Interactions between plants and soil nutrient cycling under elevated CO$_2$
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Interactions between plants and soil nutrient cycling under elevated CO$_2$

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Interactions between plants and soil nutrient cycling under elevated CO₂

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Abstract

The atmospheric concentration of the greenhouse gas CO$_2$ is rising and may stimulate plant production and soil C input. If soil C input rates exceed soil C respiration rates under elevated CO$_2$, global warming may be mitigated by long-term soil C sequestration. However, whether soils will serve as CO$_2$ sinks is still debated, since it is uncertain how elevated CO$_2$ will affect the interactions between plant growth and soil nutrient cycling.

In the first part of this dissertation, I explored how long-term elevated CO$_2$ affects soil C inputs versus SOM decomposition, and how these changes ultimately feedback to soil C sequestration. This research was carried out in a Free Air Carbon dioxide Experiment (FACE) in Switzerland that had been exposed to elevated CO$_2$ and N fertilization treatments for 10 years. The isotopic label of the applied CO$_2$ and N allowed for tracing new C and N dynamics in the system. In addition, I summarized available data related to plant growth and soil nutrient cycling from long-term CO$_2$-enrichment experiments using the statistical tool Meta analysis. By incubating litter and soil derived from Swiss FACE, I concluded that the impact of elevated CO$_2$ on litter quality and litter decomposition rates was minor. Therefore, elevated CO$_2$ is not expected to affect soil C contents through its impact on litter quality and decomposition. The Meta analysis showed that the main driver of soil C sequestration is not SOC decomposition, but soil C input through plant growth, which is strongly controlled by nutrient availability. If soil nutrient availability was high, soil C input outweighed C decomposition leading to net C sequestration. However, if soil nutrient availability was low, soil C input rates lagged behind soil C decomposition rates due to CO$_2$-induced nutrient immobilization, which had reduced plant growth. Thus, for soil C sequestration under elevated CO$_2$ ample soil nutrient availability is required. In the Swiss FACE experiment however, soil C sequestration did not increase under elevated CO$_2$, despite high fertilization rates, concurrent increases in plant growth, and relatively low decomposition rates. This may be due to frequent harvests and shows that the potential for soil C sequestration in individual agro-ecosystems is still uncertain, due to management practices that can affect soil C input and/or soil C decomposition.

The potential for soil C sequestration in individual unfertilized/natural ecosystems is also unclear, since unexplained processes appear to prevent N limitation in some of these FACE systems. These processes may occur in the rhizosphere, which is often overlooked, but plays a vital role in mechanistically coupling plant production and soil nutrient cycling. In the second part of this dissertation I focused on how rhizodeposition affects microbial regulation of soil N availability. Elevated CO$_2$ stimulated the amounts of root-derived C and N substrates entering the soil, but without specific exudation of amino acids. Enhanced rhizodeposition was accompanied by a proportional increase in root production, suggesting that rhizodeposition under
elevated CO₂ only increases when root biomass production is stimulated. The increase in rhizodeposition of N under elevated CO₂ comprised a significant portion of the plant assimilated N, and was quickly immobilized by microbes upon entering the soil. This shows another pathway by which elevated CO₂ may enhance nutrient limitation in low N-input systems. Alternatively, elevated CO₂ may alleviate N limitation by stimulating rhizodeposition induced decomposition, leading to the release of N retained in stable SOM pools. This dissertation shows that increased rhizodeposition of C under elevated CO₂ may be responsible for sustained plant growth in low nutrient input FACE systems. Since this mechanism did not increase plant tissue N concentrations, and does not contribute to a net gain of ecosystem N, however, it is not expected to offset nutrient limitation under elevated CO₂ in the future (i.e. decades to centuries).

A third aim of this dissertation was to increase the understanding of plant specific responses to elevated CO₂. Therefore, I compared the responses of plants with genetic similarity but contrasting C allocation patterns, so reducing the number of plant traits that can explain a plants' response to elevated CO₂. In addition, C allocation to roots is a key plant trait for explaining differential responses in C and N cycling as it affects both rhizodeposition and nutrient uptake. I showed that agronomic selection has resulted in a morphological tradeoff, where C allocation to organs associated with C assimilation compared to organs associated with nutrient uptake is favoured in modern cultivars. As a result modern cultivars are more likely to increase shoot biomass production under elevated CO₂ than their wild relatives in fertilized ecosystems. On the other hand, greater root production and N uptake rates indicate a greater potential for sustained plant growth and soil C sequestration under elevated CO₂ for the wild compared to the cultivated genotypes in low N-input systems. These data showed that sink strength is an important trait for controlling plant responses to elevated CO₂.

In conclusion, elevated CO₂ can increase soil C sequestration when sufficient nutrients are available. The extent of the increase however is still unclear in agro-ecosystems, due to a set of management practices that affect soil C decomposition and soil C input. In unfertilized ecosystems, simultaneous increases in N demands of microbes and plants reduce nutrient availability. Increased C allocation to roots under elevated CO₂ will benefit nutrient acquisition and C sequestration in low N systems but this mechanism is expected to be transient. Therefore, in natural ecosystems soil C sequestration is likely to be constrained in the future (i.e. decades to centuries) by progressive nutrient limitation.
Preface

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1. General introduction

1.1 Relevance

The exchange of carbon (C) between global C reservoirs, including the biosphere, geosphere, hydrosphere, and atmosphere constitutes the C cycle. Carbon dioxide (CO₂) is the dominant form of C in the atmosphere. The concentrations of atmospheric CO₂ are naturally regulated by both physical and biological processes, either fixing or releasing CO₂ in the other C reservoirs. These give-and-take fluxes of C were balanced to provide for a more or less constant atmospheric CO₂ concentration for the last 650,000 y (i.e. 180 to 300 ppm as determined from ice cores), until anthropogenic activities began to tip the scales (Alley et al., 2007).

Human activities such as the abundant use of fossil fuels for energy and deforestation add about 7.1 Gt C y⁻¹ of CO₂ to the atmosphere (Schimel et al., 2000). Measurements of atmospheric CO₂ levels (since 1957) suggest that of the total 7.1 Gt C annually released by human activities, approximately 3.2 Gt C remain in the atmosphere, resulting in a rising atmospheric CO₂ concentration (Schimel et al., 2000). As a result, the global atmospheric concentration of CO₂ has increased from a pre-industrial value of about 280 ppm to 379 ppm in 2005, and it is expected to continue increasing to 700 ppm by the year 2050 (Alley et al., 2007).

Carbon dioxide is a greenhouse gas that helps regulate our climate. When solar radiation is absorbed by oceans and land, it warms the earth surface. As a result, the earth emits infrared radiation, which is trapped by greenhouse gases, thereby warming the atmosphere. This phenomenon is called the greenhouse effect and without it, the average temperature on earth would be lower by approximately 30°C. However, the present positive imbalance between emissions and absorption of CO₂ stimulates the greenhouse effect, leading to global warming. Climate models predict that the global surface temperatures are likely to increase by 1.1 to 6.4 °C between 1990 and 2100 (Alley et al., 2007). Such increases in global temperatures are projected to increase sea levels (Wild et al., 2003), change the amount and pattern of precipitation (Narisma et al., 2007), stimulate glacier retreat (Barry, 2006), enhance species extinctions (Root et al., 2003) and increase the ranges of disease vectors (Alley et al., 2007).

It has been proposed that the biosphere can play a role in mitigating global warming, since vegetation assimilates atmospheric CO₂, which is subsequently sequestered in both plant tissues and soil (Gifford, 1994). However, whether sufficient amounts of CO₂ can be sequestered to effectively reduce the atmospheric CO₂ concentration depends on a set of complex interactions between plants and soil nutrient cycling. This dissertation sets out to investigate how elevated CO₂ impacts plants-soil interactions with regard to C and N cycling, and how such interactions affect net soil C sequestration.
1.2 Background

1.2.1 Plant-soil interactions

Plants supply the soil with carbon (C) assimilated from atmospheric CO₂ by photosynthesis. Upon death of plants or parts thereof, this C is used by soil biota, leading to the decomposition of plant litter and release of nutrients necessary for plant growth. These plant-soil relationships consist of complex interactions at the above- and belowground interface, providing feedbacks regulating ecosystem processes. Feedback in the context of soil-plant interactions posits that a change in soil conditions causes changes in the plant component, which in turn causes further change in the soil, and *vice versa*. That is, the results of the process will affect the cause of the process and increases (positive feedback) or decreases (negative feedback) the magnitude of the effect. Plant-soil feedback processes are not only essential to many ecological processes, they are also important in regulating the magnitude of the ecosystem response to environmental changes. Feedbacks in the plant-soil system will not only affect the biosphere’s response to anthropogenic environmental change, but can themselves create large-scale feedbacks that influence the magnitude of anthropogenic perturbations of atmospheric CO₂ concentrations (Ehrenfeld *et al.*, 2005).

1.2.2 Soil C input

The organic C found in the soil is mainly plant-derived. The rate of soil C input depends primarily on the rate of plant growth, driven by photosynthesis. Photosynthesis supplies plants with the C needed for the production of biomass and its metabolism. The fundamental feature of photosynthesis is the conversion of inorganic substrates (CO₂, H₂O and light-energy) into organic products (glucose). The rate of photosynthesis is mainly affected by the concentration of CO₂, the intensity of light, and temperature. The impact of CO₂ on photosynthesis is not equal for all plants, since plants have evolved different photosynthetic pathways, of which the C₃- and C₄-pathways are most common. The universal photosynthetic enzyme is RUBISCO, that acts in CO₂ fixation (carboxylation), but which can also act as an oxygenase. In C₃ plants, O₂ competes with CO₂ for the active sites on the RUBISCO enzyme and subsequent metabolism. This process is called photorespiration and results in the release of CO₂. Photorespiration inevitably reduces the CO₂ gain in C₃ plants. Therefore, photosynthesis is often limited by CO₂ in C₃ plants, and their growth rates benefit from increased atmospheric CO₂ levels. The C₄ photosynthetic pathway is an adaptation that enables higher photosynthetic rates compared to the C₃ pathway under high O₂ concentrations. In C₄ plants CO₂ is fixed by the enzyme phosphoenolpyruvate (PEP) carboxylase, which has a high affinity for CO₂ and enhances the affinity of RUBISCO for CO₂. Hence, C₄ plants reveal no photorespiration and consequently have higher rates of photosynthesis than C₃ plants, especially at high irradiance and temperature. Photosynthetic rates of C₄ plants, however, do usually not benefit much from higher
levels of atmospheric CO$_2$, due to the already efficient use of CO$_2$ by PEP-carboxylase and RUBISCO.

In addition to soil C input derived from plant litter, plant C can enter the soil as 'rhizodeposition', which includes sloughing of living cells and root exudation (i.e. the loss of low weight molecular substances from roots) (Lynch & Whipp, 1990, Darrah, 1996). Root derived soil C input is an important controlling mechanism of plant-soil interactions, since it affects soil processes more directly than shoot derived C input. Namely, rhizodeposits are the preferred “food-source” for soil microbes, making them particularly important for regulating decomposition processes (Darrah, 1996). In addition, rhizodeposits function as cementing agents, by strongly adsorbing to inorganic materials, thereby helping to stabilize aggregates (Tisdall & Oades, 1979). As such, root derived C input may play an important role in soil C sequestration. In fact, recent studies have reported that roots are more important than shoots in the formation of aggregates and stabilization of aggregate-associated SOM (Gale et al., 2000; Puget & Drinkwater, 2001; Wander & Yang, 2000; Rasse et al., 2005). They found that, in the short-term, more root-derived (2.7–22.6%) than shoot-derived (1.1–3.7%) C was stabilized as particulate organic matter. Information on root derived soil C input and its impact on soil C sequestration, however, is characterized by its paucity, mainly due to the technical difficulties associated with measuring rhizodeposition and cycling of rhizodeposits in soils.

The relative amount of root- versus shoot-derived C entering the soil varies with plant species and depends largely on C partitioning, which is defined as the relative allocation of the assimilated C to the various plant organs during growth. The partitioning of dry matter in young plants in a constant environment is allometric: i.e. the relative growth rates of root and shoot stay at a constant ratio during progressive growth (Minchin et al., 1994; Farrar & Gunn, 1998), and this ratio is determined genetically. This ratio can however, change in response to environmental perturbations, thereby changing the dynamic interactions between plants and soil. The degree to which plants change C allocation in response to environmental perturbations is also genetically determined (Minchin et al., 1994; Farrar & Gunn, 1998).

### 1.2.3 Soil C decomposition

The amount of C that can ultimately be sequestered in the soil as soil organic matter (SOM) will depend on the balance between the rate of soil C input and the rate of soil C output through decomposition. Decomposition is the process in which organic macromolecules are converted into their inorganic constituents by micro-organisms, such as nutrients and CO$_2$. Carbon dioxide is a product of microbial respiration and will be lost from the soil and re-released into the atmosphere. Some of the decomposition products however, will be protected from further decomposition and these products play an important role in the formation of stable SOM. Stable SOM formation is the key to C sequestration in ecosystems.
An important controller of the decomposition rate is the intrinsic chemical properties of plant litter (i.e. litter quality). In most ecosystems the decomposition rate is correlated with the concentration of nutrients relative to the amount of C (i.e. C:N ratio) that are initially present in the litter (Aerts, 1997). Decomposition is generally faster when the litter contains high concentrations of the nutrients N, P and K. Carbon entering the soil through rhizodeposition is especially prone to high decomposition rates, since most rhizodeposits serve as an easily accessible substrate for microbial consumption (Janzen & Bruinsma, 1993; Jensen, 1996; Mayer et al., 2003). As a result, rhizodeposition is a particularly important process for the coupling of plant growth to soil nutrient cycling (Paterson et al., 2003).

1.2.4 Soil N dynamics
Nitrogen (N) is the fourth most common element in plant composition and it is crucial for sustaining photosynthesis. Nitrogen is primarily taken up from the soil solution as NO$_3^-$ or NH$_4^+$ and it is often a limiting nutrient for plant nutrition. As much as mineral N controls plant production, plants can affect the availability of mineral N. Namely, plant-derived soil C inputs influence microbial activity, which is are responsible for converting organic forms of N to inorganic forms most available for plant growth. During the decomposition of plant organic materials, the soil microbial community transforms most of the N present in the various substrates into microbial biomass. When substrates are exhausted, microbes turn over (i.e. they die or get preyed upon). Materials released from dead microbes can be used by other microbes or plants, whereas resistant spores may be stabilized in the soils. The process, by which N is incorporated into microbial biomass, and subsequently into more stable SOM, is called N immobilization. In contrast, the release of N from organic forms to inorganic forms is termed N mineralization.

Whether net immobilization or mineralization prevails in a soil depends largely on the C:N ratio of the plant material entering the soil. Generally, if the plant C:N ratio exceeds 25:1, N will be immobilized by microbes from the mineral N pool, or net N mineralization will be reduced. This results in competition for mineral N between plants and microbes, and can lead to a decrease in plant production. On the contrary, when the C:N ratio of SOM is in the order of 10:1, the microbes will release mineral N, resulting in higher soil N availability and possible enhanced plant production.

1.2.5 Plant-soil interactions under elevated CO$_2$
Elevated atmospheric CO$_2$ can stimulate photosynthesis and plant production (Ainsworth and Long, 2004). The increased C assimilation by plants and its subsequent sequestration in the soil may counterbalance the rise in CO$_2$ emissions (Gifford, 1997). Enhanced soil C sequestration under rising levels of CO$_2$ however, can only occur if (a) increases in soil C input are sustained (Taylor and Lloyd, 1992; Friedlingstein et al., 1995; Kicklighter et al., 1999), and (b) if the rate of soil C decomposition lags behind the
increase in soil C input (Raich and Schlesinger, 1992). However, it is unclear how elevated CO$_2$ will affect soil C input and decomposition in the long-term. Namely, the interaction between soil C input and decomposition is not linear, since the processes are inter-reliant: i.e. soil C input affects decomposition and decomposition affects soil nutrient availability, which feeds back to the plant growth response to elevated CO$_2$. So far, research has generated contrasting results with regard to the impact of elevated CO$_2$ on nutrient cycling and plant growth, with both positive and negative feedbacks in the C and N cycles. Figure 1.1 depicts the relations between plants and soil nutrient cycling that may be affected by elevated CO$_2$, and that have been studied for this dissertation.

![Fig. 1.1 Conceptual diagram with arrows representing the flow of nutrients between the plant and soil variables that can explain the impact of elevated CO$_2$ on ecosystems.](image)

Increased soil C inputs stimulated soil N mineralization in some studies but reduced it in others. For example, Zak et al. (1993) showed that increased C inputs under elevated CO$_2$ stimulated the growth of soil microbial biomass, thereby increasing rates of N mineralization. Diaz et al. (1993), on the other hand, found that increased C inputs under elevated CO$_2$ stimulated competition between the soil microbial biomass and plants for soil N, leading to a decline in soil N availability. In addition, Oren et al. (2001) showed that increased N fertilization may offset the decline in N availability under elevated CO$_2$. Thus, it remains unclear how initial increases in soil C input under elevated CO$_2$ feed back to microbial regulation of N availability. In addition, it is uncertain how the increase or decline in N availability ultimately feeds back to soil C sequestration. If sufficient N is available, enhanced plant growth and soil C input under elevated CO$_2$ are likely to be sustained, resulting in net soil C sequestration (Luo et al., 2004). However, it has also been argued that ample soil N availability may simultaneously enhance soil C decomposition (Niklaus et al., 1998). In that case, the increased CO$_2$ respiration rates could counterbalance a possible increase in soil C accumulation. The uncertainty surrounding the coupling of soil C inputs to soil
microbial N transformations, and the role of N availability for the potential of soil C sequestration, makes it difficult to predict whether the soil can act as a C sink to mitigate elevated atmospheric CO2.

Field studies are of vital importance to accurately predict the impact of long-term elevated CO2 on plant production and soil C and N cycling. The most appropriate field studies for such research endeavors are Free Air Carbon dioxide Enrichment (FACE) experiments. The introduction of FACE techniques has allowed for long-term CO2 fumigation studies under realistic growing conditions (Rogers et al., 1983; Hendrey, 1993). Due to methodological difficulties however, the key components of ecosystem responses to elevated CO2 often reside out of sight in such field studies; the belowground system of roots, soil and associated micro-organisms. Since, the living root system is essential for the coupling of plant production to soil nutrient cycling, laboratory studies specifically set-up to mechanistically link the root-system to soil C and N cycling should be combined with field studies.

The source of different ecosystem responses to elevated CO2 is most likely the plant, since elevated CO2 indirectly affects soil C and N cycling by directly impacting plant production. Therefore, much of the uncertainty surrounding soil C sequestration may be removed if we begin to understand the reasons behind varying plant responses to elevated CO2. So far, results have shown somewhat consistently that C4 plants respond to a lesser extent to elevated CO2 than C3 plants, which can be ascribed to the different photosynthetic pathways. However, individual responses to elevated CO2 of plant species within these functional groups (i.e. C3 versus C4 plants) usually differs (Hooper & Vitousek, 1997; Tilman et al., 1997; Nowak et al., 2003). In attempts to predict plant species specific responses to elevated CO2, plants have been classified within broad groups, relying on a broad suite of related plant traits that can generalize how species respond to environmental changes (Eviner & Chapin, 2003). For example, studies have been conducted using fast and slow growing plant species, plant species from different life forms (e.g. woody versus herbaceous species) (Paterson et al., 1995; Crookhanks et al., 1998). However, these classifications have not succeeded at finding a common plant trait that can explain the variable responses of plants to elevated CO2.

1.3 Aim

The aim of this dissertation is threefold. It aims at: (1) increasing the certainty surrounding the predictions of soil C sequestration under elevated CO2, by determining how long-term elevated CO2 affects the interactions between plant growth and decomposition processes, and how these changes ultimately feedback to soil C sequestration. (2) Advancing our current knowledge of N cycling in the rhizosphere under elevated CO2, by examining how CO2-induced changes in rhizodeposition of C and N affect microbial regulation of soil N availability. (3) Increasing the understanding of plant specific responses to elevated CO2, by comparing the responses of plants with
genetic similarity but contrasting C allocation patterns, so reducing the number of variables with regard to plant traits that can explain a plants' response to elevated CO2.

1.4 Outline and methodology

For Chapters 2, 3 and 4, I used soil and plant material collected from a long-term FACE experiment situated in Switzerland to predict the long-term effects of elevated CO2 on SOM dynamics and soil C sequestration. The Swiss FACE experiment has been used to study the impact of elevated CO2 and N fertilization on L. perenne and T. repens ecosystems and was in operation for 10 consecutive years. The CO2 applied to the systems was depleted in 13C, which allowed for accurately measuring relatively small C inputs into the large pool of soil C already present. In addition, applying 15N labeled fertilizer permitted to determine how elevated CO2 has affected fertilizer N dynamics since inception of the experiment. Chapter 2 describes how 9 years of elevated CO2 and N fertilization affected the quality and decomposition of L. perenne and T. repens plant materials. By incubating plant materials produced under ambient CO2 in a soil exposed to elevated CO2 and vice versa for 90 days under laboratory conditions, I was able to assess whether differences in the rate of decomposition were controlled by plant material quality or by changes in soil microbial dynamics and if they led to net soil C sequestration. In addition, the use of 13C-depleted CO2 in the FACE fumigation gas revealed the distribution and dynamics of old and newly sequestered soil C in the different SOM pools. This enabled me to examine the assumptions made in multi-pool SOM models considering different turnover times of SOM under elevated atmospheric CO2. In Chapter 3, I used the same 90 day incubation to determine how the decomposition of 15N enriched L. perenne and T. repens plant material grown at ambient and elevated CO2 and low- and high- N fertilizer additions for 9 years affected N mineralization rates. In Chapter 4, I collected 15N enriched soil from the Swiss FACE experiment 10 years after inception, to determine how elevated CO2 had affected soil C and fertilizer-derived N retention in stable SOM pools. I conducted long-term incubations with repetitive leaching to separate the 15N fertilizer into stable and labile pools via biological fractionation. In addition, I measured soil CO2 efflux during the incubation to be able to quantify the amount of C incorporated in labile versus stable soil C pools.

