climateresearch.html, and http://iridl.ldeo.columbia.edu/SOURCES/.NOAA/.NCEP/.CPC/.GSOD/.MONTHLY/
[2] Data from: (Oren *et al.*, 2001b), (Finzi *et al.*, 2006) and (Reich *et al.*, 2006a).

5.3. Effects of long-term elevated CO_2 on photosynthetic capacity

5.3.1. Changes in photosynthetic capacity in long-term elevated CO_2 at both BioCON and Duke FACE sites

Because the strong positive relationship between photosynthesis and nitrogen arises from the major investment of N in photosynthetic proteins (Evans, 1989), V_{cmax} is strongly correlated with N (Medlyn *et al.*, 1999). In contrast to photosynthesis, carboxylation rates reflect the biochemical function of the plant, independent of climatic variables or stomatal responses. There were distinct relationships between V_{cmax} and N for each functional group examined (e.g. grasses, forbs and pine, Fig. 18) and two out of three functional groups had significantly different relationships in elevated CO_2 compared to ambient CO_2.

Whereas there was no elevated CO_2 effect on the photosynthetic capacity (e.g., V_{cmax}) in grasses ($P > 0.1$), both non-leguminous forbs and pine species showed significant differences in slopes between ambient and elevated CO_2 treatments of their respective V_{cmax}-N_{area} relationships (Fig. 18). Slopes were less steep in elevated CO_2 (42% less steep in forbs and 51% less steep in pines) compared to ambient CO_2. Both functional groups showed lower photosynthetic capacity in elevated CO_2, accompanied by a reduction in leaf N_m (Fig. 19). Forbs had a higher photosynthetic capacity in ambient CO_2 than C_3 grasses but reduced to the same level as the C_3 grasses when exposed to elevated CO_2. Both forbs and pine reduced mean photosynthetic capacity significantly in elevated CO_2 ($P = 0.03$ for forbs and $P = 0.06$ for pines across N treatments, Fig. 19), whereas grasses did not ($P > 0.1$). This trend corresponded to no reduction in leaf N_{mass} in grasses, but significant reductions in pines (-12%, $P = 0.017$) and forb species (-18%, $P = 0.04$) in elevated CO_2 (Fig.19).

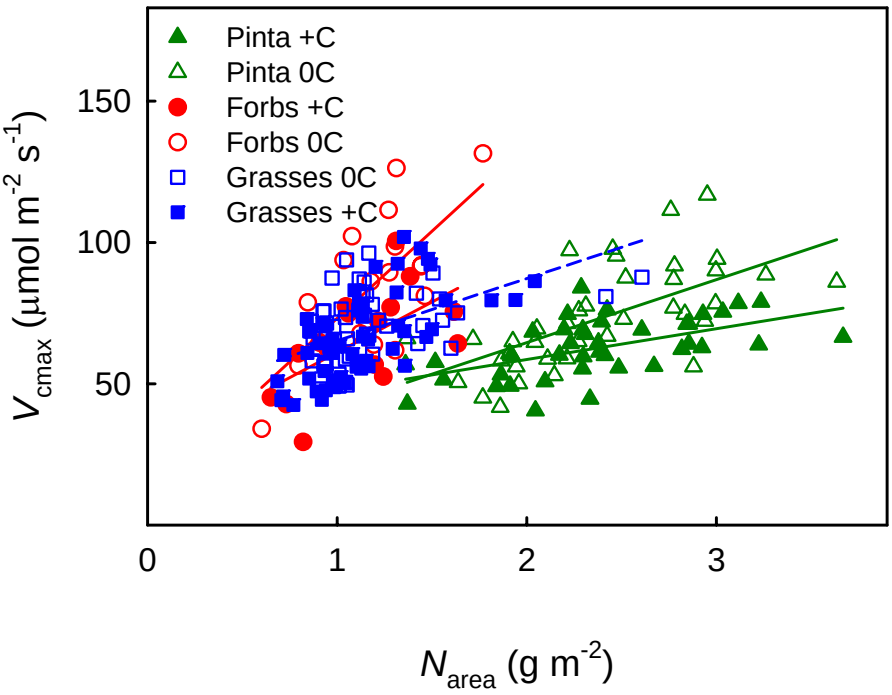

Figure 18: Relationship between maximum carboxylation rate, V_{cmax} and leaf N_{area} in ambient (0C, open symbols) and elevated CO_2 (+C, filled symbols) treatments for eight species from two FACE sites categorized in three functional groups: *Pinus taeda* (pinta, triangles), non-leguminous forbs (circles) and C_3 grasses (squares). Relationships were significant (P<0.0001) and are as follows: Pinta: V_{cmax} = 10.91 N_{area} + 36.91 in elevated CO_2, R^2 = 0.40 and V_{cmax} = 22.29 N_{area} + 19.98 in ambient CO_2, R^2 = 0.40. Non-leguminous forbs: V_{cmax} = 35.65 N_{area} + 25.49 in elevated CO_2, R^2 = 0.35 and V_{cmax} = 61.49 N_{area} + 11.74 in ambient CO_2, R^2 = 0.47. C_3 Grasses across both CO_2 treatments: V_{cmax} = 22.04 N_{area} + 43.12, R^2 = 0.26. All species are represented on a surface area basis.