In Chapter 5, I used meta-analysis to determine the effect of CO2 enrichment on interactions between plant growth and soil nutrient cycling across some plant functional types and ecosystem management practices. Hence, I compiled all the available data from long-term CO2-fumigation field experiments on plant production and a number of soil characteristics related to soil C and N cycling. Meta-analytic methods enable placing confidence limits around effect sizes; therefore they provide a robust statistical test for overall CO2 effects across a number of different studies (Curtis and Wang, 1998). Moreover, they allow testing for significant differences in the CO2 response between categories of studies (Hedges and Olkin, 1985).
In Chapter 6, I present a series of experiments aiming to determine the extent to which agronomic selection of Medicago truncatula is associated with differences in C allocation between roots and shoots, and how such differences affect their morphological and physiological responses to different levels of CO2. In separate tests I (1), tried to distinguish individual M. truncatula accessions with low versus high root: Shoot (R:S) ratios, (2) tested whether M. truncatula accessions selected for agronomic purposes responded more strongly to elevated CO2, and (3) determined differences between the genotypes in the quality of amino acid efflux from the roots under elevated CO2.

For Chapters 7 and 8, I conducted microcosm studies to examine how changes in rhizodeposition of C and N affect microbial regulation of soil N availability. In addition, I tested whether differences in growth responses between C3 and C4 crops translated into differences in rhizosphere responses to elevated CO2. Moreover, I investigated how agronomic selection affected the response of these crops to elevated CO2. In Chapter 7, I aimed at determining how elevated CO2 affects rhizodeposition and the cycling of rhizodeposited N under C3 and C4 plants. In addition, I tested how cultivated genotypes of Triticum turgidum and Zea mays versus their wild relatives responded to elevated CO2, and how inter- and intraspecific variation between C3 and C4 plants controlled N cycling of rhizodeposits under elevated CO2. Therefore, I conducted an N transfer experiment, in which I measured rhizodeposition of 15N and cycling of the rhizodeposits between plants and microbes for 4 weeks in a microcosm experiment. In Chapter 8, I examined how elevated CO2 affects rhizodeposition of C and how that feeds back to microbial N release from recalcitrant SOM under both wild and cultivated genotypes of Triticum durum (wheat). An airtight growth chamber allowed me to use continuous labelling with 13C for measuring rhizodeposition during a 4 week study. In addition, I used soil that had received 15N fertilization for 10 consecutive years to determine N mineralization from SOM and concomitant plant N uptake. To ensure that the 15N was predominantly present in the more stable SOM pools, the soil was leached with a weak nutrient solution containing all nutrients except for N for 6 months prior to the study.

Chapter 9 contains the general discussion and conclusions of this dissertation. A References Section is provided followed by a Summary.
2. Decomposition of soil and plant carbon from pasture systems after nine years of exposure to elevated CO₂: Impact on C cycling and modeling.

Abstract
Elevated atmospheric CO₂ may alter decomposition rates through changes in plant material quality and through its impact on soil microbial activity. This study examines whether plant material produced under elevated CO₂ decomposes differently from plant material produced under ambient CO₂. Moreover, a long-term experiment offered a unique opportunity to evaluate assumptions about C cycling under elevated CO₂ made in coupled climate-SOM models. *Trifolium repens* and *Lolium perenne* plant materials, produced under elevated (60 Pa) and ambient CO₂, at two levels of N fertilizer (140 vs. 560 kg ha⁻¹ y⁻¹) were incubated in soil for 90 days. Soils and plant materials used for the incubation had been exposed to ambient and elevated CO₂ under FACE-conditions and had received the N-fertilizer for 9 years. The rate of decomposition of *L. perenne* and *T. repens* plant materials was unaffected by elevated atmospheric CO₂ and rate of N fertilization. Increases in *L. perenne* plant material C:N ratio under elevated CO₂ did not affect decomposition rates of the plant material. If under prolonged elevated CO₂ changes in soil microbial dynamics had occurred, they were not reflected in the rate of decomposition of the plant material. Only soil respiration under *L. perenne*, with or without incorporation of plant material, from the low N fertilization treatment was enhanced after exposure to elevated CO₂. This increase in soil respiration was not reflected in an increase in the microbial biomass of the *L. perenne* soil. The contribution of old and newly sequestered C to soil respiration, as revealed by the Δ¹³C-CO₂ signature, reflected the turnover times of SOM-C pools as described by multi-pool SOM models. The results do not confirm the assumption of a negative feedback induced in the C cycle following an increase in CO₂, as used in coupled climate-SOM models. Moreover, this study showed no evidence for a positive feedback in the C cycle following additional N fertilization.

2.1 Introduction

During the past two centuries, the atmospheric CO$_2$ concentration has increased by 31%, mainly due to fossil fuel burning and land-use change (Houghton & Ding, 2001). Of all anthropogenic greenhouse gases, CO$_2$ is an important agent of potential future climate warming (Houghton, 1996). In addition to attempts to reduce the emissions of CO$_2$ and concomitant effects on global warming, the potential for C sequestration in soils has gained attention.

The C balance of an ecosystem is a function of the rates of C input and decomposition of soil organic matter (SOM) (Olson, 1963). Soils will act as a net source or sink for atmospheric CO$_2$, depending on the dynamics of SOM in the long term. Carbon sequestration is sustained only if C input continuously increases (Taylor & Lloyd, 1992; Friedlingstein et al., 1995; Kicklighter et al., 1999) and soil C mineralization lags behind the increase in soil C input (Raich & Schlesinger, 1992).

The impact of elevated CO$_2$ on the control mechanisms of plant material decomposition and C cycling has been intensively investigated and summarized by Norby & Cotrufo (1998), Torbert et al. (2000) and Norby et al. (2001). Diaz et al. (1993) proposed a negative feedback mechanism, where increased C input to the soil from increased productivity in elevated atmospheric CO$_2$ caused C and nutrient accumulation in SOM. On the contrary, Zak et al. (1993) found decomposition rate to increase after exposure of litter to elevated CO$_2$, suggesting that a positive feedback might occur, which would increase rates of C and N cycling through the ecosystem. Responsiveness to CO$_2$ is influenced by N input, since systems with high atmospheric N inputs can show sustained responses to CO$_2$ (Hall et al., 2000; Comins & McMurtrie, 1993). Additional N deposition will result in an increased plant growth and C input to the soil (Vitousek & Howarth, 1991; Bergh et al., 1999). Thus, if a negative feedback mechanism is imposed by elevated atmospheric CO$_2$, it might be offset by N fertilization (Oren et al., 2001).

During the last 25 years, SOM models have been used as tools for evaluating the impact of global change on ecosystems (Parton et al., 1992). A variety of SOM models has been developed to simulate the dynamics of SOM over long time periods (Paul & van Veen, 1978; Jenkinson & Rayner, 1977; van Veen & Paul, 1981). The models split organic matter into different pools; an active SOM pool, consisting of live soil microbes and microbial products with a turnover time of 1.5 y, a slow SOM pool with resistant plant material that has a turnover time of 25 y and a passive SOM compartment in which SOM has a turnover time of 1000 y (Parton et al., 1987). The flows of C are controlled by the inherent maximum decomposition rate of the different pools and the water and temperature decomposition factors (Parton et al., 1987).

Attempts have been made to link SOM models to general circulation models (Parton et al., 1992; Melillo et al., 1993). These coupled climate-SOM models are employed to study the long-term effect of climate change and elevated CO$_2$ on SOM.
dynamics and the potential for soil C sequestration (Comins & McMurtrie, 1993; Cao & Woodward, 1998; Cox et al., 2000). The models are process-based and therefore have the potential to accurately describe how important ecosystem processes interact under future climate change scenarios (McGuirre et al., 1992). The models predict global warming to cause a net loss of soil C due to higher decomposition rates (Burke et al., 1991; Jenkinson et al., 1991; Hall et al., 2000; Trumbore et al., 1996). However, these predicted losses in soil C are offset by predicted increases in C input resulting from increased atmospheric CO₂ levels (Hall et al., 2000). Coupled climate-SOM models predict a negative feedback in the C cycle if CO₂ is increased under a given temperature and moisture regime (Aber, 1992; Parton et al., 1993; Hall et al., 2000). Consequently, elevated atmospheric CO₂ might increase soil organic C storage (Kirschbaum, 2000).

This study aims to determine the effects of prolonged elevated atmospheric CO₂ and N fertilization on quality and decomposition of plant material in a pasture soil exposed to long-term elevated CO₂ concentrations. A Free Air Carbon Dioxide Enrichment (FACE) field site in Switzerland had been exposed to elevated CO₂ (600 ppm) and N fertilization for 9 years. Plant materials produced under elevated- and ambient- CO₂ concentrations were incubated in soil exposed to ambient- or elevated- CO₂ concentrations. By incubating plant materials produced under ambient CO₂ in a soil exposed to elevated CO₂ and vice versa, we were able to assess whether differences in the rate of decomposition were controlled by plant material quality or by changes in soil microbial dynamics and if they led to a positive or negative feedback mechanism in the C cycle.

The use of ¹³C-depleted CO₂ in the FACE fumigation gas revealed the distribution and dynamics of old and newly sequestered soil C in the different SOM pools. This enabled us to examine the assumptions made in multi-pool SOM models considering different turnover times of SOM under elevated atmospheric CO₂.

### 2.2 Materials and methods

**Site description**

The Swiss Free Air Carbon Dioxide Enrichment (FACE) facility, at Eschikon, Switzerland studies the impact of elevated CO₂ concentrations in a grassland ecosystem. The site consists of six FACE rings, laid out in a split-split-plot design. Three of the rings are exposed to ambient levels of CO₂; the other three rings are fumigated with ¹³C-depleted CO₂ (60 Pa CO₂ ± 10% over 92% of the fumigation time). The additional CO₂ was ¹³C depleted, leading to a δ¹³C signal of approximately −21‰ for CO₂ in the fumigated rings against −8‰ for ambient CO₂. The fumigated rings are enriched daily (12 hours per day) from March to November each year. Fumigation stops in autumn when the temperature drops below 5°C and is started again in the spring when the temperature rises above 5°C. The experiment has been in operation since May 1993. The plots consist of monocultures of *L. perenne* and *T. repens*. Nitrogen fertilizer
(NH₄NO₃) is applied 4 times a year at rates of 140 or 560 kg N ha⁻¹ y⁻¹. The swards were cut six times in 1993 and eight times in 1994 and 1995. All the cut material was removed from the plots at each harvest. Since 1996 the swards have been cut 5 times a year. The soil is classified as a fertile, eutric Cambisol. For further details of the experimental site, see Zanetti et al. (1996).

The dry matter yields of the L. perenne swards during the first cut in 2002 were 148 and 138 g m⁻² for the low-N fertilization- and ambient- and elevated- CO₂ treatments, respectively. For the high-N fertilization and ambient- and elevated- CO₂ treatments, dry matter yields were 213 and 261 g m⁻², respectively. For the T. repens swards, dry matter yields were 122 and 115 g m⁻² for the low-N fertilization- and ambient- and elevated- CO₂ treatments, respectively. For the high-N fertilization and ambient- and elevated- CO₂ treatments, dry matter yields were 128 and 117 g m⁻², respectively (Aeschlimann, personal communication).

**Soil and vegetation sampling**

In March 2002, soil samples were taken to a depth of 10 cm from each of the rings. A soil-core with a diameter of 1 cm allowed us to take 20 homogeneously distributed sub-samples per subplot. The individual soil cores taken from a subplot were mixed together and sieved to 10 mm to facilitate future soil preparation. Subsequently, the sieved soil was dried at 40°C for 24 hours prior to shipment from Switzerland to California.

One might argue that drying the soil prior to incubation has implications for mineralization rates in the later stages of the experiment (Magid et al., 1999). However, the topsoil of these grasslands is periodically exposed to a 40°C temperature and numerous drying and rewetting events occur annually (www.fb.ipw.agrl.ethz.ch). Additionally, only multiple drying events prior to decomposition studies have been shown to alter mineralization rates in the long term (Fierer & Schimel, 2002) and a consistent effect of drying and rewetting cycles on microbial community structure has yet to be reported (Fierer et al., 2003). Moreover, all treatments were exposed to the same drying event and therefore the results are directly comparable. Based on these arguments, we contend comparison of C mineralization rates between the soils is still valid.

Plant material of L. perenne and T. repens, produced under ambient and elevated CO₂ concentrations and high and low N fertilization, was collected during the first cut in May 2002. The total aboveground biomass of the field plots was harvested (shoot biomass above a cutting length of 4.5 cm) and a sub-sample was taken for chemical analysis and for the incubation study. The plant material was dried at 65°C for 48 hours.

As we only sampled the plant material once, its C:N ratio, and other characteristics, may have been unique. Also, the initial drying might have resulted in some changes in the chemical composition of the plant material. However, it was conducted consistently across all treatments and still allows us to compare the decomposition rates of the plant materials.
**Plant material and soil C and N**

Sub-samples of the plant material and soils were ground in a ball mill and total C and N and their isotopic composition were determined at the UC Davis Stable Isotope Facility (see below) and their C:N ratios were calculated.

**Incubation of plant material in soil exposed to ambient and elevated CO$_2$**

Dry soil was ground using a mortar and a pestle, sieved (<2 mm), and roots (>2 mm) were removed. This pre-treatment of the soil resulted in a homogeneous soil for all incubations, reducing variability in decomposition rates resulting from differences in soil structure. The plant material was ground (<1 mm) and 0.4 g was mixed with 40 g of soil in 120 ml specimen cups. All combinations of plant material produced under ambient and elevated CO$_2$ and soil exposed to ambient and elevated CO$_2$ were used in the incubation study (Table 2.1).

**Table 2.1** Additions of plant materials to the soils for the 90 day incubation experiment, resulting in 16 different treatments of plant material incubated in soil.

<table>
<thead>
<tr>
<th>Soil Type</th>
<th>Species</th>
<th>CO$_2$ treatment</th>
<th>N treatment</th>
<th>Type of plant material incorporated in the soil</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T. repens</td>
<td>Ambient</td>
<td>Low</td>
<td>T. repens</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Low</td>
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<td>Low</td>
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<tr>
<td></td>
<td></td>
<td>Low</td>
<td>High</td>
<td></td>
</tr>
<tr>
<td>L. perenne</td>
<td>Ambient</td>
<td>Low</td>
<td>Low</td>
<td></td>
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<tr>
<td></td>
<td>Low</td>
<td>Low</td>
<td>Elevated</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Elevated</td>
<td>High</td>
<td></td>
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</tbody>
</table>

In addition to the field replicates (3), all incubations were carried out in duplicate (2 laboratory replicates). Moreover, duplicates of unamended soils (control soils) were incubated along with the litter-amended soils, to account for CO$_2$ evolution from the pre-existing soil C. Water-holding capacity of the soils was determined by calculating the difference in weight of soils at saturation point and oven-dry weight (100°C). Water was added to obtain 60% of saturation. Specimen cups were placed in 1L Mason jars and 5 mL of water were added to the bottom of the jar to slow soil drying. A septum in the
lid allowed air samples to be removed with a 12 mL syringe. Control jars (5) with no soil present were included to determine the background levels of CO$_2$ and its $^{13}$C isotopic composition. CO$_2$-samples were collected in 12 mL vacutainers (Labco Unlimited, Buckinghamshire, UK).

Soil CO$_2$ evolution was measured at 0 (immediately following the first wetting event), 1, 2, 3, 5, 8, 15, 30, 60 and 90 days at 20°C. Following gas sampling, the caps were removed and the mason jars were flushed outside the building for 30 min. To promote flushing and air exchange, jars were placed under a fan.

**CO$_2$ and $^{13}$C-isotope analyses**

The concentration of CO$_2$ and its PDB-$^{13}$C signature were determined at the University of California–Davis Stable Isotope Facility using a continuous flow, isotope mass spectrometer (PDZ Europa TGII trace gas analyzer and Geo 20-20 isotope ratio mass spectrometer, Cheshire UK). Carbon mineralization data are expressed on the basis of oven-dry (40°C) weight of soil.

Results of the C isotope analyses are expressed in $\delta$ units (‰). The $\delta^{13}$C values were determined in relation to Vienna-Pee Dee Belemnite.

$$\delta^{13}C = 1000 \left( \frac{R_{sample}}{R_{standard}} - 1 \right)$$

(2.1)

where $R = ^{13}C / ^{12}C$.

To calculate the amount of CO$_2$-C derived from plant material produced under elevated CO$_2$ concentrations, incubated in soil formerly exposed to ambient CO$_2$, the following mass balance was used (Denef et al., 2002):

$$Q_t \delta_t = Q_p \delta_p + Q_s \delta_s + Q_b \delta_b$$

(2.2)

where $Q_t =$ the total amount of CO$_2$-C; $\delta_t =$ its isotopic composition, $Q_p =$ the amount of CO$_2$-C derived C from the plant material; $\delta_p =$ the isotopic composition of plant material; $Q_s$ is the amount of CO$_2$-C derived from the unlabeled soil; $\delta_s =$ the isotopic composition of the unlabeled soil; $Q_b =$ the CO$_2$-C amount in the control blank jar; $\delta_b =$ the isotopic composition of CO$_2$ in the control blank jar. The CO$_2$-C derived from the plant materials ($Q_t$) during the incubation was quantified by subtracting soil respiration ($Q_s + Q_b$) from the respiration of soil with incorporated plant materials ($Q_t$).

The $^{13}$C depletion of plants under elevated CO$_2$ was sufficient to allow us to examine the stability of new C sequestered under prolonged exposure to elevated CO$_2$. In addition, the $^{13}$C isotope signal introduced in the elevated CO$_2$ treatment allowed us to partition C mineralization in ambient and elevated soils during the incubation experiment and to follow the dynamics of old versus newly sequestered C. Our study offered a unique opportunity to evaluate the assumptions made by multi-pool SOM-models considering the different turnover times of SOM.
The C budget for the FACE soil during incubation was calculated with the isotopic mixing model. The fraction of C turnover in the old and new SOM pools in the FACE soils was calculated as follows (Balesdent et al., 1987):

$$ f_{\text{CSOM}} = \frac{\delta_2 - \delta_0}{\delta_1 - \delta_0}, \quad (2.3) $$

where $\delta_2$ and $\delta_0$ are $\delta^{13}$C values for SOM pools in the elevated and ambient CO$_2$ treatments, respectively and $\delta_1$ is the average $\delta^{13}$C value of L. perenne and T. repens roots and litter (Van Kessel et al., 2000a).

**Microbial biomass**

Soil microbial biomass C was estimated using the fumigation-extraction method (Vance et al., 1987). A third of the incubated soils was sampled destructively at day 22; two replicate soil samples were taken from each specimen cup, subsequently each of them was mixed with 80 ml of 0.5 M K$_2$SO$_4$ and shaken for 45 min prior to filtration (Whatman #1). One of the 2 subsamples (20 g dry weight) was extracted instantly after harvesting. The other subsample (16 g dry weight) was exposed to chloroform fumigation for 48 h prior to the same extraction procedure.

Microbial C was defined as organic C extracted from a fumigated soil minus organic C extracted from a non-fumigated soil (Ec), using an extraction efficiency coefficient, k, of 0.45 (Wu et al., 1990; Joergensen, 1996). Organic C was measured in the soil extracts as dissolved organic carbon (DOC), using the UV Persulfate TOC Analyzer Phoenix 8000MT (Teledyne Tekmar, Mason, Ohio).

**Statistical analysis**

Addition of T. repens and L. perenne plant material produced under ambient- or elevated- CO$_2$ concentrations to soil exposed to ambient or elevated CO$_2$, resulted in four different treatments (Table 1). A four-way comparison was made within the low- and high- N fertilization treatment for the two species.

The results were analyzed using the Mixed Model in the SAS system for Windows™ V8. An ANOVA was conducted with blocks (i.e. the field plots) as random effects and treatments (i.e. CO$_2$, N and species treatments) as fixed effects. Statistical tests were performed on cumulative respired CO$_2$ at the end of the incubation. Differences between means were tested using Least Significant Differences (LSD). The levels of significance were $P<0.05$ and $P<0.01$. 
2.3 Results

Total respiration

Total CO₂ efflux was the same in all *T. repens* plant – soil treatment combinations; no significant effects of N fertilization rate or CO₂ treatment were found (Figs. 2.1 a, b). When *L. perenne* plant material produced at the high rate of N fertilization was added to soil total CO₂ efflux was also not affected by the previous treatment of elevated CO₂ in either plant or soil (Fig. 2.1 d). However, when *L. perenne* plant material produced under the low rate of N fertilization was added to the soil, total CO₂ efflux was significantly higher (P < 0.0001) in the soil that had been exposed to prolonged elevated CO₂ than to ambient CO₂ (Fig. 2.1c). In this low N input *L. perenne* system, the increase in cumulative CO₂ efflux of the FACE soil amounted to a 20% increase above the ambient soil with either ambient or elevated-CO₂ plant materials incorporated.

![Graphs showing total cumulative CO₂ efflux](image)

**Fig. 2.1 a, b, c, d** Total cumulative CO₂ efflux from soils plus incorporated *L. perenne* and *T. repens* plant materials during a 90-day incubation; plant materials and soils had been exposed to ambient or elevated atmospheric CO₂ and low- and high- N fertilization treatments for 9 years. Values are means with SEM indicated by the error bars.
**Contribution of soil to total respiration**

Although a trend for higher values in elevated soil was apparent, CO$_2$ efflux from *T. repens* control soil (without plant materials added) was not significantly affected by the history of CO$_2$ and N fertilization (Figs. 2.2a, b). Similarly, respiration from *L. perenne* soil was not significantly affected by prior atmospheric treatment under a high rate of N fertilization (Fig. 2.2d). However, *L. perenne*-soil under low N fertilization management and exposed to elevated CO$_2$ respired more than soil exposed to ambient CO$_2$ ($P < 0.043$); increasing with 20% (Fig. 2.2c).

**Fig. 2.2 a, b, c, d.** Total cumulative C efflux during a 90-day incubation from unamended soils collected from *T. repens* and *L. perenne* swards. The soils had been exposed to elevated- and ambient- atmospheric CO$_2$ and low- and high- N fertilization treatments for 9 years. Values are means with SEM indicated by the error bars.

**Plant material quality and decomposition**

Both elevated CO$_2$ and the low-N treatment significantly increased the C:N ratios of the *L. perenne* plant material (Table 2.2). In contrast, N fertilization and elevated CO$_2$ had no significant effect on the C:N ratio of *T. repens* (Table 2.2). The C:N ratios of *L. perenne* plant materials were generally significantly higher than the C:N ratios of *T. repens* plant materials. However, the net rate of decomposition of *L. perenne* and *T. repens* plant materials was not significantly affected by either the CO$_2$ or N fertilizer treatments (Figs. 2.3 a-d).
Table 2.2 Total C and N, $^{13}$C- signature and the C:N ratio of *L. perenne* and *T. repens* plant materials harvested in March 2002 from the Swiss FACE experiment, as affected by atmospheric CO$_2$ and N fertilization treatments. Values are means ± SEM (n=3), *, ** significant at the 0.05 and 0.01 level, respectively.

<table>
<thead>
<tr>
<th>Species</th>
<th>CO$_2$</th>
<th>N</th>
<th>Total C (g C kg$^{-1}$)</th>
<th>$\delta^{13}$C (‰)</th>
<th>Total N (g N kg$^{-1}$)</th>
<th>C:N</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>T. repens</em></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ambient</td>
<td>Low</td>
<td>439± 9.5</td>
<td>-27.7± 0.1</td>
<td>49.9± 2.7</td>
<td>8.8± 0.3</td>
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<tr>
<td></td>
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<td>High</td>
<td>431± 5.3</td>
<td>-28.6± 0.1</td>
<td>52.9± 2.6</td>
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<td></td>
<td>Elevated</td>
<td>Low</td>
<td>440± 3.3</td>
<td>-35.6± 0.1</td>
<td>46.4± 3.6</td>
<td>8.5± 0.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>High</td>
<td>446± 4.9</td>
<td>-35.9± 0.1</td>
<td>48.9± 1.5</td>
<td>9.1± 0.2</td>
</tr>
<tr>
<td><em>L. perenne</em></td>
<td></td>
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<tr>
<td></td>
<td>Ambient</td>
<td>Low</td>
<td>429± 0.8</td>
<td>-29.5± 1.0</td>
<td>19.1± 1.0</td>
<td>22.6± 1.2</td>
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<td>High</td>
<td>435± 4.6</td>
<td>-29.3± 0.3</td>
<td>28.7± 3.1</td>
<td>13.5± 2.4</td>
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<tr>
<td></td>
<td>Elevated</td>
<td>Low</td>
<td>426± 1.1</td>
<td>-36.8± 0.3</td>
<td>16.0± 0.9</td>
<td>26.8± 1.6</td>
</tr>
<tr>
<td></td>
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<td>High</td>
<td>427± 3.7</td>
<td>-36.5± 0.2</td>
<td>27.3± 1.8</td>
<td>15.8± 0.9</td>
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Source of variation ANOVA

<table>
<thead>
<tr>
<th>T. repens</th>
<th>CO$_2$</th>
<th>N</th>
<th>CO$_2$*N</th>
<th>Species</th>
</tr>
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<tbody>
<tr>
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<td>ns</td>
<td>ns</td>
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<table>
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<th>CO$_2$</th>
<th>N</th>
<th>CO$_2$*N</th>
<th>Species</th>
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<tbody>
<tr>
<td></td>
<td>ns</td>
<td>ns</td>
<td>*</td>
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</table>


Nine years of elevated CO\(_2\) significantly depleted the \(^{13}\)C of \(L\). \(perenne\) and \(T\). \(repens\) plant materials, for both N treatments (Table 2.2). On average, plant materials produced under elevated CO\(_2\) concentrations were 7.4 ‰ more depleted than plant materials produced under ambient atmospheric CO\(_2\). The isotopic difference between the isotopic signature of the plant material and the control soil was on average 10-12‰ (Table 2.2 and 2.3).

**Microbial biomass**

The additions of plant materials produced under elevated or ambient CO\(_2\) concentrations did not lead to a significant increase in soil microbial biomass C in any of the soils after 22 days of incubation. Moreover, soil microbial biomass was not affected by the rate of N fertilization. Microbial biomass then ranged from 267 (± 50 SE) to 453 µg C g\(^{-1}\) soil (± 99 SE) in ambient- and elevated-CO\(_2\) \(T\). \(repens\) soils, respectively, and from 328 (± 26 SE) to 477 µg C g\(^{-1}\) soil (± 105 SE) respectively in ambient- and elevated-CO\(_2\) \(L\). \(perenne\) soils.
Soil C mass balance

After 9 years, no significant effects of CO\textsubscript{2} and N fertilizer treatments or plant species on total soil C were found. However, total C in the soils exposed to elevated was consistently higher than total C in soils exposed to ambient CO\textsubscript{2} (Table 2.3). Nine years of elevated CO\textsubscript{2} did significantly deplete SOM in $^{13}$C under both plant species and for both rates of N fertilizer (Table 2.3). On average, soils exposed to elevated CO\textsubscript{2} were 3.1‰ depleted in $^{13}$C compared to soils exposed to ambient CO\textsubscript{2}.

Using the change in the $^{13}$C isotopic signature, the fraction of new C in the soil (< 9 yr old) was calculated to be 0.23 (Table 2.4). The relative respiration rate of new C respired from the soils exposed to elevated CO\textsubscript{2} was significantly higher than the relative respiration rate of old C in both L. perenne and T. repens soils under low and high N fertilization rates (Fig. 2.4). Respiration of new C was significantly higher for L. perenne soil compared to T. repens soil for both rates of N fertilization (Table 2.4). The differences in respiration of newly sequestered C between T. repens and L. perenne soil after 90 days of incubation amounted to 31% for the low N- and 34% for the high N-fertilization treatment.

![Graphs showing the decomposition of new and old C over time for T. repens and L. perenne soils exposed to elevated CO\textsubscript{2}.](image)

Fig. 2.4 a, b, c, d Relative contribution of old (>9 y) and newly (<9 y) sequestered C to respiration rates of T. repens and L. perenne soils exposed to elevated CO\textsubscript{2} for 9 years (the line was fit using an exponential decay function) Values are means with SEM indicated by the error bars.
Table 2.3 Total C and N, $^{13}$C- signature and the C:N ratio of *L. perenne* and *T. repens* soils (0-10 cm depth, bulk density: 1.2 g cm$^{-3}$) harvested in March 2002 from the Swiss FACE experiment, as affected by atmospheric CO$_2$ and N fertilization treatments. Values are means ± SEM (n=3), *, ** significant at the 0.05 and 0.01 level, respectively.

<table>
<thead>
<tr>
<th>Species</th>
<th>CO$_2$</th>
<th>N</th>
<th>Total C (Mg C ha$^{-1}$)</th>
<th>$^{13}$C (‰)</th>
<th>Total N (Mg N ha$^{-1}$)</th>
<th>C:N</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>T. repens</em></td>
<td>Ambient</td>
<td>Low</td>
<td>32.6± 4.8</td>
<td>-25.9± 0.4</td>
<td>3.5± 0.5</td>
<td>9.2± 0.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>High</td>
<td>33.3± 6.1</td>
<td>-26.2± 0.4</td>
<td>3.5± 0.6</td>
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Source of variation (ANOVA)

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| Species      | CO$_2$N*species | ns |    | ns | ns |

Table 2.4 Fractions of old and new C in *L. perenne* and *T. repens* soils exposed to elevated CO$_2$ and the proportions of old and new C respired, as affected by N fertilization rate. Soils were harvested in March 2002 from the Swiss FACE experiment. Values are means ± SEM (n=3), *, ** significant at the 0.05 and 0.01 level, respectively.

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<th>Fractions of old and new C</th>
<th>Proportion C respired</th>
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<td>Mg C ha$^{-1}$</td>
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Source of variation (ANOVA)

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Chapter 2

2.4 Discussion

**Total respiration: changes in plant material quality or soil**

The experimental design used to study the impact of increasing CO₂ levels on decomposition has a major impact on the generated results. Norby *et al.* (2001) showed in a synthesis that the effects of CO₂ on litter chemistry or decomposition were usually smallest under experimental conditions similar to natural field conditions. Also, short-term changes in litter chemistry and decomposition might differ from longer-term responses (Finzi and Schlesinger, 2002). It has therefore been argued that long-term measurements of C cycling under elevated CO₂ are necessary (Canadell *et al.*, 1996; Norby *et al.*, 2001). This incubation study was performed with soil and plant materials derived from a long-term FACE experiment, offering the opportunity to study the impact of prolonged exposure of elevated CO₂ on decomposition and subsequent C cycling in a pasture soil.

Elevated CO₂ did not increase the C:N ratios of either *T. repens* or *L. perenne* plant material produced under low and high rates of N fertilizer. With the exception of the *L. perenne* system that had received a low rate of N fertilization, total respiration of soils plus plant material was unaffected by whether the soil had been exposed to elevated or ambient CO₂, or by whether the incorporated plant material was produced at ambient or elevated CO₂ concentration. However, in the low-N treatment of the *L. perenne* soil exposed to elevated CO₂, a significant increase in C mineralization was found with either ambient- or elevated-CO₂ plant materials. This suggests that the difference in total respiration reflected a change in soil rather than in plant material quality. Calculations of the CO₂-C derived from plant materials confirmed this deduction; no changes in plant material decomposition were detected for any of the plant materials in any of the soils. The ¹³C signature of plant material produced under elevated atmospheric CO₂ also allowed us to accurately calculate plant material decomposition using a ¹³C mass balance approach (Equation 2). The C:N ratio of *L. perenne* plant material under low N fertilization increased under elevated CO₂, though the increase was relatively small and it did not alter the decomposition rate of the plant material. These results indicate that 9 years of exposure to elevated CO₂ did not alter the quality of plant material and its rate of decomposition.

Numerous recent studies have evaluated the impact of elevated CO₂ on plant material quality, decomposition and C cycling (Gorissen *et al.*, 1995; Cotrufo *et al.*, 1998; King *et al.*, 2001). No consistent pattern has emerged, which makes the potential for net C sequestration in soils under elevated CO₂ concentrations difficult to predict (Norby and Cotrufo, 1998). Studies have shown that decomposition under elevated CO₂ is not necessarily altered by changes in litter quality (Weatherly *et al.*, 2003; Norby and Cotrufo, 1998; Gorissen & Cotrufo, 2000). Our results reaffirm the conclusion that responses of litter quality to elevated CO₂ might not be a good indicator of plant material decomposition (Franck *et al.*, 1997; Sowerby *et al.*, 2000; Ross *et al.*, 2002).
CO₂-driven changes in soil respiration.

Soil from the *L. perenne* sward following 9 years of exposure to elevated CO₂ and low N fertilization showed significantly higher respiration than the soil under *L. perenne* receiving ambient CO₂. Soil respiration of the *L. perenne* sward following high N fertilization and of the *T. repens* swards, following both high and low N fertilization, were not enhanced by elevated CO₂. Several studies (Oren *et al.*, 2001; Niklaus *et al.*, 1998) conclude that the absence of microbial responses to enhanced soil C cycling originates from mineral nutrient limitations on microbial processes. Our results do not support the hypothesis that a positive microbial response to elevated CO₂ is to be expected only when N is not limiting. We observed a response of increased C mineralization only in the low-N, high-CO₂ treated soil. However, in spite of the low and high N fertilization rates, total N was similar in all soils. Moreover, no significant effects on soil C:N ratios were detected. As soils were fertilized with P and K to avoid plant nutrient deficiencies (Daepp *et al.*, 2000), it is unlikely that the differences in decomposition rates resulted from differences in nutrient availability.

Although our results point out that there was increased microbial activity in response to elevated CO₂ in the low-N soil under *L. perenne* we did not find any differences in microbial biomass C between the ambient- and elevated-CO₂ plots. Measuring microbial biomass alone might not have been sufficient for explaining changes in respiration rates. In agreement with our results, Schortemeyer *et al.* (1996) earlier found no significant effects of CO₂ concentrations on microbial biomass C in soil under *L. perenne* or *T. repens* in this same FACE experiment. They did find a positive effect of elevated CO₂ on microbial numbers in the rhizosphere of *L. perenne* in samples taken in the spring after 1.5 years of CO₂ enrichment. In another experiment, Montealegre *et al.* (2002) showed changes in soil microbial activity, numbers and community composition under elevated CO₂. Another explanation for the increased respiration rates might be related to the pretreatment of the soil. Six *et al.* (2001) observed for the *L. perenne* soil an increase in macroaggregation and total intra-aggregate particulate organic matter-C (iPOM-C) and total particulate organic matter-C (POM-C) under elevated CO₂. During pretreatment of the soil macroaggregates might have been broken up, which can lead to a release of SOM-C, especially recently stabilized SOM-C such as iPOM-C; this could have contributed to the increased respiration of the *L. perenne* soil under elevated CO₂.

New soil C input and dynamics after 9 years

Relative decomposition of newly sequestered C in the *L. perenne* system was about twice as high as decomposition of newly sequestered C in the *T. repens* system. SOM models assume that the C:N ratio is a key factor, which controls the split of plant material into structural (resistant to decomposition) and metabolic (readily decomposable) material (Parton *et al.*, 1987). The C fraction of plant material is assumed to flow directly to the slow soil C pool as structural material decomposes (Parton *et al.*, 1987).
The models thus predict decomposition to decrease as the C:N ratio of plant material increases. The faster turnover of newly sequestered SOM of *L. perenne* than of *T. repens* contradicts this prediction. This may have resulted from pretreatment of the soils. However, in some elevated CO₂ experiments decomposition was more rapid in systems with plant material having a higher C:N ratio (Franck et al., 1997; Ross et al., 2002), possibly because of an increase in non-structural carbohydrates (Lewis et al., 1994; Rogers et al., 1994). Non-structural carbohydrates are an easily available carbon and energy source for the microorganisms in the rhizosphere and thus they are prone to fast turnover (Hütsch et al., 2000). This could have contributed to the turnover rate of *L. perenne* material being faster than that of *T. repens* (Gifford et al., 2000). This is further supported by the observation that there were no significant differences in total soil C detected between any of the soils, although C inputs to the soil were higher under *L. perenne* than under *T. repens* (Van Kessel et al., 2000a). These results after 9 years of elevated atmospheric CO₂ corroborate earlier findings of no significant changes in total C after 4, 6 and 8 years of exposure to elevated CO₂ (Van Groenigen et al., 2002; Van Kessel et al., 2000b).

Several studies have estimated SOM turnover and pool structure using stable ¹³C-depleted isotopes (Balesdent et al., 1987; Martin et al., 1990). We used the technique to evaluate if the contributions of the newly sequestered C-to-C turnover concurred with the assumptions made in SOM models. New C (<9 y) was distributed between the active and slow SOM pools, whereas old C (>9 y) was mainly distributed over the slow and passive soil C pools (Six et al., 2001; Van Groenigen et al., 2002, 2003). The relative fraction of new soil C was significantly smaller than the fraction of old C and new C was mineralized at a significantly higher rate. Thus, the relative contribution of old and newly sequestered C to soil respiration reflected the different turnover times of the various SOM pools as described by multi-pool SOM models. A limitation of our study is that we cannot directly compare the ambient- and the elevated-CO₂ treatments. However, we have no evidence that CO₂ affects SOM dynamics in the different soil C pools.

**Implications for modeling**

Coupled climate-SOM models assume that an increased CO₂ concentration in the atmosphere increases the rates of photosynthesis (Melillo et al., 1993) and increases the litter C:N ratios. When tissues produced at elevated CO₂ are shed, they are assumed to decay more slowly than those from an ambient CO₂ atmosphere (Aber, 1992; Hall et al., 2000). Results generated from this study do not comply with the assumed negative feedback following CO₂ fertilization in the climate-SOM models since soil C respiration was not reduced in the elevated-CO₂ treatments. This is supported by other longer-term decomposition studies. Finzi & Schlesinger (2002) found no evidence that plant and microbial processes following 4 years of elevated CO₂ resulted in systematic changes in mass loss during decomposition. Dukes & Hungate (2002) also concluded that elevated CO₂ does not consistently decrease litter quality and decomposition in a Californian
SOM decomposition under elevated CO2

grassland ecosystem. Our findings show that an increase in atmospheric CO2 concentration alone may not lead to a net gain of soil C.

In addition, the suggested positive feedback associated with N fertilization in a CO2-enriched atmosphere (Cao & Woodward, 1998; Mellilo et al., 1993; Comins & McMurtie, 1993) is not supported by our data. However, this is probably related to the relatively high fertilization rates in both fertilization treatments of our study. Nevertheless, our results emphasize the importance of a basic understanding of belowground processes controlling the direction of C cycling feedbacks and ultimately the potential for net soil C sequestration.

2.5 Conclusions

The rate of decomposition of *L. perenne* and *T. repens* plant materials was unaffected by atmospheric CO2 concentrations, or the rate of N fertilization. It is remarkable that all the plant materials decomposed at very similar rates despite C:N ratios ranging from 8 to 27. The contribution of old and newly sequestered C to soil respiration reflected the turnover times of C in the SOM pools, as described by multi-pool SOM models. Our data suggest that N fertilization in combination with elevated CO2 does not necessarily lead to a positive feedback (i.e., the release of more C to the atmosphere). If limited to the impact of atmospheric CO2 on soil C cycling, the results do not support the assumed negative feedback in climate-SOM models on C cycling that is predicted to occur under elevated CO2. The small, non-significant differences between the amounts of total soil C measured in the different treatments at the FACE sites provide further evidence of a lack of a negative or positive feedback under prolonged elevated CO2.
3. Prolonged elevated atmospheric CO$_2$ does not affect decomposition of plant material.

Abstract
Prolonged elevated atmospheric CO$_2$ might alter decomposition. In a 90-day incubation study, we determined the long-term (9 years) impact of elevated CO$_2$ on N mineralization of *Lolium perenne* and *Trifolium repens* plant material grown at ambient and elevated CO$_2$ and low- and high- $^{15}$N fertilizer additions. No significant differences were observed in $^{15}$N-NO$_3^-$ recovery rates between any of the treatments, except an N addition effect was observed for *L. perenne* (0.4 versus 0.5 $^{15}$N-NO$_3^-$ day$^{-1}$ in high versus low N). The results suggest that elevated CO$_2$ did not change plant N mineralization in any of the soils, because of a surplus of available N in the fertilized and leguminous systems, and because of insignificant plant responses to elevated CO$_2$ in the low soil N availability systems.

Chapter 3

3.1 Introduction

Land-use changes and large-scale combustion of fossil fuel have contributed to increased atmospheric CO$_2$ concentrations (Houghton & Ding, 2001). Elevated atmospheric CO$_2$ might change nutrient cycling in ecosystems by changing litter input and chemistry thereby affecting decomposition rates (Schlesinger, 1997). The impact of elevated CO$_2$ on the control mechanisms of plant material decomposition has been investigated (Torbert et al., 2000; Norby et al., 2001), but results have been inconsistent and contradictory. Zak et al. (1993) found evidence for a negative feedback in the N cycle under elevated CO$_2$, while Diaz et al. (1993) proposed a positive feedback. However, this positive feedback may only be sustained when additional N is applied (Oren et al., 2001). Long term experiments are essential for determining changes in mineralization processes under elevated CO$_2$ (Canadell et al., 1996; Finzi & Schlesinger, 2002). By using plants and soil exposed to ambient and elevated CO$_2$ and low and high $^{15}$N fertilization treatments for 9 years in the Swiss Free Air CO$_2$ Experiment (FACE), we aimed at determining the impact of prolonged elevated atmospheric CO$_2$ and N fertilization on plant material decomposition.