Despite these patterns of down-regulation in the photosynthetic capacity of pines and non-leguminous forbs in elevated CO_2, there was still significant enhancement of net photosynthesis in elevated CO_2. The photosynthetic enhancement is largest in grasses (+ 57%, $P < 0.0001$), where no down-regulation occurred versus a smaller enhancement in pines (+ 31%, $P = 0.0011$) and no significant enhancement in forbs (+9%, $P = 0.58$) across nitrogen treatments (Fig. 19). This suggests that the concentrations of elevated CO_2 contribute directly to the overall response via direct effects such as the repression of photorespiration at the leaf-level and a more efficient carboxylation enzyme (Nowak *et al.*, 2004; Ainsworth & Rogers, 2007) and that the direct positive effect of elevated CO_2 on photosynthesis is larger than the indirect down-regulation effect.

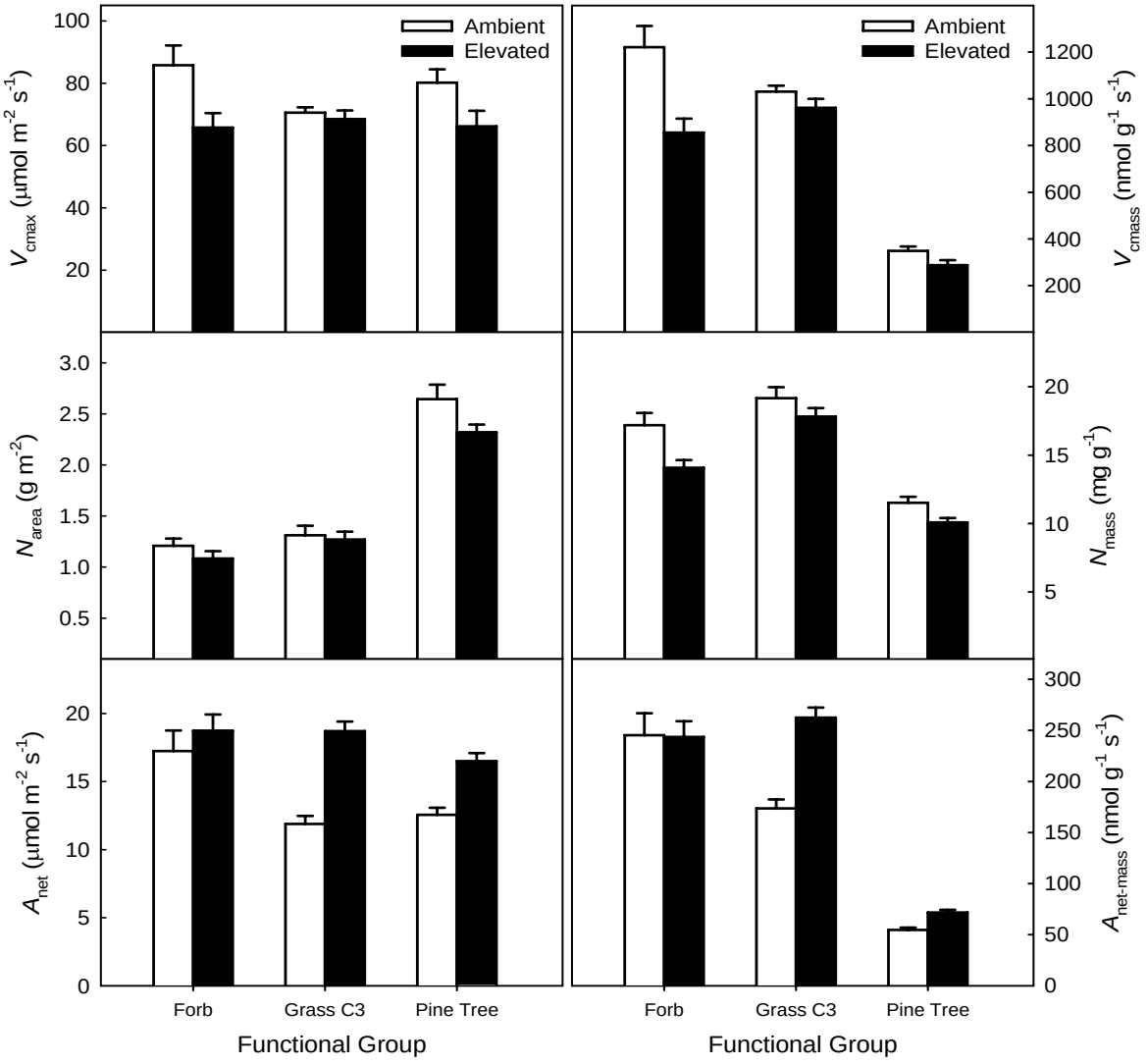

Figure 19: Means and standard errors of maximum carboxylation rate (V_{cmax}, top), foliar N (middle) and net photosynthesis (bottom) on an area basis (left panels) and mass basis (right panels) in ambient (open bars) and elevated CO_2 (filled bars) for each functional group (e.g. Forbs, C_3 grass or Pine tree) representing eight species studied in this dissertation at two FACE sites with 6-9 years of elevated CO_2 exposure.