3.2 Materials and methods

The Swiss FACE experiment was established at Eschikon in 1993, Switzerland consists of six FACE rings, laid out in a split-split-plot design. Three of the rings are under ambient CO$_2$; the other three rings are fumigated with CO$_2$ (60 Pa CO$_2$ ± 10%). The subplots consist of monocultures of L. perenne and T. repens, that were cut 5 times a year. The harvested biomass was removed from the plots. Nitrogen fertilizer ($^{15}$NH$_4$$^{15}$NO$_3$) was applied 4 times a year at rates of 140 or 560 kg N ha$^{-1}$ y$^{-1}$. The high and low N treatments contained 0.4 and 1.6 atom%$^{15}$Nexcess in 1994 and 1995 and 0.3 and 1.3 atom%$^{15}$Nexcess from 1996-2000, respectively. For further details of the experimental site, see Zanetti et al. (1996).

In March of 2002, 20 soil samples per plot were taken to a depth of 10 cm. Shoot biomass of L. perenne and T. repens, were collected during the first cut in May of 2002. The sampling and incubation protocol have been previously described (de Graaff et al., 2004). The soil was ground, sieved (<2 mm), and roots (>2 mm) were removed. The plant material was ground (<1 mm) and 0.4 g of the $^{15}$N enriched plant material was mixed with 40 g of unlabeled soil for incubation. Plant materials produced under ambient and elevated CO$_2$ were mixed with soil exposed to ambient and elevated CO$_2$ and vice versa (Table 1). Water was added to obtain 60% of water holding capacity.

Soil N mineralization was measured at 0, 22, 50 and 90 days at 20°C, by extracting 20 g of each soil with 60 mL 0.05 M K$_2$SO$_4$. The solution was shaken for 30 minutes, extracted and analyzed by colorimetry for NO$_3$ content (Forster, 1995). The amounts of $^{15}$N were determined by diffusing N according to Stark & Hart (1996). Isotopic composition of N was measured by an automated N/C analyser-isotope ratio mass
N mineralization under elevated CO$_2$.

spectrometer (ANCA-IRMS, Europa Scientific Integra, UK). The amount of dilution (D) of the N isotope pool that occurred during the incubation was calculated as follows:

\[ D_n = \frac{\text{atom}\%^{15}\text{N}_{\text{final}} - \text{atom}\%^{15}\text{N}_{\text{background}}}{\text{atom}\%^{15}\text{N}_{\text{initial}} - \text{atom}\%^{15}\text{N}_{\text{background}}} \quad (3.1) \]

Background atom\% for $^{15}$N was 0.3663.

A Mixed Model ANOVA in the SAS system for Windows™ V8 was conducted with blocks (i.e. the field plots) as random effects and treatments (i.e. CO$_2$, N and species treatments) as fixed effects. Differences between means were tested using Least Significant Differences (LSD). The level of significance was $P<0.05$.

### 3.3 Results

Mineralization of plant material N as revealed by recovery of $^{15}$N-$\text{NO}_3^-$ was not affected by CO$_2$ treatment for both $L$. $perenne$ and $T$. $repens$ (Fig. 3.1a, b). At all sampling times during the incubation percentages of $^{15}$N-$\text{NO}_3^-$ recoveries were equal, independent of ambient plant materials were added to elevated soils, or vice versa (Fig. 3.1a, b). At day 90, recoveries ranged from 29.8\% (± 1.7) to 31.5\% (± 0.8) for $T$. $repens$ under low N fertilization and from 29.7\% (± 1.3) to 32.9 (± 1.2) for $T$. $repens$ under high N fertilization treatments (Fig. 3.1b).

Fig. 3.1 a, b Percent recovery of $^{15}$N as $^{15}$N-$\text{NO}_3^-$ from (a) $L$. $perenne$ and (b) $T$. $repens$ plant materials incubated in soils exposed to ambient and elevated CO$_2$. Measurements are taken at 3 different time points (day 22, 50 and 90) during the incubation experiment. Values are means across experimental units with SEM indicated by the error bars.
Chapter 3

For *L. perenne* recoveries ranged from 21.6% (± 1.7) to 22.6% (± 1.9) and from 28.0% (± 1.8) to 30.4% (± 1.6) under low and high N fertilization treatments, respectively (Fig. 3.1a). In addition, the $^{15}$N recovery rates were dependent on plants species and N treatments rather than CO$_2$ treatment (Table. 3.1). *T. repens* plant materials decomposed at the highest rates followed by *L. perenne* plant material exposed to the high N fertilization treatment. *L. perenne* plant material following the low N fertilization treatment decomposed at the lowest rate (Table. 3.1).

Table 3.1 Percent recovery rates of $^{15}$N as $^{15}$N-NO$_3^-$ from *T. repens* and *L. perenne* plant materials following low and high N fertilization and ambient and elevated CO$_2$ incubated in soils exposed to ambient and elevated CO$_2$. Measurements are taken at days 22, 50 and 90 of the incubation experiment. Values are means ± SEM (n=3). ** Significant P< 0.05.

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<th>Plant-CO$_2$</th>
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Source of variation ANOVA

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</table>
3.4 Discussion

At the Swiss FACE experiment elevated CO₂ has increased harvestable biomass (up to 32%) and N demand in plants (Hartwig et al., 2002; Schneider et al. 2004; Aeisschlimann personal Communication.). This increased N uptake may cause down-regulation of soil N nutrition, leading to greater demand for N by the microbial community (Cotrufo et al., 1998). In addition, plant C to N ratios may be increased under elevated CO₂. If elevated CO₂ induces such changes, N mineralization will likely decrease (Soussana et al., 1996; Hu et al., 2001). However, the results of this study show that 9 years of elevated CO₂ did not change N mineralization of plant materials. This may partially be explained by the minimal response of litter C to N ratios (de Graaff et al., 2004) to elevated CO₂, which has also been observed in other FACE studies (Norby & Cotrufo, 1998; King et al., 2001; Weatherly et al., 2003). In addition, microbial activity may have not changed under elevated CO₂; Gloser et al. (2000) did not observe a change in the amount of available N in the L. perenne swards after four years of elevated CO₂. Finally, Schneider et al. (2004) did find increases in the amounts of harvestable biomass and in the proportions of soil N immobilized in plant tissues of L. perenne, but only when additional N was applied. These data suggest that elevated CO₂ did not reduce plant N mineralization in any of the soils, because of a surplus of available N in the fertilized and leguminous systems, and because of insignificant plant responses to elevated CO₂ in the low N systems. This study corroborates other FACE studies showing insignificant responses of ecosystem N cycling to elevated CO₂ (Finzi et al., 2002; Finzi & Schlessinger, 2003; Holmes et al., 2003; Zak et al., 2003; Johnson et al., 2004). In the same experiment, after 7 years of exposure to elevated CO₂, Richter et al. (2003) found no changes in gross N mineralization using the ¹⁵N dilution technique in intact soil cores. Using a different approach based on the physical fractionation of particulate organic matter, van Groenigen et al. (2002) also concluded that soil N dynamics were unaffected by 8 years of elevated CO₂ concentrations. By using yet a different method, we conclude that 9 years of elevated CO₂ induced neither a positive nor a negative feedback in the N cycle. In these agricultural soils, management practices seem to exhibit a greater impact on soil N cycling than prolonged elevated CO₂.
4. The impact of long-term elevated CO$_2$ on C and N retention in stable SOM pools.

Abstract

Elevated atmospheric CO$_2$ frequently increases plant production and concomitant soil C inputs, which may cause additional soil C sequestration. However, whether the increase in plant production and additional soil C sequestration under elevated CO$_2$ can be sustained in the long-term is unclear. One approach to study C-N interactions under elevated CO$_2$ is provided by a theoretical framework that centers on the concept of Progressive Nitrogen Limitation (PNL). The PNL concept hinges on the idea that N becomes less available with time under elevated CO$_2$. One possible mechanism underlying this reduction in N availability is that N is retained in long-lived soil organic matter (SOM), thereby limiting plant production and the potential for soil C sequestration. The long-term nature of the PNL concept necessitates the testing of understanding mechanisms in field experiments exposed to elevated CO$_2$ over long periods of time. The impact of elevated CO$_2$ and $^{15}$N fertilization on $L$. perenne and $T$. repens monocultures has been studied in the Swiss FACE experiment for 10 consecutive years. We used a biological fractionation technique to determine how elevated CO$_2$ affects the accumulation of N and C into stable SOM pools. Elevated CO$_2$ significantly stimulated retention of fertilizer-N in the stable pools of the $L$. perenne soils receiving low and high N fertilization rates by 18% and 22%, respectively, and by 45% in the $T$. repens soil receiving the low N fertilization rate. However, elevated CO$_2$ did not significantly increase stable soil C formation. The increase in N retention along with an absence of labile and stable C formation under elevated CO$_2$, suggests that elevated CO$_2$ has induced PNL in the Swiss FACE experiment. However, plant production increased under elevated CO$_2$, indicating that the additional N supply through fertilization prohibited PNL at this site. Therefore, it remains unresolved why elevated CO$_2$ did not increase labile and stable C accumulation in these systems.

4.1 Introduction

The atmospheric CO$_2$ concentration has been increasing continuously since the industrial revolution, and it is expected to continue rising due to extensive burning of fossil fuels and land-use changes (Houghton & Ding, 2001). Elevated atmospheric CO$_2$ will likely affect agro-ecosystem functioning through its direct impact on photosynthesis. Indeed, plant production has increased by 20% on average under elevated CO$_2$ (Ainsworth & Long, 2005; de Graaff et al., 2006b). Gifford (1997) suggested that increased C assimilation by plants and its subsequent sequestration in the soil may counterbalance the rise in CO$_2$ emissions. However, enhanced soil C sequestration under rising levels of CO$_2$ can only occur if increases in soil C input are sustained (Taylor & Lloyd, 1992; Friedlingstein et al., 1995; Kicklighter et al., 1999) and soil C mineralization lags behind the increase in soil C input (Raich & Schlesinger, 1992).

The rates of both soil C input and mineralization are strongly controlled by soil N availability; however, the impact of elevated CO$_2$ on soil N mineralization processes is uncertain (Norby & Cotrufo, 1998; Zak et al., 2000). Most studies have observed that elevated CO$_2$ initially stimulates plant production and concomitant soil C inputs, but these enhanced soil C inputs either stimulated or reduced soil N mineralization. For example, Zak et al. (1993) showed that the increased C inputs under elevated CO$_2$ stimulated the growth of soil microbial biomass, thereby increasing rates of N mineralization. Whereas, Diaz et al. (1993), found that increased C inputs under elevated CO$_2$ stimulated competition between the soil microbial biomass and plants for soil N, leading to a decline in soil N availability. In addition, Oren et al. (2001) showed that increased N fertilization may offset the decline in N availability under elevated CO$_2$. Thus, it remains unclear how initial increases in soil C input under elevated CO$_2$ affect microbial N transformation processes.

Another topic of debate is how the increase or decline in N availability ultimately controls soil C sequestration. Results of a recent meta analysis showed that if plenty of N is available, enhanced plant growth and soil C input under elevated CO$_2$ are likely sustained, resulting in net soil C sequestration (de Graaff et al., 2006b; Reich et al., 2006; van Groenigen et al., 2006). However, it has also been argued that ample soil N availability may simultaneously enhance soil C mineralization (Niklaus et al., 1998). In that case, the increased CO$_2$ respiration rates could off-set the increase in soil C accumulation.

To explain how elevated CO$_2$ affects soil N availability and soil C sequestration, Luo et al. (2004) proposed a conceptual framework which can be used to study C-N interactions under elevated CO$_2$ conditions. They projected that an increase in C influx into an ecosystem under elevated CO$_2$ stimulates two processes that are critical for regulating long-term ecosystem N dynamics: (1) increased demand for N to support
Soil C and N retention under elevated CO2

stimulated plant growth and (2) enhanced sequestration of N into plant biomass and long-lived SOM pools. The latter process can decrease soil N availability for plant growth and serves as the core mechanism in driving progressive N limitation (i.e. PNL). If PNL occurs, enhanced plant growth and soil C sequestration can not be sustained under elevated CO2. Nevertheless, if N sequestration is compensated for by additional N supply through N-fertilization or N deposition, it is possible that N will not limit C accumulation at all.

To test whether elevated CO2 does in fact increase incorporation of N and C into long-lived SOM pools, data on soil C and N dynamics under elevated CO2 from long-term field experiments are required (Luo et al., 2004). Free Air Carbon dioxide Enrichment (FACE) techniques allow for long-term CO2 fumigation studies in a field situation (Rogers et al. 1983; Hendrey, 1993). The impact of elevated CO2 and N fertilization on L. perenne and T. repens monocultures has been studied in the Swiss FACE experiment for 10 consecutive years. Therefore, this experiment offers a good opportunity to test the validity of the PNL concept. It has already been established that elevated CO2 has not significantly increased soil C and N sequestration during the 10 years of this experiment (van Kessel et al., 2006). Yet, trends eluding to changes in soil C and N accumulations under elevated CO2 have been found (Six et al., 2001; van Groenigen et al., 2003), and the reason that such changes have not been detected may be a result of the difficulty to detect statistically significant changes in total soil C and N pools, since these pools with long residence times are large but change slowly (Hungate et al. 1996, Schlesinger and Lichter 2001).

The long-term use of 15N fertilizer in the Swiss FACE experiment offers a unique opportunity to assess with greater sensitivity whether elevated CO2 stimulates N accumulations in stable soil pools. Indeed, using physical fractionation, Van Groenigen et al. (2003) did detect an increase in the amount of fertilizer-N recovered in the SOM pools associated with the mineral fraction (mSOM) for both species receiving a high N fertilization rate and exposed to elevated CO2. This finding suggests that elevated CO2 increased N sequestration, which supports the PNL-concept. In this study we used long-term incubations to separate the 10 year long 15N-fertilizer addition into stable and labile SOM-N pools. In addition we measured soil CO2 efflux during the incubation to be able to define C incorporated in labile and stable soil C pools. Based on the PNL concept, we hypothesized that sustained increases in plant production under elevated CO2, will stimulate the incorporation of C and fertilizer-N into stable SOM pools.

4.2 Materials and methods

Site description and sampling procedure

The Swiss Free Air Carbon dioxide Enrichment (FACE) field site at Eschikon, Switzerland was established in 1993, and consists of six FACE rings, laid out in a split-split-plot design. Three of the rings were exposed to ambient atmospheric CO2.
concentrations, while the other three rings were exposed to elevated atmospheric CO₂ concentrations (60 Pa CO₂ ± 10% over 92% of the fumigated time). The subplots consisted of monocultures of *Lolium perenne* and *Trifolium repens*, that were cut 5 times a year, after which the harvested biomass was removed from the plots. Nitrogen fertilizer (¹⁵NH₄¹⁵NO₃) was applied 4 times a year at rates of 140 or 560 kg N ha⁻¹ y⁻¹. The low and high N treatments contained 0.3841 and 0.1789 atom% ¹⁵N excess in 1995 and 1.0602 and 0.2890 atom% ¹⁵N excess from 1996-2000, respectively. The soil is classified as a fertile, eutric Cambisol (Zanetti et al., 1996).

In March of 2003, 20 soil samples were taken to a depth of 10 cm, from each of the treatment plots. A soil core with a diameter of 7 cm allowed us to take 4 homogeneously distributed subsamples per subplot. Individual soil cores taken from a subplot were composited and sieved to 10 mm to facilitate future soil preparation. Subsequently, the sieved soil was air dried for 48 h prior to shipment. Following shipment, the soil was ground, sieved (<2 mm), and roots (>2 mm) were removed.

**Incubation with repeated leaching**

Subsamples of the soils (70 g) were incubated in plastic filters (Falcon Filter model 7111; Becton Dickinson Labware, Lincoln Park, NJ, USA) at 35°C (Kaye et al., 2002). A glass fiber filter (Whatman GF/A, Whatman Inc., Ann Arbor, MI, USA), and an “extra thick” glass fiber prefilter (Gelman Sciences, Ann Arbor, MI, USA) were used to replace the filter originally in the filter unit. Water-holding capacity of the soils was determined by calculating the difference in weight of soils at saturation point and oven-dry weight (100 °C). Water was added to obtain 60% of water holding capacity. Subsequently, the filter units were sealed in airtight 2L jars fitted with septa. Ten ml of water was added to the bottom of the jar to prevent the soil from drying. Three jars containing the filter unit, but no soil were included for background N and ¹⁵N measurements.

To determine the labile soil N pool size, the soils were leached at days: 1, 8, 25, 43, 58, 86, 112, 145, 175 and 220, with a leaching solution containing all essential nutrients except for N (Stanford and Smith, 1972; Nadelhoffer, 1990; Kaye et al., 2002). At each leaching, 120 ml of the N-free leaching solution was added to the top of the filter, allowed to equilibrate with the soil for 45 min., and then drawn through the filter with a weak vacuum until all the leachate was collected (Kaye et al., 2002). The leachates were collected in 120 ml specimen cups and frozen until further analyses for ammonium (NH₄⁺), nitrate (NO₃⁻) and nitrite (NO₂⁻). After the last leaching event, a 20 g subsample was taken from each of the soils and extracted with 100 ml K₂SO₄ (0.5 M), after which the labile N not yet leached from the soil was determined.

**Labile N analyses**

Labile N was defined as the sum of the amounts of NH₄⁺, NO₃⁻ and NO₂⁻ in the leaching solutions plus the residual labile N (NH₄⁺, NO₃⁻ and NO₂⁻) determined by K₂SO₄
extraction. The leaching solutions and extracts were analyzed colorimetrically for mineral N concentrations (Forster, 1995).

Total fertilizer-N present as labile N was determined by analyzing the \( ^{15}N \) content of each of the solutions after each leaching event. The \( ^{15}N \) determination was performed by diffusing N from the leaching solutions and extracts onto acidified disks sealed in Teflon tape (Stark & Hart, 1996). A 10 ml subsample of each of the solutions was transferred to a 20 ml plastic scintillation vial. Both MgO and Devarda’s alloy were added to convert NO\(_3^–\) and NH\(_4^+\) to NH\(_3\), and collected on 2 disks (Whatman #42 filterpaper) containing 7 \( \mu l \) of K\( \text{H}_2\text{SO}_4 \) and sealed in Teflon tape. After 5 days of diffusion, facilitated by gentle shaking, the disks were dried in an oven, packed in tin capsules, and analysed for isotopic composition by an automated N/C analyser-isotope ratio mass spectrometer (ANCA-IRMS, Europa Scientific Integra, UK) at the UC Davis stable Isotope facility.

**Labile and stable fertilizer derived N calculations**

The atom% \( ^{15}N \) of samples was compared to \( ^{15}N \) standards and corrected for N in diffusion reagents using the \( ^{15}N \) pool dilution method as described by Stark & Hart (1996). The mass of fertilizer derived N residing in the labile pool was calculated using the following equations (Kaye et al., 2002):

\[
N_o = N_a + N_n \quad (4.1)
\]

Rearranging:

\[
N_a = N_o - N_n \quad (4.2)
\]

\[
N_o*^{15}N_o = N_a*^{15}N_a + N_n*^{15}N_n \quad (4.3)
\]

Substituting from Eq. (4.2):

\[
N_o*^{15}N_o = N_a*^{15}N_a + (N_o - N_n)*^{15}N_n \quad (4.4)
\]

Rearranging:

\[
N_a = (N_o*^{15}N_o - N_n*^{15}N_n)/(^{15}N_a - ^{15}N_n) \quad (4.5)
\]

where \( N_o \) is the mass of labile N, \( N_n \) is the mass of fertilizer derived N in the labile pool, \( N_a \) is the mass of labile native soil N, \( ^{15}N_o \) is the atom% \( ^{15}N \) excess in the leachate sample, \( ^{15}N_n \) is the atom% \( ^{15}N \) excess of the added N, and \( ^{15}N_a \) is the atom% \( ^{15}N \) of the native soil N (0.368%).

The amount of fertilizer N in the stable pool was determined by subtracting the total amount of fertilizer derived N in the labile pool (\( N_a \)) at termination of the incubation, from the amount of fertilizer derived N in the soil prior to incubation. The amount of fertilizer derived N in the soil prior to the incubation was calculated using similar equations:
where $N_t$ is the mass of total N, $N_f$ is the mass of the fertilizer N in the total N pool, $N_n$ is the mass of native N in the total N pool, $\frac{15N_t}{15}N_t$ is the atom\% $^{15}$N excess in the total N sample, $\frac{15N_f}{15}N_f$ is the atom\% $^{15}$N excess of the added N, and $\frac{15N_n}{15}N_n$ is the atom\% $^{15}$N of the native soil N (0.368\%).

### Labile and stable soil C pools

The labile C pool size was estimated by measuring soil CO$_2$ evolution at days: 1, 2, 3, 5, 9, 15, 26, 44, 62, 95, 121, 150, 186 and 220 in the incubation jars. A septum in the lid of the jars allowed gas samples (12 mL) to be removed with a syringe and collected in 12 mL vacutainers (Labco Unlimited, Buckinghamshire, UK). Control jars (three) without soil present were included to determine the background levels of CO$_2$. Following gas sampling, the caps were removed and the Mason jars were flushed in open air for 30 min. To promote flushing and air exchange, jars were placed under a fan. Labile C was defined as the sum of all CO$_2$-C respired during the incubation. Stable C was defined as total C minus labile C.

### Statistical analyses

The results were analyzed using the Mixed Model in the SAS system for Windows V8. An ANOVA was conducted with blocks (i.e. the field plots) as random effects and treatments (i.e. CO$_2$, N and species treatments) as fixed effects. Statistical tests were performed on cumulative respired CO$_2$ and total labile and stable C and N at the end of the incubation. Differences between means were tested using least significant differences. The level of significance was $P < 0.05$.