Table 8: Overview of species and sites used for Figure 20 including their geographical location, functional group, type of experiment (OTC = Open-Top Chamber, FACE = Free-air CO_2 Enrichment) and duration of CO_2 exposure in years.

Species	**Functional Group**	**Site**	**Latitude**	**Type of Experiment**	**Duration of CO_2 exposure**	**Reference**
Betula alleghaniensis	Deciduous	Harvard University Concord Field Station, MA	42°30'N, 72°10'W	OTC	3	Bauer *et al*., 2001
Quercus rubra	Deciduous	Harvard University Concord Field Station, MA	42°30'N, 72°10'W	OTC	3	Bauer *et al*., 2001
Acer rubrum	Deciduous	Harvard University Concord Field Station, MA	42°30'N, 72°10'W	OTC	3	Bauer *et al*., 2001
Pinus strobus	Evergreen	Harvard University Concord Field Station, MA	42°30'N, 72°10'W	OTC	3	Bauer *et al*., 2001
Picea rubens	Evergreen	Harvard University Concord Field Station, MA	42°30'N, 72°10'W	OTC	3	Bauer *et al*., 2001
Acer rubrum	Deciduous	Duke Forest, Orange County, NC	35°58'N, 79°05'W	FACE	7	Springer & Thomas, 2007
Carya glabra	Deciduous	Duke Forest, Orange County, NC	35°58'N, 79°05'W	FACE	7	Springer & Thomas, 2007
Cercis canadensis	Deciduous	Duke Forest, Orange County, NC	35°58'N, 79°05'W	FACE	7	Springer & Thomas, 2007
Liquidambar styraciflua	Deciduous	Duke Forest, Orange County, NC	35°58'N, 79°05'W	FACE	7	Springer & Thomas, 2007
Pinus taeda	Evergreen	Duke Forest, Orange County, NC	35°58'N, 79°05'W	FACE	9	Crous *et al.*, 2008
Pinus radiata	Evergreen	Christchurch, New Zealand	43°32′S, 172°42′E	OTC	4	Griffin *et al.*, 2000
Populus x euramericana	Deciduous	Viterbo, Tuscania, Italy (PopFACE)	42°22' N, 11°48'E	FACE	4	Calfapietra *et al.*, 2005
Popoulus nigra	Deciduous	Viterbo, Italy (PopFACE)	42°22' N, 11°48'E	FACE	5	Liberloo *et al.*, 2007
Fagus sylvatica	Deciduous	Bordeaux, France		OTC	2	Liozon *et al.*, 2000
Polygonum sachalinense	Forb	Yuno-kawa, Japan	40°41'N 140°55'E	Natural CO_2 vent		Onoda *et al.*, 2007
Plantago asiatica	Forb	Nyuu, Japan	38°32'N 139°59' E	Natural CO_2 vent		Onoda *et al.*, 2007
Agrostis capillaris	C_3 grass	Institute of Grassland and Environmental research, North Wyke, UK	-	Growth chamber	2	Davey *et al,*. 1999
Lolium perenne	C_3 grass	Institute of Grassland and Environmental research, North Wyke, UK	-	Growth chamber	2	Davey *et al.*, 1999
Quercus myrtifolia	Evergreen	NASA Kennedy Space Center, FL (Meritt Island)	28°38'N 80°42'W	OTC	5	Hymus *et al.*, 2002

Quercus geminata	Evergreen	NASA Kennedy Space Center, FL (Meritt Island)	28°38'N 80°42'W	OTC	5	Hymus *et al.*, 2002
Pinus sylvestris	Evergreen	University of Antwerp, Belgium	51°10'N, 4°24' E	OTC	>1	Jach & Ceulemans, 2000
Betula pendula	Deciduous	Glencorse, Edinburgh, UK	55°31'N, 3°12'W	OTC	3	Rey & Jarvis, 1998
Lolium perenne	C_3 grass	Eschikon Experimental Station, Switzerland	47°27'N, 8°41' E	FACE	2	Rogers *et al.*, 1998
Acer saccharum	Deciduous	Rhinelander, Wisconsin	45°30'N, 89°38'W	FACE	5	Ellsworth *et al.*, 2004
Betula papyrifera	Deciduous	Rhinelander, Wisconsin	45°30'N, 89°38'W	FACE	5	Ellsworth *et al.*, 2004
Liquidambar styraciflua	Deciduous	Duke Forest, Orange County, NC, USA	35°58'N, 79°05'W	FACE	6	Ellsworth *et al.*, 2004
Populus tremuloides	Deciduous	Rhinelander, Wisconsin	45°30'N, 89°38'W	FACE	5	Ellsworth *et al.*, 2004
Quercus rubra	Deciduous	Headley III, UK	52°08'N, 00°50'W	OTC	>1	Medlyn *et al.*, 1999
Quercus petraea	Deciduous	Headley III, UK	52°08'N, 00°50'W	OTC	>1	Medlyn *et al.*, 1999
Quercus robur	Deciduous	Headley III, UK	52°08'N, 00°50'W	OTC	>1	Medlyn *et al.*, 1999
Fagus sylvatica	Deciduous	TUB Fagus II, Berlin	52°28'N, 13°18'E	Mini-FACE	4	Medlyn *et al.*, 1999
Pistacia lentiscus	Evergreen	Macchia, Italy	42°22'N, 11°32'E	OTC	3	Medlyn *et al.*, 1999
Philyrea angustifolia	Evergreen	Macchia, Italy	42°22'N, 11°32'E	OTC	3	Medlyn *et al.*, 1999
Solidago rigida	Forb	BIOCON, Minnesota, USA	45°27'N, 93°11'W	FACE	9	Crous *et al.*, submitted
Anemone cylindrica	Forb	BIOCON, Minnesota, USA	45°27'N, 93°11'W	FACE	9	Crous *et al.*, submitted
Achillea millefolium	Forb	BIOCON, Minnesota, USA	45°27'N, 93°11'W	FACE	9	Crous *et al.*, submitted
Bromus inermis	C_3 grass	BIOCON, Minnesota, USA	45°27'N, 93°11'W	FACE	9	Crous *et al.*, submitted
Agropyron repens	C_3 grass	BIOCON, Minnesota, USA	45°27'N, 93°11'W	FACE	9	Crous *et al.*, submitted
Poa pratensis	C_3 grass	BIOCON, Minnesota, USA	45°27'N, 93°11'W	FACE	9	Crous *et al.*, submitted
Koeleria cristata	C_3 grass	BIOCON, Minnesota, USA	45°27'N, 93°11'W	FACE	9	Crous *et al.*, submitted

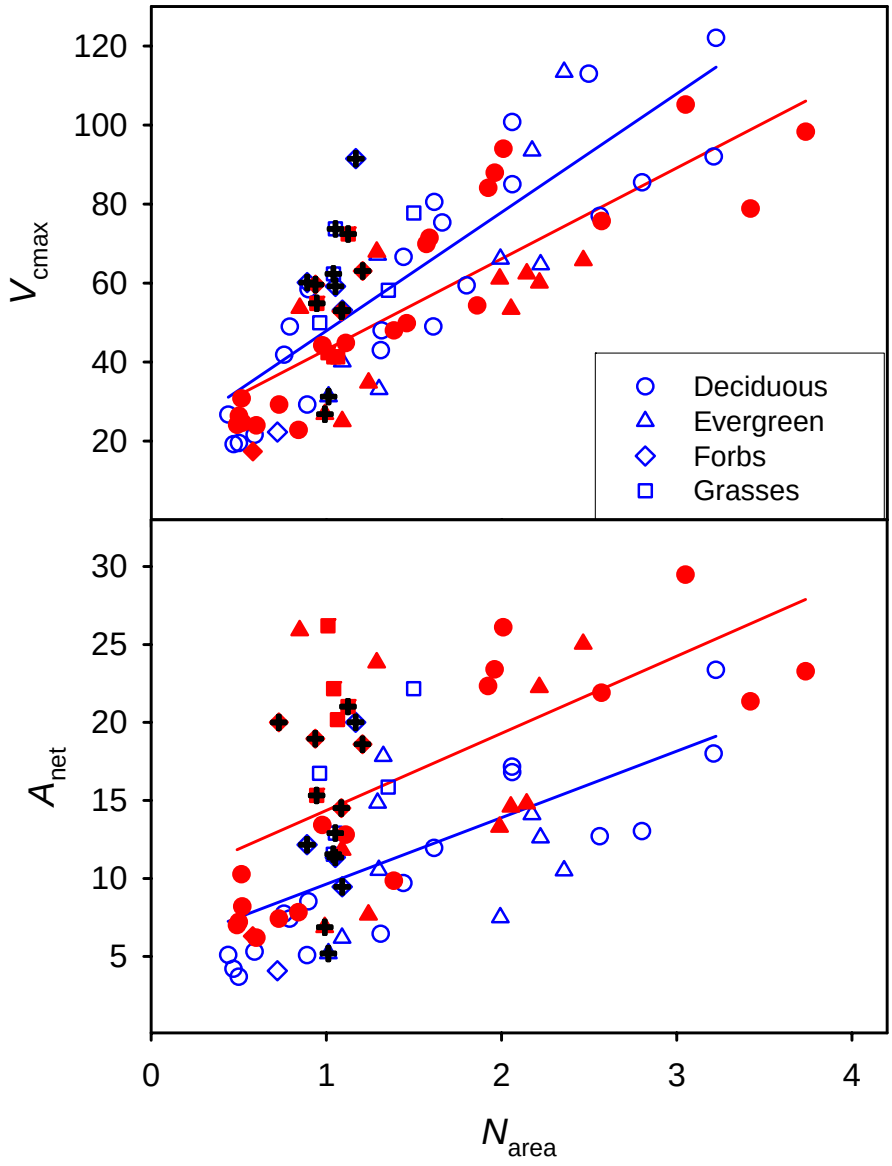

Figure 20: Relationships of V_{cmax} (upper panel) and A_{net} (lower panel) as a function of N_{area} across species in table 8. Each point represents a species at a given site in ambient (0C, open symbols) or elevated CO_2 (+C, filled symbols). Species were categorized in four different functional groups: deciduous trees (circles), evergreen trees (triangles), non-leguminous forbs (diamonds) and C_3 grasses (squares). Plus signs indicate my own data from 6-9 years of elevated CO_2 exposure at two nutrient-poor FACE sites. Equations of the linear regressions were: $V_{cmax} = 22.99\ N_{area} + 20.19$ in elevated CO_2, $R^2 = 0.66$ and $V_{cmax} = 30.01\ N_{area} + 17.90$ in ambient CO_2, $R^2 = 0.66$; $A_{net} = 4.94\ N_{area} + 9.42$ in elevated CO_2, $R^2 = 0.34$ and $A_{net} = 4.27\ N_{area} + 9.42$ in ambient CO_2, $R^2 = 0.36$.