### 4.3 Results

#### Total labile and stable soil N

After 220 days of incubation, the total amount of labile N leached from the soils amounted to an average of 10.9\% of total soil N and was not significantly affected by CO$_2$-, N-, or plant species treatments (Table 4.1). The total amount of N residing in the
stable pool after 220 days of incubation amounted up to 89.1% of total soil N and was also not affected by CO₂ concentrations, N fertilization rates or plant species (Table 4.1).

**Fig. 4.1** (a) Labile fertilizer derived N in *L. perenne* soils following 10 years of ambient versus elevated CO₂ and low versus high ¹⁵N-N fertilization rates. (b) Labile fertilizer derived N in *T. repens* soils following 10 years of ambient versus elevated CO₂ and low versus high ¹⁵N-fertilization rates. (c) Stable fertilizer derived N in *L. perenne* soils following 10 years of ambient versus elevated CO₂ and low versus high ¹⁵N-fertilization rates. (d) Stable fertilizer derived N in *T. repens* soils following 10 years of ambient versus elevated CO₂ and low versus high ¹⁵N-fertilization rates. Values are means with SEM (n=3) indicated by the error bars.

**Labile and stable fertilizer derived N**

The total amount of fertilizer derived N in the soils prior to the incubation was on average 9.7% of the total soil N content and was significantly affected by the N- and species treatments, but not by the CO₂ treatments (data not shown, but equivalent data reported in van Kessel et al. (2006)). Incubating the soils for 220 days with repeated leaching revealed that, on average, labile fertilizer-N was 27.6% of the total fertilizer-N pool; the remaining 72.4% was stable fertilizer-N. Elevated CO₂ did not change the total amount of fertilizer derived N leached from the soils compared to ambient CO₂.
(Fig. 4.1a and b; Table 4.2). However, significantly more fertilizer derived N was leached from the L. perenne than from the T. repens soils (Fig. 4.1a and b; Table 4.2). In addition, significantly more labile fertilizer derived N was leached from the soils receiving high N fertilization rates compared to the soils receiving low N fertilization rates (Fig. 4.1a and b; Table 4.2).

Total amounts of stable fertilizer derived N residing in the soils at day 220 were significantly affected by CO2-, species- and N- treatments. Stable fertilizer derived N contents were significantly lower for both T. repens and L. perenne soils when the soils had received low N fertilization rates (by 34.8% on average) (Fig. 4.1c and d; Table 4.2). Elevated CO2 significantly stimulated stable fertilizer N formation in the L. perenne soils receiving low and high N fertilization rates and T. repens soils receiving low N fertilization treatments (Fig. 4.1c and d; Table 4.2).

**Fig. 4.2** (a) Cumulative CO2 respiration during 220 days incubation from L. perenne soils following ambient versus elevated CO2 and low versus high N fertilization treatments. (b) Cumulative CO2 respiration during 220 days incubation from T. repens soils following ambient versus elevated CO2 and low versus high N fertilization treatments. Values are means with SEM (n=3) indicated by the error bars.
**Microbial activity**

Elevated CO$_2$ did not alter cumulative microbial CO$_2$ respiration in the *L. perenne* soils receiving the low and high N fertilization rates (Fig. 4.2a; Table 4.2). Cumulative CO$_2$ respiration from *T. repens* soils was equal for the soils receiving high N fertilization rates in both ambient and elevated CO$_2$ treatments (Fig. 4.2b; Table 4.2).

Also, the CO$_2$ respiration from the *T. repens* soils receiving the low N fertilization rates was similar under both ambient and elevated CO$_2$ (Fig. 4.2b; Table 4.2). Cumulative CO$_2$ respiration from the *T. repens* soils receiving the low N fertilization rates under elevated CO$_2$ was significantly smaller than the respiration from the soils receiving the high N fertilization rates under both ambient and elevated CO$_2$ (Fig. 4.2b; Table 4.2). Cumulative CO$_2$ respiration from the *T. repens* soils previously exposed to elevated CO$_2$ and receiving low N fertilization rates was significantly lower than cumulative CO$_2$ respiration from the *L. perenne* soils exposed to elevated CO$_2$ and receiving high N fertilization rates (Fig. 4.2a and b; Table 4.2).

The amount of labile C respired from the soil during 220 days of incubation amounted to 8.1% of total soil C on average, and was not affected by any of the treatments (Table 4.3). The amount of more stable soil C was on average 91.9% of total soil C, and was also not affected by exposure to ambient or elevated CO$_2$, low or high N fertilization rates or plant species (Table 4.3).

**Table 4.1** Total soil N contents, labile soil N contents, and stable soil N contents in *T. repens* and *L. perenne* soils following 10 years of low and high N fertilization and ambient and elevated CO$_2$. Values are means ± SEM (n=3). * Significant at the 0.05 level of probability.

<table>
<thead>
<tr>
<th>Species-treatment</th>
<th>CO$_2$-treatment</th>
<th>N-treatment</th>
<th>total N (mg g$^{-1}$ soil)</th>
<th>labile N (mg g$^{-1}$ soil)</th>
<th>stable N (mg g$^{-1}$ soil)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>L. perenne</em></td>
<td>Ambient</td>
<td>Low</td>
<td>3.25 ± 0.05</td>
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<td>2.91 ± 0.02</td>
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<td>High</td>
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<tr>
<td><em>T. repens</em></td>
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<td>2.58 ± 0.21</td>
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<td>High</td>
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<td>0.38 ± 0.03</td>
<td>3.09 ± 0.18</td>
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<td>Elevated</td>
<td>Low</td>
<td>3.49 ± 0.18</td>
<td>0.40 ± 0.05</td>
<td>3.09 ± 0.13</td>
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<td></td>
<td>High</td>
<td>3.39 ± 0.48</td>
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Source of variation ANOVA

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Table 4.2 Analysis of variation associated with the means and SEM-values represented in graph 4.1a-d and 4.2 a-b.

<table>
<thead>
<tr>
<th>Source of variation ANOVA</th>
<th>Labile fertilizer derived N</th>
<th>Stable fertilizer derived N</th>
<th>CO₂ respiration</th>
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<tr>
<td>CO₂</td>
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<tr>
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<tr>
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<tr>
<td>CO₂*N</td>
<td>ns</td>
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</tr>
</tbody>
</table>

Table 4.3 Total soil C contents, labile soil C contents, and stable soil C contents in *T. repens* and *L. perenne* soils following 10 years of low and high N fertilization and ambient and elevated CO₂. Values are means ± SEM (n=3), * Significant at the 0.05 level of probability.

<table>
<thead>
<tr>
<th>Species-treatment</th>
<th>CO₂-treatment</th>
<th>N-treatment</th>
<th>total C (mg g⁻¹ soil)</th>
<th>labile C (mg g⁻¹ soil)</th>
<th>stable C (mg g⁻¹ soil)</th>
</tr>
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<td><strong>L. perenne</strong></td>
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<tr>
<td>Ambient</td>
<td>High</td>
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<td>29.92 ± 1.09</td>
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<td>27.48 ± 0.93</td>
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<td>Low</td>
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<td>28.74 ± 0.37</td>
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<td>26.33 ± 0.47</td>
</tr>
<tr>
<td>Elevated</td>
<td>High</td>
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<td>30.79 ± 3.62</td>
<td>2.48 ± 0.39</td>
<td>28.30 ± 3.28</td>
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<tr>
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<td>38.78 ± 1.33</td>
<td>2.82 ± 0.11</td>
<td>35.95 ± 1.25</td>
</tr>
<tr>
<td><strong>T. repens</strong></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Ambient</td>
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<td>2.22 ± 0.16</td>
<td>24.53 ± 0.89</td>
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<td>29.08 ± 1.01</td>
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<td>26.76 ± 0.72</td>
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<tr>
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<td>1.90 ± 0.12</td>
<td>28.92 ± 2.75</td>
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<tr>
<td></td>
<td>Low</td>
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<td>26.92 ± 4.82</td>
<td>2.52 ± 0.08</td>
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Source of variation ANOVA

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<th>ns</th>
<th>ns</th>
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<td>ns</td>
</tr>
<tr>
<td>CO₂ * Species * N</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
</tbody>
</table>
4.4 Discussion

**N dynamics under elevated CO₂**

We found that elevated CO₂ significantly stimulated retention of fertilizer-N in the stable SOM pools in both the *L. perenne* and *T. repens* soils. The percentage of fertilizer derived stable N residing in the soil at the end of the incubation, relative to the total amount of fertilizer derived N at the inception of the incubation was, on average, 70% for the soils exposed to ambient- and 75% for the soils exposed to elevated CO₂. This suggests that a major fraction of the fertilizer derived N had been stabilized in the soils exposed to both ambient and elevated CO₂. Earlier results on N stabilization in SOM have also suggested that substantial quantities of N are incorporated into stable organic pools. For example, after a long-term incubation with repeated leaching, Kaye et al. (2002) found that 2 years after a ¹⁵N tracer was applied, half of the retained N resided in the stable pool of a grassland soil.

Our data corroborate the results of van Groenigen et al. (2003), who investigated the impact of elevated CO₂ on N dynamics in the Swiss FACE swards using physical fractionation techniques. They found that elevated CO₂ increased the amount of fertilizer-N recovered in the SOM pools associated with the mineral fraction (mSOM) for both species in the high N treatments. Since, the mSOM-pool is considered the more stable SOM pool, an increase of N in these pools also suggests a potential for increased N retention under elevated CO₂. The physical fractionation technique used by van Groenigen et al. (2003) did however not estimate soil N stability directly, instead, our laboratory incubations allowed for directly determining whether the added fertilizer-N is isolated from the plant-microbe internal cycle, since N availability to microbes is directly estimated (Robertson and Paul 1999). Our results and those of van Groenigen et al. (2003) however, do compliment each other and both support the hypothesis that elevated CO₂ increases N retention into long-lived SOM pools.

Leaching of fertilizer derived N was significantly higher in the soils receiving high- compared to low N fertilization rates. Although this finding is not surprising, leaching of the labile fertilizer derived N was significantly higher in the *L. perenne*, compared to the *T. repens* soils. This suggests that the amounts of labile fertilizer N susceptible to mineralization were larger in the *L. perenne* compared to the *T. repens* soils, which might be related to differences in losses of fertilizer-N originally applied to the systems. Indeed, van Kessel et al. (2006) reported a total loss of 43.4% of applied ¹⁵N fertilizer for *L. perenne* versus 65.8% for *T. repens* systems, leaving more fertilizer N in the *L. perenne* systems available for mineralization.

Elevated CO₂, however, did not reduce leaching of fertilizer derived N in our incubation. This corroborates the results from earlier studies on N mineralization in the Swiss FACE experiment; both gross N mineralization rates measured by using the ¹⁵N dilution technique in intact soil cores and mineralization rates of soil and plant material during a 90 day laboratory incubation experiment were not affected by elevated CO₂.
(Richter et al., 2003; de Graaff et al., 2006a). Yet, the lack of a difference in leaching of fertilizer derived N between soils exposed to ambient and elevated CO₂ was unexpected, since at termination of the incubation the amount of stable fertilizer derived N residing in the soils exposed to elevated CO₂ was greater than the amounts in the soils exposed to ambient CO₂. The explanation for the discrepancy between the lack of differences in loss of labile fertilizer-N and the simultaneous increase in stable fertilizer-N may be found in the insignificant differences in initial fertilizer-N contents in the soils. Indeed, the initial fertilizer-N contents in the soils showed a trend towards more fertilizer derived N retained in the soils exposed to elevated CO₂. Even though the amounts of fertilizer derived labile N were not significantly different between ambient and elevated CO₂ treatments, the differences were sufficient to cause a shift from insignificant differences between ambient and elevated CO₂ in the amounts of stable fertilizer derived N retained in the soil at inception of the incubation, to significant differences in stable fertilizer-N at termination of the incubation. The greater initial fertilizer derived N recovery in response to elevated CO₂, although not significant, did suggest a stimulative effect of CO₂ on N retention. Our data now provide direct evidence of increased N stabilization under elevated CO₂.

**Linking soil N dynamics to soil C dynamics under elevated CO₂**

A partial indicator of PNL is N mineralization; a decrease in N mineralization is likely to indicate incipient PNL (Luo et al., 2004). None of the studies conducted at the Swiss FACE site have detected either a significant increase or decrease in N mineralization rates (de Graaff et al., 2006a, Richter et al., 2003). However, in this present study we found significantly more fertilizer derived N accumulated in the stable SOM pool under elevated compared to ambient CO₂, which supports the PNL concept. In addition, elevated CO₂ did not affect soil C sequestration; neither labile- nor stable C were significantly affected by CO₂ concentration, N-fertilizer rates, or species. These results suggest that PNL could have prevented soil C sequestration under elevated CO₂ in the Swiss FACE experiment.

However, even though we found increased fertilizer N retention under elevated compared to ambient CO₂ treatments, elevated CO₂ had increased the harvestable biomass production of *L. perenne* in the high N fertilizer treatment by 28% and biomass production of *T. repens* by 11–14% in the low and high N fertilizer treatments (Aeschlimann et al., 2005). This suggests that the stimulated N sequestration under elevated CO₂ was sufficiently compensated for by the additional N supply through N fertilization and symbiotic N₂ fixation to sustain enhanced plant production. Thus, additional N inputs had prevented PNL from occurring at the Swiss FACE site. By contrast, Daepp et al. (2000) argued that plant production in the *L. perenne* system receiving low N fertilization rates and elevated CO₂ was limited by N availability. However, Luo et al. (2004) proposed that negligible growth responses to elevated CO₂, and low levels of N accumulation in the soil, will not automatically elicit PNL. Hence,
PNL cannot explain the lack of soil C sequestration under elevated CO₂ in the Swiss FACE experiment.

Another reason for the lack of soil C sequestration could be that the stable SOC pool was saturated (Kool et al., 2007). When Six et al. (2001) used physical fractionation to detect changes in soil C sequestration under elevated CO₂, they observed a significant increase in macro aggregation. Macro aggregates, have relatively high turnover rates, therefore increased incorporation of C in macro-aggregates does not necessarily enhance long-term C stabilization (Six et al., 2001). Concurrently, the fraction of new C increased only in the first three years of the Swiss FACE experiment and remained relatively stable in the following years (de Graaff et al., 2004, van Kessel et al., 2006). This suggests that during the FACE experiment, the majority of the new C was incorporated in soil pools with fast turnover times, and that net soil C sequestration under elevated CO₂ may have been prohibited by the capacity of the soil to retain C in SOM pools with slow turnover times. However, in that case higher rates of decomposition under elevated CO₂ should have offset the increase in soil C input. Nevertheless, both this study and another study conducted at the Swiss FACE experiment (de Graaff et al., 2004) found no increases in decomposition rates under elevated CO₂. Therefore it is unlikely that higher soil C inputs were counter balanced by increased CO₂ efflux derived from labile soil C pools and that saturation of the stable soil C pool limited net soil C sequestration in this FACE experiment.

Finally, the removal of the harvested biomass significantly reduced litter input to the soil, thereby reducing soil C sequestration rates. Even though, the root biomass of both plant species was significantly increased by elevated CO₂ (Hebeisen et al., 1997), which is an important contributor to soil C inputs (de Graaff et al., 2006b). Nonetheless, the ratio of root- over shoot-derived C decreases with time: from 6.1 after 5 months to 2.9 after 12 months and to 1.8 and 1.2 after 18 months of addition of labeled plant material (Six et al., 2002). Thus, lower shoot-derived soil C inputs could have been responsible for the lack of soil C storage in the long term.

Apparentely, the predictive power of the PNL concept for soil C sequestration in individual ecosystems is ambiguous, since management practices in fertilized agro ecosystems may reduce soil C input or increase decomposition rates under elevated CO₂, thereby counter balancing the stimulating effect of fertilizer applications on net soil C sequestration. In addition, the saturation of the stable SOM pool may prevent net soil C sequestration under elevated CO₂.

4.5 Conclusions

Elevated CO₂ increased fertilizer derived N retention in stable SOM pools. However, C accumulation in stable SOM pools was not stimulated by elevated CO₂. The increase of N retention in stable SOM pools in conjunction with the lack of additional stable C formation under elevated CO₂, suggest that elevated CO₂ may have induced PNL in the Swiss FACE experiment. However, elevated CO₂ still stimulated plant productivity
compared to ambient CO₂, indicating that the increased formation of stable fertilizer derived N formation under elevated CO₂ was sufficiently compensated for by the additional N supply through N fertilization and symbiotic N₂ fixation to sustain enhanced plant productivity. If under elevated CO₂, N availability does not limit plant growth in the long-term, significant soil C sequestration is expected. Additional soil C storage in this experiment may have been prohibited by management practices reducing soil C input, or any increases in soil C sequestration may have not been detected due to large variability between field-plots. These data, suggest that the predictive power of the PNL theory for soil C sequestration in individual ecosystems is ambiguous.
5. Interactions between plant growth and soil nutrient cycling under elevated CO2: a Meta-Analysis.

Abstract

Free Air Carbon dioxide Enrichment (FACE) and Open Top Chamber studies (OTC) are valuable tools for evaluating the impact of elevated atmospheric CO2 on nutrient cycling in terrestrial ecosystems. Using meta-analytic techniques, we summarized the results of 116 studies on plant biomass production, soil organic matter dynamics and biological N2 fixation in FACE and OTC experiments. The objective of the analysis was to determine whether elevated CO2 alters nutrient cycling between plants and soil and if so, what the implications are for soil carbon (C) sequestration. Elevated CO2 stimulated gross N immobilization by 22%, whereas gross and net N mineralization rates remained unaffected. In addition, the soil C:N ratio and microbial N contents increased under elevated CO2 by 3.8% and 5.8%, respectively. Microbial C contents and soil respiration increased by 7.1% and 17.7%, respectively. Despite the stimulation of microbial activity, soil C input still caused soil C contents to increase by 1.2% per year. Namely, elevated CO2 stimulated overall above- and belowground plant biomass by 21.5% and 28.3%, respectively, thereby outweighing the increase in CO2 respiration. In addition, when comparing experiments under both low and high N availability, soil C contents (+2.2% per year) and above- and belowground plant growth (+20.1% and +33.7%) only increased under elevated CO2 in experiments receiving the high N treatments. Under low N availability, above- and belowground plant growth increased by only 8.8% and 14.6%, and soil C contents did not increase. Nitrogen fixation was stimulated by elevated CO2 only when additional nutrients were supplied. These results suggest that the main driver of soil C sequestration is soil C input through plant growth, which is strongly controlled by nutrient availability. In unfertilized ecosystems, microbial N immobilization enhances acclimation of plant growth to elevated CO2 in the long-term. Therefore, increased soil C input and soil C sequestration under elevated CO2 can only be sustained in the long-term when additional nutrients are supplied.

5.1 Introduction

The atmospheric CO₂ concentration has increased from 280 µmol mol⁻¹ in pre-industrial times to the current level of 365 µmol mol⁻¹ and it is expected to exceed 700 µmol mol⁻¹ by the end of this century (Houghton & Ding, 2001). Elevated atmospheric CO₂ directly affects ecosystems by stimulating plant growth (Kimball & Idso, 1983; Drake et al., 1997; Ainsworth & Long, 2005). Gifford (1994) suggested that increased C assimilation by plants and its subsequent sequestration in the soil may counterbalance CO₂ emissions. However, enhanced C sequestration under rising levels of CO₂ can only occur if increases in soil C input are sustained (Taylor & Lloyd, 1992; Friedlingstein et al., 1995; Kicklighter et al., 1999) and soil C mineralization lags behind the increase in soil C input (Raich & Schlesinger, 1992).

During the last 25 years, SOM models have been used as tools for evaluating the impact of global change on ecosystems (Parton et al., 1992; Paul & van Veen, 1978; Jenkinson & Rayner, 1977; van Veen & Paul, 1981). Such models split SOM into an active, slow and passive pool, with a turnover time of 1.5 y, 25 y and 1000 y, respectively (Parton et al., 1987). They have been linked to climate models and predict that any losses in soil C due to a rising temperature will be offset by an increase in C sequestration resulting from increased atmospheric CO₂ levels (Hall et al., 2000).

However, results on soil C sequestration have been inconsistent, with studies showing an increase (Rice et al., 1994), no change (van Kessel et al., 2005), or even a decrease (Cardon et al., 2002; Calabritto et al., 2002; Hoosbeek et al., 2004) in soil C contents under elevated CO₂. To explain these conflicting results, many studies have investigated the impact of elevated CO₂ on the control mechanisms of soil C sequestration, i.e. SOM input through plant production and soil C and N dynamics driven by microbial decomposition of SOM.

Soil C input is primarily governed by photosynthesis, which generally increases under elevated CO₂ (Ainsworth & Long, 2005). However, the increased C assimilation under elevated CO₂ may eventually become down-regulated since plants need to maintain a balance between N and other resources controlling photosynthesis (Rogers & Humphries, 2000). Indeed, under limited N supply photosynthetic acclimation is more marked, as the capacity of the sinks in plants is too small to utilize the additional photo-assimilates produced under elevated CO₂ (Rogers & Humphries, 2000). Thus, an increase in photosynthesis and concomitant soil C input under elevated CO₂ can partially be inhibited when soil mineral nutrient availability is not sufficient to support plant growth (Vitousek & Howarth, 1991; Bergh et al., 1999).