5.3.2. Could declining slopes between V_{cmax} and N_{area} in elevated CO_2 be a more general phenomenon across sites?

To assess how general the observed changes in the V_{cmax}-N_{area} relationship in elevated CO_2 were across sites, I collected data from the literature including 35 C_3 species across 17 different sites (Table 8). Means of photosynthesis, leaf nitrogen concentration and maximum carboxylation rates were collected in ambient and elevated CO_2 for four different functional groups to plot V_{cmax}-N_a relationships in each CO_2 treatment. These functional groups included non-leguminous forbs, C_3 grasses, evergreen and deciduous trees. To be consistent with previously collected data and former meta-analyses, I included data from upper canopy sunlit leaves in nutrient-poor conditions. When seasonal data were reported, I included data measured during a similar time of the year as my own data (e.g. May or June) and the duration of CO_2 exposure had to be at least one year. When more than one year was reported, the latest measurements were included. Each point in Fig. 20 represents a different species in ambient or elevated CO_2 at a given site. Though many elevated CO_2 studies are available, only a handful of studies with CO_2 exposure of more than one year reported both the photosynthetic capacity (V_{cmax}, J_{max}) and leaf N (on area basis) in addition to photosynthesis rates.

Across four functional groups and 17 species per CO_2 treatment, there was a trend towards a declining slope in the V_{cmax}-N_a relationship in elevated CO_2 conditions (Fig. 20). Though only weakly significant ($P = 0.0749$), the slope value of the V_{cmax}-N_a relationship in elevated CO_2 was 24% lower than that in ambient CO_2 conditions, potentially suggesting less N-investment towards photosynthetic components. However, despite reduced N invested into Rubsico, net photosynthesis was enhanced in elevated CO_2 by 34% across functional groups ($P = 0.0077$, Fig. 20 lower panel).

Both the intercept ($P < 0.0001$) and the effect of CO_2 treatment ($P = 0.0315$) were highly significant (Fig. 20 upper panel). This signifies a strong direct effect of elevated CO_2 on Rubisco, where Rubisco is reduced in long-term elevated CO_2 exposure. The change in intercept is important because it reflects the structural investment in the leaf in order to photosynthesize. As slopes decline in elevated CO_2, intercepts tend to increase, potentially indicating a higher investment of N in structural components in elevated CO_2

(Hikosaka *et al.*, 1998). It has been hypothesized that there is a trade-off between N allocation to photosynthetic components and investment into structural components such LMA (Leaf mass per area) (Onoda *et al.*, 2004; Hikosaka *et al.*, 2005). LMA is often used as a structural biomass index (Reich *et al.*, 1991; Wright & Cannon, 2001) and is negatively correlated with the use of photosynthesis per unit nitrogen (Poorter & Evans, 1998). There was a negative correlation in my data (not shown), supporting the trade-off between structural components and N allocation to photosynthesis. This potential trade-off could be further investigated as it may point to changed N distribution patterns in the leaf.

5.3.3. The ratio of electron transport rate (J_{max}) to the carboxylation rate (V_{cmax})

Theory predicts that as CO_2 concentrations rise, the metabolic control of light-saturated photosynthesis by Rubsico (V_{cmax}) declines in elevated CO_2 whereas the control by the RubP regeneration rate (J_{max}) increases (Long & Drake, 1991). As Rubsico is more efficient in elevated CO_2, a change in J_{max}:V_{cmax} ratio would indicate a change in N allocation between photosynthetic components because less N would need to be invested into Rubisco (Drake *et al.*, 1997; Long *et al.*, 2004). In an optimization model, Medlyn (1996) found that a change in J_{max}:V_{cmax} ratio was a good indicator of re-allocation of leaf nitrogen. The optimal J_{max}:V_{cmax} ratio was predicted to increase by 40% when atmospheric CO_2 concentrations doubled (Medlyn, 1996). This theory is based on the assumption that plants optimize allocation of nitrogen within the plant given that N is the most limiting element in terrestrial ecosystems (Sage, 1994). Therefore, down-regulation of photosynthetic capacity in elevated CO_2 is related to the re-allocation of nitrogen from non-limiting processes to more limiting processes in order to optimize nitrogen-use in elevated CO_2 (Sage, 1994; Stitt & Krapp, 1999).

Despite these theoretical predictions, many studies have not observed changes in the J_{max}:V_{cmax} ratio at different light regimes (Wullschleger, 1993) nor at elevated CO_2 concentrations (Gunderson & Wullschleger, 1994; Curtis *et al.*, 1995; Medlyn *et al.*, 1999; Crous & Ellsworth, 2004). The only exception is a recent meta-analysis conducted by Ainsworth *et al.* (2005, 2007) suggesting a diversion of J_{max} in elevated CO_2 from

V_{cmax}, potentially resulting in different J_{max}:V_{cmax} ratios. This diversion was attributed to a shift in carboxylation limitation in ambient CO_2 conditions to a limitation in RubP regeneration in elevated CO_2, in which plants would reduce their carboxylation capacity (V_{cmax}) before reducing RubP regeneration capacity (J_{max}) (Ainsworth & Long, 2005; Ainsworth & Rogers, 2007). This result could be an artifact in the meta-analysis due to higher availability of V_{cmax} measurements compared to J_{max} values reported in the literature.

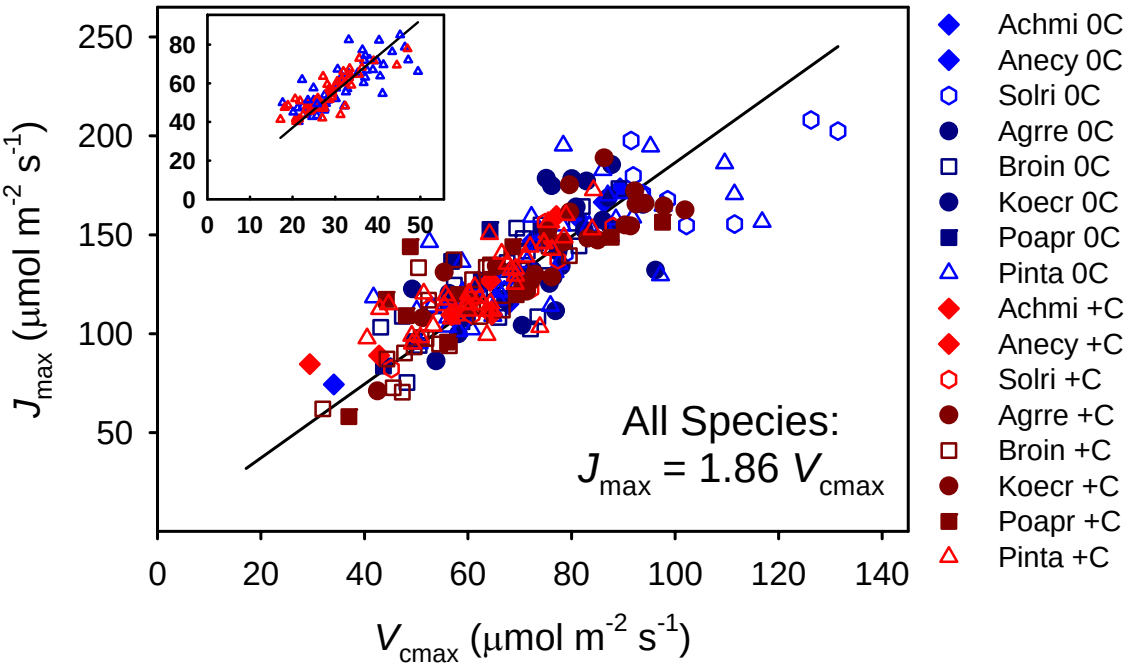

Figure 21: Relationship between J_{max} and V_{cmax} for eight species in ambient (0C) and elevated CO_2 (+C) treatments after 6-9 years of elevated CO_2 exposure in FACE. Species names were abbreviated and represent the following: Achmi = *Achillea millefolium*, Anecy = *Anemone cylindrica*, Solri = *Solidago rigida*, Agrre = *Agropyron repens*, Broin = *Bromus inermis*, Koecr = *Koeleria cristata*, Poapr = *Poa pratensis* and Pinta = *Pinus taeda*. All species are represented on a surface area basis. The inset shows the pine species on a projected-area basis.

My study showed strong evidence for a tight coordination between V_{cmax} and J_{max} because no significant difference was detected between ambient and elevated CO_2 treatments at high and low N-addition treatments (Fig. 21). Moreover, all species pooled together showed a tight line with pines at the bottom to herbaceous species at the top of

the relationship. The slope of all species pooled together remained exactly the same as the slope for pine species at Duke only, or the slope of all herbaceous species at BIOCON pooled together (J_{max} = 1.86 V_{cmax}). As suggested in previous studies (Medlyn *et al.*, 1999), there may be a universal slope for all C_3 species, indicating that V_{cmax} and J_{max} are responding to changing environmental conditions in a proportional manner. Hence, plants could adjust to long-term elevated CO_2 by reducing nitrogen invested in carboxylation enzymes and light-harvesting pigment-protein complexes and allocating the nitrogen towards other limiting components (Sage, 1994; Drake *et al.*, 1997; Long *et al.*, 2004).