Elevated CO₂ can decrease or increase soil nutrient availability, depending on the response of the soil microbial community (Norby & Cotrufo, 1998; Torbert et al., 2000; Norby et al., 2001). Diaz et al. (1993) proposed a negative feedback mechanism, where increased C input to the soil from increased productivity in elevated atmospheric CO₂ caused nutrient accumulation in SOM. On the contrary, Zak et al. (1993) found
decomposition rate to increase after exposure of litter to elevated CO2, suggesting that a positive feedback might occur, which would increase rates of nutrient cycling through the ecosystem. In addition, Oren et al. (2001) found that a negative feedback in the nutrient cycles induced by elevated CO2 can be offset when additional N is supplied to the system.

Clearly, the responses of ecosystems to elevated CO2 have been divergent and C and N dynamics in terrestrial ecosystems depend on a set of complex interactions between soil and plants. It is not clear what the relative importance is of soil C input and soil C mineralization on soil C sequestration under elevated CO2. Even if a positive feedback in the C cycle is induced by elevated CO2, it is unclear whether this will cause the system to be a source of C. Namely, a disproportionate input of C, though stimulated plant growth may counterbalance C outputs and cause the system to be a sink for C. Also, since the establishment of equilibrium between soil organic matter (SOM) input and decomposition can take up to decades or longer, we need long-term experiments under realistic field situations to predict changes in ecosystems under future CO2 levels.

The introduction of open top chambers (OTC) and Free Air Carbon dioxide Enrichment (FACE) techniques allowed for long-term CO2 fumigation studies under realistic growing conditions (Rogers et al. 1983; Hendrey, 1993). Since approximately 20 years, numerous OTC and FACE experiments have been conducted in a broad range of ecosystems. Plant growth and soil characteristics related to C and N cycling have been studied in many of these experiments, but no clear pattern has emerged that allows us to generalize about the effect of rising CO2 levels on C and N cycling through the plant-soil system (Zak et al. 2000).

Due to high spatial variability and the large size of the soil C pool compared to soil C input, the sensitivity of individual experiments to detect changes in soil C is low (Hungate et al. 1996, Six et al., 2001). A quantitative integration of results across experiments might help to overcome some of these problems. Meta-analytic methods enable placing confidence limits around effect sizes; therefore, they provide a robust statistical test for overall CO2 effects across multiple studies (Curtis and Wang 1998). Moreover, they allow testing for significant differences in the CO2 response between categories of studies (Hedges & Olkin 1985).

For this review we have compiled the available data from FACE and OTC experiments on plant biomass and a number of soil characteristics related to soil C and N cycling. Using meta-analysis, we compared the effect of CO2 enrichment on these variables across some plant functional types and ecosystem management practices. The objective of the analysis is to elucidate whether elevated CO2 alters nutrient cycling between plants and soil and if so, what the implications are for ecosystem services such as soil C sequestration.
5.2 Materials and methods

Database compilation

Data were extracted from 45 studies on plant growth, 59 studies on SOM dynamics and 13 published studies on biological N\(_2\) fixation in FACE and OTC experiments. Data on soil C, N\(_2\) fixation and root biomass were included in a previous meta-analysis (van Groenigen et al., 2006), which compared outdoor facilities (FACE and OTC) with growth chamber and greenhouse studies. In contrast, our analysis focuses solely on OTC and FACE studies. The response variables included in the meta-analysis are listed in Table 5.1. Values reported in tables were taken directly from the publication, whereas results presented in graphs were digitized and measured to estimate values for the particular pool or flux. Both above- and belowground biomass data were expressed on a dry weight per area basis. When soil density data were available, soil data reported on an area basis were converted to a weight basis. In all other cases, equal bulk soil density in ambient CO\(_2\) and elevated CO\(_2\) treatments was assumed.

To make meaningful comparisons between experiments, a number of restrictions were applied to the data. Due to a limited number of studies reporting NPP, only data on total standing above- and belowground plant biomass were included in the analysis. The sampling depth of the belowground biomass ranged from 0-10 to 0-60 cm. When studies reported belowground biomass data in multiple depths, the sum of all depths was used in order to account for the complete root system.

Table 5.1 List of response variables included in the meta-analysis, and their abbreviations used in figures.

<table>
<thead>
<tr>
<th>Response variable</th>
<th>Parameter abbreviation</th>
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<tbody>
<tr>
<td>Aboveground standing plant biomass</td>
<td>APB</td>
</tr>
<tr>
<td>Belowground standing plant biomass</td>
<td>BPB</td>
</tr>
<tr>
<td>Soil C content</td>
<td>C</td>
</tr>
<tr>
<td>Soil C to N ratio</td>
<td>C:N</td>
</tr>
<tr>
<td>Microbial C content</td>
<td>MicC</td>
</tr>
<tr>
<td>Microbial respiration, measured in short term (&lt;15 days) incubations</td>
<td>rCO2</td>
</tr>
<tr>
<td>Soil N content</td>
<td>N</td>
</tr>
<tr>
<td>Microbial N content</td>
<td>MicN</td>
</tr>
<tr>
<td>N mineralization rates, measured in short term (&lt;30 days) incubations</td>
<td>MinN</td>
</tr>
<tr>
<td>Gross N immobilization, measured by (^{15})N pool dilution methods</td>
<td>GNI</td>
</tr>
<tr>
<td>Gross N mineralization, measured by (^{15})N pool dilution methods</td>
<td>GNM</td>
</tr>
<tr>
<td>Biological N(_2) fixation</td>
<td>N(_2)</td>
</tr>
</tbody>
</table>

With regard to soil data, soil layers ranging in depth from 0-5 to 0-40 cm were included. When data were reported for several depths, the results that best represented the 0-10 cm soil layer were included. For N\(_2\) fixation, all forms of biological N\(_2\) fixation
Interactions between plants and soil nutrient cycling under elevated CO$_2$

(i.e. free-living and symbiotic bacteria, symbiotic actinomycetes and cyanobacteria) were included. Our review focuses on mineral soils, therefore, measurements on forest litter layers, marsh and rice paddies were excluded from the soil and plant biomass database. The elevated CO$_2$ levels of the experiments included in the database ranged from 430 ppm to 750 ppm. Data were not corrected for the degree of CO$_2$ enrichment. When more than one elevated CO$_2$ level was included in the experiment, only the results at the level that is approximately twice ambient CO$_2$ were included. Results from different N treatments, plant species and communities, soils and irrigation treatments within the same experiment were considered independent measurements. These studies were included separately in the database. For OTC experiments, data from the control chambers rather than the non-chamber control plots were included as the results for ambient CO$_2$. In case these were available, data for blower controls in FACE experiments were included as the results at ambient CO$_2$.

All root biomass data were obtained by soil coring. Results on C and N fluxes were all based on incubation data (laboratory and in situ). Data for microbial biomass were obtained by the fumigation-extraction method (Vance et al., 1987) or the substrate induced respiration technique (Anderson & Domsch 1978). The N$_2$ fixation data were determined by acetylene reduction, $^{15}$N dilution, or N contents of plant tissue when atmospheric N$_2$ was the only available N source.

For standing plant biomass and soil C and N contents, only the most recent data of each study were incorporated into the database. For data on microbial biomass and activities and N$_2$ fixation, time series from the most recent year of measurement were included whenever available.

Experimental conditions were summarized by a number of categorical variables: type of exposure facility, N addition and vegetation type (Table 5.2).

Table 5.2 Categorical variables used to summarize experimental conditions, and the values they could assume in the analysis of between group heterogeneity.

<table>
<thead>
<tr>
<th>Categorical variables</th>
<th>Level 1</th>
<th>Level 2</th>
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<tr>
<td>Facility</td>
<td>OTC</td>
<td>FACE</td>
</tr>
<tr>
<td>Low versus high N treatments within studies</td>
<td>Low N</td>
<td>High N</td>
</tr>
<tr>
<td>Vegetation</td>
<td>Herbaceous</td>
<td>Woody</td>
</tr>
</tbody>
</table>

We analyzed the interaction between CO$_2$ and soil N availability by comparing studies that had received low N versus high N treatments within the same experiment. For some of the response variables such experiments were underrepresented, in which case we compared between studies receiving low (0-30 kg ha$^{-1}$ y$^{-1}$) or high (>30 kg ha$^{-1}$ y$^{-1}$) levels of N fertilizer. To make statistically meaningful comparisons within categories using meta-analysis, we decided that we need at least 10 data points from at least 5 different studies. With regard to the N$_2$ fixation data, we compared studies receiving no mineral fertilization to studies receiving additional mineral (non-N) fertilization.
Vegetation was characterized as either herbaceous or woody. The duration of each experiment (i.e. years of CO2 fumigation) was also included in the database.

**Statistical analyses**

The data set was analyzed with meta-analytic techniques described by Curtis & Wang (1998) and Ainsworth et al. (2002), using the statistical software MetaWin 2.0 (Rosenberg et al., 2000). The natural log of the response ratio (\(r= \text{response to elevated CO}_2 / \text{response to ambient CO}_2\)) was used as a metric for above- and belowground biomass, C:N ratio’s, microbial biomass and activity, soil N mineralization and immobilization rates, and N2 fixation. It is reported as the percentage change under elevated CO2 (\([r-1]^{*100}\)).

In the short term (e.g., decadal), increases in soil C following a rise in soil C input are approximately linear over time (Schlesinger, 1990). As the average duration of CO2 exposure in the meta-analysis was 3.4 years, we assumed linear accumulation of soil C and N and the natural log of the time-adjusted response ratio \(r_t = (r-1)/\text{yr} + 1\) was used as a metric. Soil C and N results are reported as the percentage change per year under elevated CO2 (\([r_t-1]^{*100}\)).

In conventional meta-analyses, each individual observation is weighted by the reciprocal of the mixed-model variance (Curtis & Wang 1998). However, such an analysis requires that the standard deviations of individual studies are known. For a large proportion of the observations, these data were not available. Thus, studies were weighted by experimental replication, using the function \(F_N = (n_a*n_e)/(n_a+n_e)\) (Hedges & Olkin, 1985; Adams et al., 1997), where \(n_a\) and \(n_e\) represent the number of replicates under ambient and elevated CO2, respectively. We weighted observations of soil C and N by experimental duration and replication, using the function \(F_C = (n_a*n_e)/(n_a+n_e) + (\text{yr}^*\text{yr})/(\text{yr}+\text{yr})\), with \(n_a\) and \(n_e\) as before, and \(\text{yr}\) as the length of the study in years. We choose this metric because well-replicated and long-term studies provide more reliable estimates of effects on soil C and N (Hungate et al., 1996).

Bootstrapping techniques were used to calculate confidence intervals on mean effect size estimates for the whole data set and for categories of studies (Adams et al., 1997). The number of iterations used for bootstrapping was 4999. The CO2 effect on a response variable was considered significant if the 95% confidence interval did not overlap 0. Means of categories were considered significantly different if their 95% confidence intervals did not overlap.
5.3 Results

Plant growth

Both above- and belowground standing biomass increased significantly under elevated CO₂ by 21.5% and 28.3%, respectively (Fig. 5.1a). Aboveground plant growth was stimulated significantly more under elevated CO₂ for woody species (+30.5%) compared to herbaceous species (+12.6%) (Fig. 5.1a). A comparable response of woody and herbaceous plant production was observed for belowground biomass, however the difference was not significant (Fig. 5.1a).

![Graph](image)

**Fig. 5.1** (a) Percentage response of above- and belowground plant biomass production to elevated CO₂. (b) Percentage response of above- and belowground plant biomass production to elevated CO₂ in low and high N fertilizer treatments.

In contrast to root growth, the aboveground response of plant growth to elevated CO₂ was significantly different between FACE and OTC experiments. The aboveground biomass increase under FACE conditions was 16.5%, whereas CO₂ stimulated plant growth by 27.9% under OTC conditions (Fig. 5.1a; Table 5.3). However, the CO₂ pressure used in OTC experiments, particularly for woody species, was generally higher than in FACE experiments (Fig. 5.2). Also, OTC experiments were heavily biased
toward woody species, while herbaceous species made up most of the database for FACE experiments (Fig. 5.2).

**Fig. 5.2** Percentage response to elevated CO$_2$ of woody and herbaceous plants as a function of the CO$_2$ concentrations to which the plants are exposed in Free Air Carbon Dioxide (FACE) and Open Top Chamber (OTC) experiments.

Within experiments that included N availability treatments, high N availability significantly increased the response of aboveground plant growth to elevated CO$_2$. Aboveground biomass increased by 8.4% under elevated CO$_2$ following low N availability treatments, but the response to elevated CO$_2$ was significantly higher (+20.1%) under high soil N availability (Fig. 5.1b; Table 5.3).

The response of root biomass to elevated CO$_2$ was not significantly different between low and high N availability treatments. However, only in the high N availability treatments did root biomass significantly increase under elevated CO$_2$ (Fig. 5.1b). The continuous variable time did not affect the CO$_2$ response of above- and belowground plant growth in any of the N availability classes (data not shown).

**Soil C dynamics**

Total soil C increased significantly by 1.2% per year under elevated CO$_2$, but the CO$_2$ response depended on soil N availability (Fig. 5.3; Table 5.3). Under low N availability soil C contents were significantly lower than under high N availability (P=0.05). In addition, in experiments under low N availability, soil C contents were unaffected, whereas experiments under high soil N availability showed a significant CO$_2$ response of +2.1% (Fig. 5.3; Table 5.3). None of the other categorical variables affected the CO$_2$ response for soil C contents in the N availability classes. Across the whole soil C data set, the CO$_2$ response did not differ between herbaceous and woody species. Yet, elevated CO$_2$ did not significantly affect soil C contents under woody species (+0.89% y$^{-1}$).
Interactions between plants and soil nutrient cycling under elevated CO₂

1), whereas soil C contents under herbaceous species were significantly increased (+1.4% y⁻¹) (Fig. 5.3).

**Fig. 5.3** The yearly response (% y⁻¹) of soil C contents to elevated CO₂: overall response and categorized soil N availability treatment.

Averaged over all experiments, the soil C:N ratio increased by 4.1% under elevated CO₂ (Fig. 4). However, only the C:N ratio of the woody species increased significantly under elevated CO₂ (+7.5%) (Fig. 5.4; Table 5.3). No interactions between CO₂ and N availability treatments were observed for the C:N ratio (data not shown).

**Fig. 5.4** The percentage response of soil C:N ratios to elevated CO₂: overall response of and categorized by type of vegetation.

Microbial respiration and microbial C increased by 17.1% and 7.7% under elevated CO₂, respectively (Fig. 5.5). Due to a low number of soil microbial studies conducted under woody species (only 5), we were unable to test whether any differences existed in CO₂ response with regard to microbial respiration and microbial C contents, between woody and herbaceous species. The percentage response to elevated CO₂ of either
microbial respiration, or microbial C contents, was not affected by N fertilization treatments (Fig. 5.5; Table 5.3).

Soil N dynamics

Soil N concentrations did not change under elevated CO₂ (Fig. 5.6). Under herbaceous species, however, total N increased by 1.0% per year in the high CO₂ environments, which is a significant difference from woody species, which did not respond at all to elevated CO₂ (Fig. 5.6; Table 5.3). The response of total soil N to elevated CO₂ was unaffected by N availability treatments (Fig. 5.6; Table 5.3).

The effect of elevated CO₂ on microbial N pools and fluxes was characterized by large confidence intervals, indicating large differences among studies in the magnitude of CO₂ responses (Fig. 5.7a & b). Gross and net N mineralization were not significantly affected by elevated CO₂, whereas gross N immobilization and microbial N contents increased under elevated CO₂ by 22% and by 6%, respectively (Fig. 5.7a & b). No interactions between CO₂ and N availability treatments were found for microbial N contents (Fig. 5.7b; Table 3). Due to a limited number of studies (ranging from 3 to 5) conducted on gross and net soil N mineralization and gross N immobilization, we were unable to test whether any differences existed their CO₂ response under different N fertilization treatments (Fig. 5.7b).

Averaged over all studies biological N₂ fixation did not increase under elevated CO₂. However, N₂ fixation was significantly stimulated (+51%) by elevated CO₂ when additional non-N nutrients were supplied (Fig. 5.8; Table 5.3).
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Fig. 5.6 The yearly response (% y⁻¹) of soil N contents to elevated CO₂: overall response and categorized by type of vegetation and soil N availability treatment.

Fig. 5.7 (a) The percentage response of microbial N contents to elevated CO₂: overall response and categorized by low and high N fertilization treatments. (b) The overall percentage response of gross and net N mineralization and gross immobilization to elevated CO₂.
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Fig. 5.8 The percentage response of biological N$_2$ fixation to elevated CO$_2$, overall response and categorized by low and high N fertilization treatments.

Table 5.3 Analysis of variance, showing differences in percentage response to elevated CO$_2$ within the categorized response variables. Differences in response are considered significant when P < 0.05.

<table>
<thead>
<tr>
<th>Response variable</th>
<th>Categorial variable</th>
<th>P-value</th>
</tr>
</thead>
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</tr>
<tr>
<td>APB</td>
<td>vegetation</td>
<td>0.005</td>
</tr>
<tr>
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<td>soil N availability</td>
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</tr>
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<td>soil N availability</td>
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</table>

5.4 Discussion

*Plant growth*

Overall, elevated CO$_2$ stimulated aboveground standing biomass by 21.5%, but the increase was significantly greater for woody than for herbaceous species (Fig. 1a). This corroborates the results of Ainsworth & Long (2005), who found an increase in aboveground plant growth of 20% in a meta-analysis on FACE experiments. Additionally, of all functional groups, they found that trees showed the largest response.
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to elevated CO₂. Several other meta-analyses found no systematic differences between
the growth response of herbaceous and woody plants to elevated CO₂ (Poorter & Pérez-
Soba, 2001; Curtis & Wang, 1998). The discrepancy between results is probably caused
by differences in studies included in the meta-analyses, i.e. field versus pot studies. It
has been shown that the biomass increase of herbaceous plants in field studies is
considerably smaller than the increase observed in growth chamber studies (Cure &
Acock, 1986; Amthor, 2001; Jablonski et al., 2002). Conversely, constraints in growing
conditions caused by pots may strongly reduce the growth response to elevated CO₂,
particularly for trees (Norby et al., 1999). The relatively strong growth response to
elevated CO₂ of woody compared to herbaceous plants in our analysis may be attributed
to the young age of many of the woody species in FACE and OTC experiments
(Ainsworth & Long, 2005). Also, most experiments on woody species were conducted
in OTC’s, which showed a stronger growth response (Fig. 5.2). However, it should be
pointed out that woody species showed a stronger CO₂ response for plant growth than
herbaceous species in both FACE and OTC experiments. Thus, the stronger CO₂
response for woody compared to herbaceous species was not an artifact caused by
experimental design.

Why did woody species show a stronger response to elevated CO₂ in OTC
experiments than in FACE experiments? Firstly, OTC experiments generally apply
greater CO₂ concentrations than FACE experiments (Fig. 5.2). This greater CO₂
concentration is expected to cause a strong growth response, when we assume a linear
correlation between plant growth and atmospheric CO₂ concentrations (Kimball et al.,
2002). Indeed, we found a significant correlation (P<0.01) between the relatively high
CO₂ pressures in OTC experiments and growth responses of woody species to elevated
CO₂ (Fig. 2). A second explanation may be that the majority of the data derived from
FACE experiments were associated with older trees grown in forest stands, while more
studies in OTC experiments were conducted on individual seedlings and young trees.
When trees are grown individually, while they are young and there are no constraints
on leaf area production, very large CO₂ responses can occur (Norby et al., 1999).
Therefore, care should be taken in extrapolating the observed large growth responses of
woody species to longer timeframes than current experiment durations (Norby et al,
1999). To obtain realistic information concerning forest responses to elevated CO₂, we
need long-term experiments conducted under natural conditions.

Belowground biomass increased by 28.3% under elevated CO₂ (Fig. 5.1a). An
increase in root biomass is expected to result in higher amounts of C input into the soil
through rhizodeposition. Rhizodeposition includes C deposited by living roots in the
form of soluble exudates, mucilage, sloughed of root cells or root turnover (Darrah,
1996). An increase in input of root-derived materials under elevated CO₂ likely exerts a
more direct effect on soil C and N cycling than aboveground litter input, as root-
derived materials are an immediate substrate for microbial activity (Zak et al., 2000).
Our results suggest that root biomass is stimulated more than shoot biomass under
 Elevated CO₂. Thus, elevated CO₂ may have greater impact on soil C and N cycling.
through plant production than predicted from aboveground biomass data alone. However, Nowak et al. (2004) directly compared root and shoot growth responses to elevated CO\(_2\) and found that results considering the impact of elevated CO\(_2\) on root: shoot ratios have been inconsistent. Due to a lack of data on root and shoot biomass measured simultaneously within long-term experiments, our analysis can not give a definitive answer with regard to the relative response of roots versus shoots to elevated CO\(_2\).

Nevertheless, Matamala et al. (2003) suggested that not total root biomass, but fine roots are especially important in controlling soil C and N cycling. Norby et al. (1999) found that the relative effect of elevated CO\(_2\) on fine root density varied from 60–140% in field experiments with trees. Our results indicated an average 40% greater stimulation of fine root biomass under elevated CO\(_2\) compared to total root biomass (data not shown). These results, however, are based on a very small number of observations to allow making statistically meaningful comparisons. Moreover, even when ample data on fine root biomass would be available, differences in fine root longevity and biochemistry between plant species could influence microbial activity in a way that leads to highly variable responses in soil C and N cycling (Zak et al., 2000). Clearly, to gain a more basic understanding of changes in plant-soil interactions under elevated CO\(_2\), an emphasis on root and rhizosphere research in long-term field experiments is needed.