It has been suggested that N allocated away from photosynthetic components would be invested in structural components, such as the cell wall in plants grown in elevated CO_2 potentially resulting in increased leaf thickness in elevated CO_2 (Onoda *et al.*, 2004; Hikosaka *et al.*, 2005) but without affecting the J_{max}:V_{cmax} ratio. Alternatively, there are other non-photosynthetic pools that could serve as N sinks in elevated CO_2 such as root biomass (Higgins *et al.*, 2002; Handa *et al.*, 2008) and the reproductive organs (Jablonski *et al.*, 2002; Thürig *et al.*, 2003) but, results have been inconsistent. However, nitrogen distribution may not change in the most optimal way in elevated CO_2 due to other constraints such as a significant decrease in mesophyll conductance that may occur in elevated CO_2 (Medlyn, 1996). Changes in mesophyll conductance in elevated CO_2 are only documented in a handful of species (Warren, 2006; Warren & Dreyer, 2006; Flexas *et al.*, 2007) but may account for the discrepancy between the observed and theoretically predicted J_{max}:V_{cmax} ratios at elevated CO_2 (Medlyn, 1996). Given that the relationship between J_{max}:V_{cmax} did not change in long-term elevated CO_2 or N-addition, I conclude that a reduction in N investment in photosynthetic components occurred proportionally but the potential role of mesophyll conductance in elevated CO_2 may warrant further investigation.

5.4. Overall conclusions

This dissertation is framed relative to a large body of previous literature on plant responses to elevated CO_2 over the past 20 years. In the last two decades, over 2700 studies and experiments have been conducted using elevated atmospheric CO_2 exposures of native plants in various growing conditions such as growth chambers, glasshouses and open-top chambers as well as FACE using a wide variety of species (Curtis & Wang, 1998; Saxe *et al.*, 1998; Ainsworth & Long, 2005). Based on published literature reviews, I found a broad range of photosynthetic responses at a common CO_2 level, from -160% to +200% comparing to the ambient conditions. Saxe *et al.* (1998) reported a range between -50 and +200% of CO_2 enhancement at a common CO_2 level across 33 conifer and deciduous trees, whereas Curtis & Wang (1998) showed a minimum of -160 % in *Pinus mariana* up to a +64% CO_2 enhancement in *Pinus nigra*. Medlyn *et al.* (1999) reported on a range of photosynthetic enhancement in elevated CO_2 between 0-120% in tree species.

Most of these studies exposed plants to elevated CO_2 for periods up to one year, though sometimes longer periods of time were used. Different species, growing conditions and measurement methods caused large variation in the range of photosynthetic enhancement. In fact, the variation of responses within one species grown and measured under different conditions in different studies (e.g., *Picea abies*, whose response ranged from -50 to +6%; see Medlyn *et al*., 1999 and (Marek *et al.*, 2002) is also large. While a number of studies have attempted to assign causes for such variation (Medlyn *et al.*, 1999; Poorter & Perez-Soba, 2001), much of the variation among studies is unexplained. FACE studies provide a common experimental framework and a common set of measurements to better facilitate cross-comparisons among elevated CO_2 experiments than were previously possible. Free-Air CO_2 enrichment allows for natural growing conditions of plants, minimizing artifacts that have been documented for previous elevated CO_2 studies (Arp, 1991). There are no changes in light environment or effects of higher temperatures because there are no chamber enclosures, and there are no root restrictions because the plants grow in the naturally developed soil. In my work, I examined decade-long responses of eight species in different functional groups at two

major FACE experiments, conducting measurement in a consistent manner facilitating species comparisons.

This dissertation gave insight into plant responses of different functional groups to elevated CO_2 and N-addition and potential interactions between those two factors. The range of photosynthetic enhancement I found among species ranged from +9% to +57% after long-term exposure to elevated CO_2 across N addition. This range of enhancement was linked to photosynthetic capacity and leaf N to achieve insight into how and why this range of photosynthetic stimulation in elevated CO_2 occurred, focusing on changes in N allocation to photosynthetic components.

Changes in allocation of N to photosynthetic components were apparent in one-year old leaves in pine and in leaves of non-leguminous forbs as evidenced by significantly different slopes of the V_{cmax}-N_a relationship in elevated CO_2 compared to ambient CO_2 (Fig. 18). These changes in N investment to photosynthetic components may be an important adjustment to long-term elevated CO_2 exposure in low fertility sites. Moreover, N was allocated away from photosynthetic components in elevated CO_2 via a reduction in both V_{cmax} and J_{max} but without changing the J_{max}:V_{cmax} ratio (Fig. 21). My study clearly showed that after nearly a decade of exposure to elevated CO_2, both carboxylation and electron transport processes adjusted to elevated CO_2 proportionally reducing N invested in photosynthetic components, regardless of N-addition treatments or species. The maximum carboxylation and electron transport rates were mediated by separate enzyme systems and their coordinated response suggests large-scale coordination of enzyme systems in response to elevated CO_2.

Changes in N allocation to photosynthetic components could be linked to reduced concentrations of foliar N in elevated CO_2. A reduction of leaf N in elevated CO_2 is a commonly observed response (Curtis & Wang, 1998; Nowak *et al.*, 2004; Ainsworth & Long, 2005). When plants had reduced leaf N in elevated CO_2, this usually co-occurred with reduced photosynthetic capacity. Hence, smaller increases of net photosynthesis were observed in plants with reduced leaf N and reduced photosynthetic capacity. Maintenance of foliar N was key for sustaining stimulation of photosynthesis in long-term elevated CO_2. C_3 grasses did this after nearly a decade of growth in elevated CO_2, whereas forbs and pines did not. Hence, photosynthetic stimulation corresponded

negatively with the amount of down-regulation that occurred. Photosynthetic enhancement was smaller when stronger down-regulation occurred in elevated CO_2 so it was largest in grasses (+57%), then pines (+31%) and smallest in forbs (+9%). Therefore, I conclude that much of previous experimental work on CO_2 enrichment has greatly overestimated photosynthetic enhancement in native ecosystems on natural soils.

In addition to changes in N partitioning within the leaf in response to elevated CO_2, I found clear differences in CO_2 responses of forbs and C_3 grasses associated with different patterns of above and belowground allocation, resulting in a higher root:shoot ratio in grasses compared to forbs. Low root:shoot ratios in forbs may constrain additional N uptake from the soil to meet increased growth demands in elevated CO_2 showing strong down-regulation of photosynthetic capacity and an inability to maintain leaf N in forbs in elevated CO_2. Consistent with other studies forb species were more negatively affected by elevated CO_2 and N addition than other C_3 species (Zavaleta *et al.*, 2003; Suding *et al.*, 2005; Stevens *et al.*, 2006). It is possible that the inability to maintain N is preferential to a reduced N fraction allocated to photosynthetic components in order to reduce the possibility of feedback inhibition of photosynthesis in elevated CO_2 or to allow N allocation to other limiting non-photosynthetic components,

These leaf-level results were incorporated into a sun-shade model to evaluate the importance of physiological controls on daily C uptake (GPP_{day}). Different magnitudes of photosynthetic stimulation at the leaf-level were reflected in the modeled GPP_{day} where both LAI responses and adjustments in physiological capacity contributed about equal to the total daily GPP, in addition to the direct effect of elevated CO_2. The increase in LAI can partly compensate for indirect acclimation effects in long-term elevated CO_2, resulting in increased GPP in elevated CO_2 in most species. It is possible that elevated CO_2 may induce increases in canopy LAI due to photosynthetic stimulation in productive, upper canopy leaves, increased photosynthetic efficiency in the shaded portion of the canopy, or reallocation of N within the canopy to allow for more foliage. However, including indirect acclimation effects in response to elevated CO_2 was important when scaling up to the canopy in order to obtain accurate C uptake estimates because both LAI and acclimation affect the C uptake at the canopy scale in elevated CO_2.

These conclusions reveal other gaps in our knowledge and raise further questions needing to be addressed in order to gain a better understanding. Elevated concentrations of atmospheric CO_2 affect many different facets of ecosystem functioning and interactions with other factors such as increased temperature or changed water availability are likely. Though multi-factorial experiments are more difficult to maintain, the importance of interactions is evident from this dissertation and other studies (Shaw *et al.*, 2002; Reich *et al.*, 2006b; Mikkelsen *et al.*, 2008; Zavala *et al.*, 2008). Direct and indirect effects of increased atmospheric [CO_2] such as global warming could have a synergistic or antagonistic effects when they co-occur in ecosystems. Increased temperatures, decreased water availability and increased N availability are other important governing factors with the potential to modulate the elevated CO_2 response in plants. Moreover, ecosystems usually consist of co-occurring plant species of different functional groups. Species interactions with elevated CO_2 and N deposition have been observed in this dissertation and other studies (Reich *et al.,* 2006b) but it remains unclear how these factors or any combination of factors will affect species diversity and species composition. Shifts in competitive balance among species communities are likely to occur over time affecting species responses at a range of different scales. These results clearly point to the need for more multi-factorial experiments where the importance of resource availability, allocation patterns and species composition can be developed and clarified in an integrated approach (Field *et al.*, 1992; Körner, 2000).

The physiological mechanisms occurring in elevated CO_2 at the leaf-level are far from completely understood with regard to photosynthetic components. What controls the coordinated response of photosynthetic enzymes in elevated CO_2? Is reduced leaf N in elevated CO_2 the cause or consequence of changes in allocation patterns? Which feedback mechanisms link the reductions in leaf N with reduced photosynthetic components? Because sugars play a regulatory role in the expression of plant genes (Koch, 1996), a sugar-mediated feedback mechanism, reducing gene expression of photosynthetic components when excess carbohydrates are present, has been hypothesized (Long *et al*., 2004). It is clear that the molecular background of ecological mechanisms in response to elevated CO_2 still need to be clarified.

Potential changes in N allocation patterns may be a key plant response in elevated CO_2, and allocation patterns to non-photosynthetic components are particulary poorly understood. Therefore, there is a need to quantify N invested in non-photosynthetic components, especially because this quantity may represent the intercept of the photosynthesis-nitrogen relationship. As such, it could help to understand N mobilization and retranslocation responses in elevated CO_2 as well as the minimum required N needed to maintain the photosynthetic apparatus. Quantifying how elevated CO_2 affects structural components in the leaf can provide further insights into a potential trade-off between physiology and leaf structure. A redistribution of N in the plant in response to elevated CO_2 may be a key response adjusting plant growth in long-term elevated CO_2, a mechanism which will cascade through to larger scales and our estimates of C uptake from the atmosphere.

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