Under elevated CO\(_2\), N availability had a positive effect on both above- and belowground plant growth (Fig 5.1b). It has been generally accepted that N additions to systems enhance plant growth responses to elevated CO\(_2\) (Ceulemans & Mousseau, 1994; Kimball et al., 2002; Nowak et al., 2004, Ainsworth & Long, 2005). In fact, it has been proposed that increased plant growth under elevated CO\(_2\) can only be sustained in the long-term when additional N enters the system (Oren et al., 2001, Luo et al., 2004, Reich et al., 2006). Although the growth response of the aboveground biomass to elevated CO\(_2\) was still sustained under low N availability, it declined by 50% compared to systems receiving high N availability treatments. These results suggest that even in relatively short-term experiments low soil N availability strongly limits growth under elevated CO\(_2\), and that the increase in growth under elevated CO\(_2\) in unfertilized systems is expected not to be sustained in the long-term.

Soil C dynamics

The overall increase in total soil C under elevated CO\(_2\) suggests a potential for soil C sequestration. However, when taking into consideration the spatial variability in individual field experiments, the average increase of soil C by 1.2% per year is small. Indeed, only a small number of experiments reported a significant impact of elevated CO\(_2\) on soil C sequestration (Wood et al. 1994; Hagedorn et al. 2001; Prior et al. 1997; Prior et al. 2004; Rice et al. 1994; Six et al. 2001; Williams et al. 2000), while some studies showed no differences (van Kessel et al., 2006) and others found decreases in soil
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Several papers have reported a decrease in soil C contents under elevated CO₂. This decrease may have been the result of priming, which is defined as the stimulation of SOM decomposition caused by the addition of labile substrates (Jenkinson et al., 1985; Dalenberg & Jager, 1989). Priming may increase under elevated CO₂ as a result of cometabolic decomposition following a rise in microbial activity (Cheng, 1999). Several studies found that elevated CO₂ affects the decomposition of native SOM (Hoosbeek et al., 2004; Pendall et al., 2003; Cardon et al., 2001). However, as the amount of available native SOM is limited, a CO₂ induced increase in its decomposition is expected to diminish in the long-term. Indeed, isotopic data do suggest that CO₂ induced priming is a transient process (Hoosbeek, personal communication).

Soil C sequestration significantly increased under elevated CO₂ for herbaceous species only (Fig. 5.3). However, the larger growth response to elevated CO₂ of woody compared to herbaceous plants implies a greater potential for soil C storage under woody plants. Though, the high variability associated with soil C contents under woody species makes the impact of elevated CO₂ on C sequestration hard to predict (Fig. 5.4). This variability may be caused by differences in C input, which depends largely on the type (i.e. deciduous versus evergreen) and the age (i.e. size) of the woody species used and on the type of soil used (Ladegaard-Pedersen et al., 2005). Alternatively, it has been suggested that trees decrease soil nutrient availability rather quickly under elevated CO₂ (Finzi & Schlesinger, 2002; Barron-Gafford et al., 2005), which may make forests soils prone to priming (Hoosbeek et al., 2004; Calabritto et al., 2002). These factors probably contributed to the relatively strong negative and positive responses of woody species to elevated CO₂, which in combination with the small number of FACE and OTC studies conducted in forests, resulted in high variability.

In our meta-analysis, CO₂ enrichment significantly increased soil microbial C contents and microbial respiration. Microbial activity increased by 17.7% under elevated CO₂ and was not affected by N availability. As soil microorganisms are generally C-limited (Anderson & Domsch 1978), the increase in C availability, due to increased plant production and concomitant soil C input under elevated CO₂, probably contributed to the greater microbial activity. Van Groenigen et al. (2006b) showed that the increased input under elevated CO₂ strongly increased labile C pools. Since the increase in input is largely counterbalanced by microbial consumption of the shorter lived C pools, the net effect of the additional C input under elevated CO₂ on C sequestration will likely be limited.

Van Groenigen et al. (2006a) found an increase in soil C only when experiments received additional N fertilization rates of 30 kg ha⁻¹ or more. They suggested that the outcome of their analysis may be confounded by potential differences in initial soil N availability between individual experiments, possibly leading to a misconception of anticipated soil nutrient availabilities based on N fertilization rates. To avoid this confounding factor, we compared only experiments receiving both low and high N
fertilization treatments. Our results support the conclusion of van Groenigen et al. (2006a) that soil C contents increase under elevated CO2 only when high N fertilization rates are applied. Apparently, additional N fertilizer stimulates C input (+20.1% and +33.7% for above- and belowground plant growth, respectively) more than microbial activity (+22.3%), leading to greater C accumulation (+2.1% per year). Conversely, input under low N availability (+8.8% and +14.6% for above- and belowground plant growth, respectively) is counterbalanced by microbial C respiration (+14.4%). These results support models predicting that N availability may be the limiting factor for C sequestration under prolonged elevated CO2 (Hungate et al., 2003).

**Soil N dynamics**

Total N did not increase under elevated CO2, nor did net N mineralization, gross N mineralization, or N2 fixation (Figs. 5.6-5.8). The significant increase in gross N immobilization and microbial N contents under elevated CO2 suggest a greater N demand by microorganisms. Also, the significant increase in soil C:N ratios under elevated CO2 suggests a potential decrease in soil N availability. Mikan et al. (2000) found that gross N immobilization and plant N uptake by *Populus tremuloides* increased under elevated CO2, without affecting microbial N contents. Their results suggest that the effect of increased gross N immobilization on soil N availability can be compensated for by enhanced turnover of microbial N. However, we found that microbial N contents increase under elevated CO2 in long term experiments, which suggests that over time, an increase in CO2 will stimulate microbial immobilization of N.

Elevated CO2 decreased total soil N under woody species, while it increased soil N contents under herbaceous species. In line with these results, the soil C:N ratio increased significantly under woody species only. In several forest ecosystems, soil N leaching decreased under elevated CO2 (Johnson et al., 2004; Hagedorn et al., 2000; Hungate et al., 1999), suggesting a positive effect of elevated CO2 on plant N uptake. In corroboration, Barron-Gafford et al. (2005) found that increased above- and belowground biomass production of Eastern Cottonwoods in response to 4 years of elevated CO2, accelerated soil nutrient depletion. These and our data support Finzi & Schlesinger’s prediction (2002) that the productivity response of a Sweetgum forest to elevated CO2 will decline over time as nutrient supplies decrease through increased demands under elevated CO2.

Overall, N2 fixation was not affected by elevated levels of atmospheric CO2. This contradicts results from several short-term experiments investigating the impact of elevated CO2 on N2 fixation (Lee et al., 2003). We expected a positive response of legumes to elevated CO2, as increased rates of photosynthesis increase the C supply to rhizobia (Arnone & Gordon, 1990; Vitousek et al., 2002). In fact, several FACE studies found that legumes, particularly the more effectively nodulating genotypes, showed higher growth responses to elevated CO2 than other functional groups (Luscher et al., 2000; Ainsworth & Long, 2005; Poorter & Navas, 2003). Arnone (1999), however, found
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no effect of elevated CO₂ on N₂ fixation in a late successional alpine sedge community. In agreement with these results, West et al. (2005) found no increase in N₂ fixation under elevated CO₂ in three out of four legumes in a multi-species FACE experiment after 4 years of growth. These data suggest that N₂ fixation under elevated CO₂ may, at least for certain leguminous species, not be sustained in the longer term. Niklaus et al. (1998) and Hungate et al. (2004) suggest that limitation of Mo and P may possibly limit N₂ fixation in long-term FACE experiments. Indeed, our data show that N₂ fixation increases only under elevated CO₂ when additional non-N nutrients are supplied (van Groenigen et al., 2006a).

5.5 Plant-soil interactions under elevated CO₂, a synthesis.

The potential for soil C sequestration to mitigate rising levels of atmospheric CO₂ has stimulated research on C and N cycling in plant-soil systems under elevated CO₂. Results from studies investigating the impact of elevated CO₂ on soil C and N cycling or on the controlling processes of soil C and N cycling, such as microbial activity, root dynamics and plant growth, have been inconsistent (Norby & Cotrufo, 1998; Zak et al., 2000). These divergent results have generally been attributed to varying environmental and experimental factors. Meta-analysis allows for classifying ecosystem responses to elevated CO₂ and, therefore, to test for the relative importance of these subordinate factors for the potential of soil C sequestration under elevated CO₂.

Our analysis suggests that the main factor controlling the potential for soil C sequestration under elevated CO₂ is nutrient availability. The pathways by which nutrient availability controls the mechanisms responsible for soil C cycling are depicted in a conceptual diagram (Fig. 5.9). Overall, our data suggest that on average, across all systems and fertilization classes, elevated CO₂ stimulated soil C input through increased plant growth (Fig. 5.9). Even though microbial activity increased by 17.7% under elevated CO₂, the average increase of 22.5-28.3% in input offset the losses in soil C through increased C mineralization and led to an average 1.2% increase in soil C per year. These results are in agreement with Jastrow et al. (2005), who also reported a significant increase in soil C under elevated CO₂. However, when classifying ecosystem responses to elevated CO₂ by low and high N availability treatments, we found that soil C sequestration under elevated CO₂ was strongly limited by the availability of nutrients. The lack of C sequestration under low N availability was caused by a reduced response in plant growth and particularly by the reduced stimulation of root growth with elevated CO₂ in low N systems. Namely, the below- and aboveground biomass showed a 8.7-14.6% increase in the low N systems, which was not sufficient to counterbalance the 14.4% increase in microbial activity and resulted in no response of soil C (Fig. 3). In contrast, in the high N systems, the belowground biomass response (+33.7%) exceeded the increase in microbial activity (+20.1%) and the aboveground biomass increase was equivalent to the increase in microbial activity (+22.3). These results show that ample input of root derived C into
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the soil is key to sequestering C. Since CO$_2$ respiration increased under elevated CO$_2$, irrespective of soil N availability, these results suggest that not microbial activity, but rather C input determines the potential for soil C sequestration; C storage is determined by the balance between soil C input and C mineralization, of which the latter showed largely independent and the former is dependent on nutrient availability in ecosystems exposed to elevated CO$_2$.

In addition to these results, we observed an overall increase in microbial N immobilization under elevated CO$_2$. These results suggest that in the long term, elevated CO$_2$ may induce a decrease in soil N availability, which will result in a decrease in plant growth and soil C sequestration. This idea is supported by our and other data on woody plant production (Finzi & Schlesinger, 2002), showing the relatively fast depletion of available N pools under elevated CO$_2$ caused predominantly by young trees. Overall, this implies that C sequestration in both plants and soils under elevated CO$_2$ can only be sustained when additional nutrients are supplied (Fig. 5.9). This conclusion supports the theory of progressive nitrogen limitation (PNL) postulated by Luo et al. (2004). In corroboration with this theory a recent study on plant production under long-term elevated CO$_2$ and different N fertilization rates, also stresses the importance of nutrient additions to grassland systems for sustaining increased plant growth under elevated CO$_2$ (Reich et al., 2006).

In the short term, PNL can be alleviated or delayed by a number of ecosystem responses. Such responses probably explain the ~10% increase in aboveground plant production under elevated CO$_2$ and low N availability in our analysis. Firstly, priming has been observed to supply systems with sufficient nutrients to sustain increased plant growth under elevated CO$_2$ (Hungate et al., 2003). Priming has been shown to occur in nutrient poor soils (Cardon et al., 2001; Pendall et al., 2003, Fontaine et al., 2004), as a mechanism to supply N needed to sustain plant growth under elevated CO$_2$ (Zak et al., 1993). This mechanism, however, does not contribute to a net gain of ecosystem N, but rather causes redistribution of available N. Redistribution of N under elevated CO$_2$ is expected to have a limited effect on N availability and soil C sequestration in the long term (Hungate et al. 2003).

Secondly, the efficiency of plant N uptake under elevated CO$_2$ has been observed to increase due to increased fine root production (Mikan et al. 2000), or increased mycorrhizal colonization of roots (Rillig et al. 2000). However, further increases in plant growth and soil C input resulting from these adaptations, will increase competition for N between plants and microbes. Thus, CO$_2$-induced mechanisms that increase plant N uptake without a net ecosystem gain of N are self-limiting (Hungate et al. 2003).

Finally, PNL may be alleviated by the additional supply of N to the soil through N$_2$ fixation by leguminous plants (Fig. 5.9). However, our analysis revealed that symbiotic N$_2$ fixation only increased (50.8%) under longer-term elevated CO$_2$ when additional non-N nutrients were added. Thus, these results suggest that in unfertilized systems, symbiotic N$_2$ fixation will have a limited effect on preventing PNL under increased
levels of atmospheric CO₂, and that yet again nutrient availability determines the potential for C sequestration under elevated CO₂ (Fig. 5.9).

In conclusion, we expect that any rapid increases in plant production under elevated CO₂ in unfertilized systems are transient and that the potential for mitigating atmospheric CO₂ through additional soil C sequestration in such systems is minimal (Fig. 5.9). In contrast, when additional nutrients are supplied we do expect a potential for C sequestration (Fig. 5.9). The amount of C sequestered, however, seems limited as the increased C inputs are partially counterbalanced by increased C outputs through mineralization. Furthermore, many systems receiving additional nutrients are managed for production of agricultural products. Soil disturbance in these systems may cause a majority of the recently accumulated C to be re-released into the atmosphere (Paustian et al., 2000; Six et al., 2002).

![Fig 5.9 Conceptual diagram depicting the flows of C and N between plants and soil under elevated atmospheric CO₂ and low versus high nutrient availability. Soil C sequestration is a function of C input through plant growth and C output through mineralization. Nutrient availability has a limited effect on C mineralization rates under elevated CO₂. However, the stimulation of above- and below plant growth by elevated CO₂ is larger under high compared to low nutrient availability. Consequently, C input outweighs C mineralization under high nutrient availability, resulting in net C sequestration. On the contrary, when no additional nutrients are supplied the increase in C mineralization under elevated CO₂ counterbalances the increase in C input. Nitrogen fixers can supply additional N needed to sustain plant growth and support C sequestration under elevated CO₂, but only do so when other nutrients are added. Thus, additional nutrients are required for net C sequestration under elevated CO₂. Larger boxes represent larger pool sizes and thicker lines represent larger fluxes of C and other nutrients from one pool to the other.](image-url)
6. The Impact of Agronomic Selection on the Response of *Medicago truncatula* Accessions to Elevated Atmospheric Carbon Dioxide

**Abstract**

Increased atmospheric CO₂ frequently stimulates plant productivity, but considerable variation in responses to elevated CO₂ among crop plant genotypes has been observed. Firstly, this study under controlled environmental conditions tested the extent to which agronomic selection of *Medicago truncatula* Gaertner (medic) is associated with variation in root:shoot (R:S) ratios under ambient CO₂ and moderate N conditions. Comparing seedlings of 30 wild (i.e., unselected) and 30 cultivated (i.e., selected) medic accessions showed that R:S ratios in wild lines were higher (+15%, P<0.01) than those in cultivated genotypes, which was linked to a higher root biomass (+12%, P<0.01) and a slightly smaller shoot biomass (-4%) in the wild materials. Secondly, we hypothesized that agronomic selection in medic accessions facilitated larger growth responses to coordinate changes in both CO₂ and mineral nutrients than in wild lines. Indeed, tests with a subset of accessions defined by low versus high R:S ratios showed that elevated CO₂ increased both root (+20%, P<0.05) and shoot (+21%, P<0.05) biomass of the cultivated genotypes, but this occurred only when additional mineral nutrients were supplied. In contrast, elevated CO₂ had no effect on shoot growth of wild plants under either low or high N conditions, and root biomass of wild plants was increased (+27%, P<0.01) by elevated CO₂ only under low N conditions. Additional tests found that elevated CO₂ stimulated total net efflux of 12 amino acids (i.e., exudation, micromoles/g root fresh weight) only in the wild plants. These data support the concept that, when sufficiently fertilized, cultivated crop genotypes are more likely to increase production under elevated CO₂ than their wild progenitors. However, wild plants are more likely to increase belowground biomass and root exudation under elevated CO₂.

6.1 Introduction

Rising levels of atmospheric CO$_2$ are predicted to reach 700 ppm by the year 2050 (Houghton & Ding, 2001), but their possible effects on ecosystems are unclear. Elevated atmospheric CO$_2$ affects ecosystem functioning, by stimulating photosynthesis, and field studies show that the greater C uptake increases plant production by 20% on average (Ainsworth & Long, 2005; de Graaff et al., 2006). The stimulation of plant production should enhance soil C input, which in turn may increase soil C sequestration, thereby counterbalancing the rise in atmospheric CO$_2$ (Gifford, 1994). However, the extent to which increased plant growth under elevated CO$_2$ affects additional soil C storage has proven hard to predict (de Graaff et al., 2006), partly because of large variations in plant responses to elevated CO$_2$.

Plants' differential responses to elevated CO$_2$ have not been predictable from a knowledge of species' biology (Hungate et al., 1996; Paterson et al., 1995; Crookhanks et al., 1998; Zak et al., 2000). To predict plant species' responses to elevated CO$_2$, they have been classified into broad groups relying on a broad suite of related plant traits that may generalize how they respond to environmental changes (Eviner & Chapin, 2003). However, so far such classifications have provided limited understanding on how plant species mediate soil C sequestration under elevated CO$_2$ (Hungate et al., 1996, Zak et al., 2000). Using plants with genetic similarity, but contrasting C-allocation patterns may provide another approach for elucidating why plants vary in their response to elevated CO$_2$. Namely, genetic similarity reduces the number of plant traits that can influence a plants' response to climate change, and C allocation to roots is a key plant trait for explaining differential responses soil C sequestration as it affects both the magnitude of root derived soil C input, and nutrient uptake for sustained plant growth.

In this study we hypothesize that differences in the extent of selection among genotypes may be an important reason for variations in CO$_2$ responsiveness, as wild (i.e. unselected for agriculture) versus cultivated genotypes of crops are expected to have contrasting C allocation patterns. Rengel & Marschner (2005) argued that modern agriculture developed cultivars that function well in favorable soil environments but lack traits necessary for growth in low-resource environments. Indeed, several studies found that selection for increased yield under high-input agricultural systems produced cultivars with smaller root systems (Chapin et al., 1989; Siddique et al., 1990; Jackson, 1995), and that genetic improvement of yield has come mainly through selection for C partitioning to shoots (Gifford et al., 1984).

The amount of C that a plant allocates to its root versus shoot is one trait which relates to the habitat occupied by a species: generally, species from fertile sites have inherently low root:shoot (R:S) ratios, while species that develop in less favourable conditions have high R:S ratios (Chapin, 1980; Grime 1977, 1979; Grime et al., 1986). These intrinsic differences in R:S ratios can be linked to species-specific adaptation strategies to environmental change (Grime et al., 1997). Namely, differences in the
habitats in which wild versus cultivated genotypes have developed may have resulted in what Grime et al. (1997) defined as ‘acquisitive’ (competitive) and ‘retentive’ (stress-tolerant) genotypes with respect to growth rate and resource dynamics. As elevated CO\textsubscript{2} is expected to change both growth rates and resource availability (Luo et al., 2004), comparing selected genotypes of plants with their wild relatives (i.e. unselected for agriculture) offers another basis for predicting the response of plants to elevated CO\textsubscript{2}.

*Medicago truncatula* Gaertner (medic) has emerged in the last few years as a model legume species for fundamental studies of plant-microbe interactions and is increasingly being used to investigate developmental questions. Based on their origin and degree of selection, we postulate that different accessions of medic will have varying R:S ratios. Wild (i.e. unselected) lines should have root systems that can exploit more unpredictable and stressful soil environments than their cultivated (i.e. selected) relatives. Therefore, we hypothesize that wild medic accessions will have higher R:S ratios. In addition, we hypothesize that elevated CO\textsubscript{2} will stimulate aboveground biomass production of cultivated medic accessions more than that of their wild relatives due to their acquisitive nature. However, when nutrients are limiting, the impact of elevated CO\textsubscript{2} on biomass production of cultivated accessions will be limited as well.

Reynolds & D’Antonio (1996) argued that the ecological significance of measuring root versus shoot weights for determining the degree of a plants’ response to environmental changes is limited, and that both morphological and physiological plasticity should be considered. Root exudation is an important physiological process that can affect microbial regulation of soil N availability (Paterson et al., 2003). Soil N availability in-turn, is a key parameter that determines whether increased plant growth and concomitant soil C sequestration can be sustained under elevated CO\textsubscript{2} (Luo et al., 2004). A number of studies have shown that root derived soil C input increases proportionally with increasing root production under elevated CO\textsubscript{2} (Cotrufo & Gorissen, 1997; Billes et al., 1993). The ecological implications may be much greater, however, if elevated CO\textsubscript{2} would affect compound specific exudation per unit of root (Darrah, 1996). However, the latter has barely been measured due to the technological difficulties associated with measuring compound specific exudation in soil. Amino acids form an important constituent of root exudation and they are a key source of labile C and N for soil microbes (Janzen & Bruinsma, 1993). Thus, a difference in the response of specific net amino acid efflux per unit of root to elevated CO\textsubscript{2} between wild and cultivated genotypes may have significant ecological implications.

The goal of the series of experiments presented here was to determine the extent to which agronomic selection of medic is associated with differences in C allocation between roots and shoots, and how such differences relate to growth and root exudation responses to different levels of CO\textsubscript{2} and mineral nutrients. This approach may offer another basis for predicting how rising levels of atmospheric CO\textsubscript{2} may influence C sequestration in a plant species occupying both agronomic and natural ecosystems.
6.2 Materials and methods

Selection of *M. truncatula* accessions

The 180 *Medicago truncatula* Gaertner (medic) accessions suitable for these tests were generously supplied by Dr. D. Stout, USDA (Pullman, WA). Of that number, 143 were wild (unimproved) materials, and 37 were cultivated (improved) accessions. Equal numbers of wild and cultivated accessions (*i.e.* 30) were chosen from these materials. Criteria for choosing included geographical distribution and growth data (*i.e.* biomass, growth habit, winter vigor, flower days and seed production) obtained from the USDA national germplasm database. Firstly, to include accessions from all geographical regions, the accessions collected from similar geographical regions were grouped and similar numbers from within these groups were picked at random. To ensure comparable growth traits, the final selection of cultivated and wild accessions was adjusted to produce similar frequency distribution diagrams for each of the growth parameters.

Experimental designs and growing conditions

Three separate tests were conducted. The first test distinguished medic accessions with low versus high R:S ratios. The second tested whether agronomic selection in medic facilitated larger growth responses to changes in both CO\textsubscript{2} and mineral nutrients, and the third measured root exudation in wild and selected germplasms.

In each of these tests the plants were grown in controlled environment chambers using a photosynthetically active (400–700 nm) photon fluence rate of 500 $\mu$mol m$^{-2}$s$^{-1}$, a 12/12 hours (day/night) cycle, and 25$^\circ$C/20$^\circ$C (day/ night) temperature. CO\textsubscript{2} concentration, air temperature, light and vapour pressure deficit were monitored and controlled every minute. In Tests 1 and 2 plants were grown in containers (Stuewe & Sons, Inc., Corvallis, Oregon, USA) with a diameter of 2.5 cm and a depth of 16 cm. The growth medium was coarse vermiculite, which was enriched on a daily basis with the mineral nutrient solutions supplied from the bottom.

In Test 1, 60 accessions were grown in a controlled environment chamber under ambient levels (425 $\mu$mol mol$^{-1}$) of CO\textsubscript{2}. Plants were supplied daily with a 1/10th strength Hoagland solution supplied from the bottom (Hoagland & Arnon 1941). Because growth chamber space permitted only 168 conetainers, the experiment was conducted four times with eight replications of 19 different accessions and a uniform "reference plant", which was *M. truncatula* Gaertner, genotype A17, originally from the agronomic selection 'Jemalong'. A preliminary trial with A17 established that plant growth was uniform throughout the growth chamber. Since biomass production of this genotype was independent of its position in the chamber (data not shown), the plants were positioned in a complete randomized design in all of the experiments. After 2 weeks of growth the plants were harvested and their R:S ratios were determined. The 12 accessions representing the lines with the six lowest and the six highest R:S ratios
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within the wild and cultivated accessions were selected and used for further experimentation in Test 2 (Table 2). In addition, six accessions representing the lines with the three lowest and the three highest R:S ratios were selected and used for Test 3. Three wild and three cultivated accessions were represented within these plants (Table 2). Two additional trials with these accessions were conducted to test whether R:S ratios continued to differ significantly in the same direction over periods of 3 and 4 weeks.

In Test 2, plants were exposed continuously for 4 weeks to ambient (425 μmol mol⁻¹) or elevated (850 μmol mol⁻¹) atmospheric CO₂, and low (1/100th Hoagland) or high (1/4th Hoagland) mineral nutrient solution was supplied daily from the bottom. Eight replicate plants of each accession were arranged in a randomized complete block in each of two growth chambers. To avoid a possible chamber effect, CO₂ treatments along with the plants were rotated weekly between the chambers.

In Test 3, the plants were grown aseptically in hydroponic solutions for 3 weeks, under ambient (425 μmol mol⁻¹) or elevated (850 μmol mol⁻¹) atmospheric CO₂ levels. Seeds were sterilized as described previously (Phillips et al., 2004), including an antibiotic treatment. Thorough rinsing regimes were used to remove all antibiotic, and any seedlings lacking vigor, a trait associated with the continued presence of antibiotic, were discarded. Seeds were germinated for 2 days under ambient laboratory CO₂ conditions (425 mmol mol⁻¹) in the dark at 25°C on Petri plates containing 8g L⁻¹ phytagel (Sigma, St. Louis, MO, USA). Seedlings were then transferred to axenic 1-qt wide-mouth mason jars (Alltrista Consumer Products Co. http://www.homecanning.com, Product 67000) capped with a band (http://www.homecanning.com Product 40000) and sealed with a crossed-hatched layer of Micropore surgical tape (3M Corp., St. Paul, MN, USA catalogue 1530-1) for aseptic air exchange. Roots were suspended on a plastic screen in 200 mL of Fåhraeus solution containing 1.0mM KNO₃ (Fåhraeus, 1957). Four replicate mason jars, each containing four plants, were arranged in a randomized complete block in each of two growth chambers. To avoid a possible chamber effect, CO₂ treatments along with the plants were rotated weekly between the chambers. After 21 days of growth steady-state concentrations of 12 amino-acids were measured. Steady-state was established in the hydroponic solution within 48 h of replenishing nutrient solutions (i.e. day 19).

Sampling procedures

After Tests 1 and 2, the shoots and roots were harvested separately and dried at 60°C for 48 hours. Roots were carefully rinsed with water to remove the vermiculite. For consistency purposes, the aboveground portion of the plant was defined as the green part immediately above the petiole of the lowest leaf. Thus, the root portion of the plant included part of the stem below the petiole of the lowest leaf. After drying, the roots and shoots were weighed and the R:S ratios were calculated.

In Test 3, one assessment of root exudation was estimated by measuring the total steady-state concentration of 12 amino acids. To achieve a steady state at time of
sampling, mineral solutions for the plants were replenished 2 days before measuring amino-acid concentrations. Two 1-mL samples were collected from the solutions with 1.5-mL plastic pipette tips while minimizing root disturbance. At the conclusion of each experiment, sterility of hydroponic root solutions was assessed by testing for culturable microorganisms on TY agar (Beringer, 1974). Only samples showing no microbial growth were analyzed further. The amino acids were measured in root solutions by HPLC analysis of fluorescent derivatives using commercial products (Waters, Milford, MA) and techniques described previously (Phillips et al., 2004). The concentrations of amino acids were expressed on a root fresh weight basis. After amino acid sampling, the roots and shoots of the plants were harvested separately, and their fresh weight was determined. Subsequently, the roots and shoots were dried at 60°C for 48 hours. After drying the roots and shoots were weighed and the R:S ratios were calculated.

**Statistical analyses**

The procedure GLM in the SAS system for Windows™ V8 was conducted, with CO₂, mineral nutrients and genotypes as treatments. Means were compared by the Tukey test, if the analysis of variance was significant (P < 0.01, or P < 0.05).

### 6.3 Results

**Test 1: Variations in R:S ratios among 60 medic accessions**

Medic accessions grown under ambient CO₂ and moderate nutrient conditions, showed more than a three-fold variation in R:S ratios among accessions (Table 6.1). Comparing the 30 wild (i.e., unselected) with the 30 cultivated (i.e., selected) accessions, established that the mean R:S ratio in wild accessions was higher (+15%, P < 0.01) than that of cultivated accessions (Fig. 6.1a). This difference was linked to a higher root biomass (+12%, P < 0.01) and to a slightly smaller shoot biomass (-4%) in the wild materials (Fig. 6.1b).

Based on these results, six wild and six cultivated accessions were chosen for further tests (Table 6.2). The average R:S ratios of the 6 wild accessions were significantly larger than the average R:S ratios of the 6 cultivated accessions in this subset (+12%, P < 0.05). Furthermore, also based on the results obtained in Test 1, three wild and three cultivated accessions were selected to conduct Test 3 (Table 6.2). The average R:S ratios of the three wild accessions were significantly larger than the average R:S ratios of the three cultivated accessions in this subset (+34%, P < 0.05). Two additional trials with these accessions verified that R:S ratios continued to differ significantly in the same direction over periods of 3 and 4 weeks (data not shown).
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**Fig. 6.1** Root: Shoot ratios (a) and organ masses (b) of the 30 wild and 30 cultivated medic accessions studied in Test 1. Unshaded and shaded bars report cultivated and wild accessions, respectively. Values are means ± SEM (n=8). Significant differences (P ≤ 0.01) are indicated by *. 

**Fig. 6.2** Responses of the six wild and six cultivated medic accessions in Test 2. Mean shoot biomass under ambient and elevated atmospheric CO₂ with low nutrient (a) or high nutrient (b) fertilization. Mean root biomass under ambient and elevated atmospheric CO₂ with low nutrient (c) or high nutrient (d) fertilization. The numbers 425 and 850 on the x-axis represent the ambient and elevated CO₂ concentrations (µmol mol⁻¹) in the growth chambers, respectively. Shading conventions as in Fig. 6.1. Values are means ± SEM (n=8). Significant differences between CO₂ treatments (P ≤ 0.05) are indicated by *.
Test 2: Variations in R:S ratios in response to elevated CO\textsubscript{2} and low and high nutrient additions between wild and cultivated medic accessions

Further tests with the six wild and six cultivated accessions chosen from Test 1 (Table 6.2) established that when the accessions were compared under ambient versus elevated CO\textsubscript{2} and low versus high nutrient additions, elevated CO\textsubscript{2} significantly (P < 0.05) stimulated shoot biomass of the cultivated accessions, but only when ample nutrients were available (Fig. 6.2b).

In contrast, elevated CO\textsubscript{2} did not stimulate shoot biomass of the wild accessions under either low or high mineral nutrient treatments (Fig. 6.2a & b). Elevated CO\textsubscript{2} also significantly (P < 0.05) stimulated root biomass of the cultivated accessions under high mineral nutrients, but it did not stimulate root biomass of the wild plants (Fig. 6.2d). Root biomass of the wild accessions was only stimulated significantly (P < 0.05) under elevated CO\textsubscript{2} when nutrient additions were low (Fig. 6.2c). In addition, this was the only treatment in which the root biomass of the wild plants was higher than that of the cultivated plants (Fig. 6.2c). Thus, even though the root biomass of the wild plants was larger under elevated CO\textsubscript{2} than the root biomass of the cultivated plants, elevated CO\textsubscript{2} did not stimulate shoot biomass in the wild plants under low mineral nutrients (Fig. 6.2a).

**Fig. 6.3** The impact of elevated CO\textsubscript{2} on the total steady-state concentration of 12 amino acids in root hydroponic solutions. The numbers 425 and 850 on the x-axis represent the ambient and elevated CO\textsubscript{2} concentrations (\mu mol mol\textsuperscript{-1}) in the growth chambers, respectively. Shading conventions as in Fig. 6.1. Values are means ± SEM (n=6) of three wild and three cultivated medic accessions. Significant (P ≤ 0.05) CO\textsubscript{2} treatment effects are indicated by ∗.
**Test 3: Differences in "root exudation" responses to elevated CO₂ between wild and cultivated medic genotypes**

In Test 3, elevated CO₂ stimulated total net efflux of 12 amino acids (i.e., “exudation”, micromoles/g root fresh weight) only in the wild plants (Fig. 6.3). Elevated CO₂ equally affected efflux of each of the amino acids, thus the quality of exudation was not affected by elevated CO₂ (data not shown). In addition, similar to the results from test 2, elevated CO₂ stimulated both shoot and root growth in the cultivated plants, but not in the wild plants (data not shown).

**Table 6.1** R:S ratios of the 60 wild and cultivated medic accessions used in Test 1. Values are means ± SEM (n=8).

<table>
<thead>
<tr>
<th>Cultivated accessions</th>
<th>R:S ratio</th>
<th>Wild accessions</th>
<th>R:S ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>W6 6095 0.40 ± 0.02</td>
<td>PI 577631 0.46 ± 0.03</td>
<td>W6 6010 0.60 ± 0.07</td>
<td></td>
</tr>
<tr>
<td>PI 577629 0.40 ± 0.04</td>
<td>W6 5001 0.50 ± 0.03</td>
<td>PI 577628 0.60 ± 0.05</td>
<td></td>
</tr>
<tr>
<td>W6 6019 0.50 ± 0.09</td>
<td>PI 535543 0.60 ± 0.16</td>
<td>W6 6004 0.51 ± 0.04</td>
<td></td>
</tr>
<tr>
<td>W6 6009 0.52 ± 0.05</td>
<td>PI 577600 0.60 ± 0.07</td>
<td></td>
<td></td>
</tr>
<tr>
<td>W6 6020 0.53 ± 0.07</td>
<td>W6 5976 0.60 ± 0.03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PI 577609 0.55 ± 0.06</td>
<td>W6 4980 0.61 ± 0.06</td>
<td></td>
<td></td>
</tr>
<tr>
<td>W6 6015 0.57 ± 0.08</td>
<td>W6 6034 0.65 ± 0.09</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PI 469099 0.60 ± 0.04</td>
<td>PI 577603 0.69 ± 0.07</td>
<td></td>
<td></td>
</tr>
<tr>
<td>W6 6093 0.60 ± 0.09</td>
<td>PI 535545 0.70 ± 0.06</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PI 566888 0.62 ± 0.06</td>
<td>PI 384665 0.73 ± 0.20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>W6 6104 0.63 ± 0.07</td>
<td>PI 535544 0.73 ± 0.09</td>
<td></td>
<td></td>
</tr>
<tr>
<td>W6 6102 0.64 ± 0.05</td>
<td>W6 6108 0.74 ± 0.08</td>
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<td></td>
</tr>
<tr>
<td>W6 6016 0.68 ± 0.09</td>
<td>PI 577622 0.74 ± 0.07</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PI 577640 0.71 ± 0.04</td>
<td>PI 577645 0.75 ± 0.11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>W6 6014 0.72 ± 0.13</td>
<td>PI 566886 0.78 ± 0.18</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PI 577616 0.73 ± 0.13</td>
<td>W6 6119 0.78 ± 0.09</td>
<td></td>
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</tr>
<tr>
<td>PI 577610 0.74 ± 0.12</td>
<td>W6 5963 0.79 ± 0.11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PI 469100 0.79 ± 0.09</td>
<td>PI 384664 0.79 ± 0.10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>W6 6096 0.81 ± 0.14</td>
<td>W6 6110 0.80 ± 0.06</td>
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<tr>
<td>PI 577611 0.82 ± 0.07</td>
<td>PI 516946 0.82 ± 0.16</td>
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<tr>
<td>W6 6102 0.85 ± 0.06</td>
<td>PI 577637 0.83 ± 0.10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PI 469102 0.87 ± 0.07</td>
<td>PI 577627 0.85 ± 0.11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PI 517257 0.99 ± 0.04</td>
<td>PI 516935 0.85 ± 0.14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>W6 6099 1.02 ± 0.09</td>
<td>W6 6143 0.88 ± 0.06</td>
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</tr>
<tr>
<td>W6 6103 1.08 ± 0.13</td>
<td>PI 577638 0.93 ± 0.23</td>
<td></td>
<td></td>
</tr>
<tr>
<td>W6 6011 1.08 ± 0.05</td>
<td>PI 516945 1.07 ± 0.15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PI 577641 1.11 ± 0.15</td>
<td>PI 566889 1.17 ± 0.14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PI 577643 1.12 ± 0.11</td>
<td>PI 516940 1.28 ± 0.18</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 6.2 Overview of the medic accessions selected for Tests 2 and 3. R:S ratios are derived from test 1, values are means ± SEM (n=8)

<table>
<thead>
<tr>
<th>Accession number</th>
<th>Type</th>
<th>Origin</th>
<th>R:S ratio</th>
<th>Test 2</th>
<th>Test 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>W6 6095</td>
<td>cultivated</td>
<td>Australia</td>
<td>0.40 ± 0.04</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>PI 577629</td>
<td>cultivated</td>
<td>Australia</td>
<td>0.40 ± 0.05</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>PI 577631</td>
<td>wild</td>
<td>Italy</td>
<td>0.46 ± 0.06</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>W6 5001</td>
<td>cultivated</td>
<td>Australia</td>
<td>0.50 ± 0.06</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>W6 6010</td>
<td>wild</td>
<td>France</td>
<td>0.60 ± 0.08</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>PI 577628</td>
<td>wild</td>
<td>Spain</td>
<td>0.60 ± 0.08</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>PI 516945</td>
<td>wild</td>
<td>Morocco</td>
<td>1.07 ± 0.15</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>W6 6011</td>
<td>cultivated</td>
<td>Australia</td>
<td>1.08 ± 0.14</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>PI 577641</td>
<td>cultivated</td>
<td>Australia</td>
<td>1.11 ± 0.15</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>PI 577643</td>
<td>cultivated</td>
<td>Australia</td>
<td>1.12 ± 0.11</td>
<td>*</td>
<td>*</td>
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<tr>
<td>PI 566889</td>
<td>wild</td>
<td>Tunisia</td>
<td>1.17 ± 0.14</td>
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<td>*</td>
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<tr>
<td>PI 516940</td>
<td>wild</td>
<td>Morocco</td>
<td>1.28 ± 0.18</td>
<td>*</td>
<td>*</td>
</tr>
</tbody>
</table>

6.4 Discussion

Data from these analyses offer a more comprehensive basis for comparing root vs. shoot C allocation patterns than previous reports, and they show clearly that mean R:S ratios in wild lines were significantly higher than in cultivated lines (Fig. 6.1). Although these data were collected from young seedlings, further tests proved the R:S ratios for 12 accessions remained relatively constant for 2, 3 and 4 weeks, suggesting that the differences in R:S ratios were a result of different allocation patterns, rather than different developmental rates. This finding is in agreement with Minchin et al. (1994) and Farrar & Gunn (1998) who showed that the partitioning of dry matter in young plants in a constant environment is allometric. In addition, our data strongly suggest that the relative partitioning of assimilated C to roots and shoots has a genetic basis. This result is consistent with Hebert et al. (2001) who showed that partitioning of biomass between roots and shoots varied among eight maize genotypes.

Based on previous ecological theories (Chapin, 1980; Grime, 1977, 1979; Grime et al., 1986, 1991), we hypothesized that wild accessions of medic have higher R:S ratios than cultivated accessions, since agronomic selection has adapted the cultivated accessions to favorable high-input agricultural systems. The results generated in Test 1 corroborate this hypothesis and support the theory of Grime (1977), Chapin (1980), Bryant et al. (1983) and Tilman (1988) that most plants characteristic of low-resource environments share a common suite of traits, including high R:S ratios. This trait likely represents an "adaptive strategy" enabling plants to survive in stressful environments (Grime, 1977). On the contrary, in fertile, productive environments, the ability to allocate soil resources to shoot production is characteristic of competitive species (Grime, 1979; Tillman, 1988; Ryser & Notz, 1996). Thus the wild and cultivated accessions of medic demonstrate different life strategies, where wild lines are "stress
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tolerant” and cultivated lines are “competitive” with regard to growth rate and resource dynamics (Grime et al., 1977).

The hypothesis that elevated CO2 will stimulate aboveground biomass production of cultivated medic accessions more than that of their wild relatives due to their acquisitive nature, was fully supported by our results (Fig. 6.4). Our data suggest that genetic improvement in medic has resulted in higher plasticity in response to elevated CO2 with regard to the partitioning of C to organs associated with C assimilation. This resulted in higher growth rates under non-limiting conditions. This observation is compatible with the theory that plants from high-resource environments are generally more plastic with respect to growth rates and photosynthesis than plants from low-resource environments (Chapin, 1980; Stearns, 1989). This result presumably occurs because plasticity is advantageous only where resources are plentiful enough to offset the costs of adjusting morphology (Grime, 1977, 1979).

Even though elevated CO2 affected actual root and shoot growth, it did not significantly affect R:S ratios for both the wild and cultivated lines (data not shown). Differences in a plant’s capacity to modify R:S ratios needs to be considered, since for continued optimal performance under changing environmental conditions it is important for plants to adjust their relative C partitioning to roots and shoots in order to maximize their relative growth rate (Thornley, 1969, 1972; Bloom et al., 1985; van der Werf et al., 1993). Rogers et al. (1996) summarized the effects of elevated CO2 on R:S ratios and concluded that the results were highly variable. R:S ratios usually respond to deficits in resource availability (Gutschick, 1993; Cakmak et al., 1994), with the R:S response to a given factor usually diverting dry weight to the plant part that is the most limiting to growth under prevailing environmental conditions (Wilson, 1988). This is consistent with our results, and suggests that elevated CO2 may have an indirect impact on R:S ratios, namely though its impact on nutrient availability.

Elevated CO2 did not affect compound specific exudation for any of the genotypes, however it did stimulate net amino acid efflux per unit of root in the wild genotypes (Fig. 6.4). Stimulated efflux of root derived N compounds may lead to net N losses (de Graaff et al., 2007), but increased efflux of C and N compounds may also lead to enhanced microbial activity (Zak et al., 1993), thereby increasing soil N mineralization rates. The latter process could be of advantage to the wild genotypes for optimizing growth under elevated CO2 and N limiting conditions. However, net amino acid efflux is related to, but not necessarily identical with the more complex, ecologically important concepts of root exudation and rhizodeposition. The net efflux measured here in hydroponic solution reflects both influx and efflux components, but does not reflect the quantitatively larger exudation and rhizodeposition which would occur when soil particles and microorganism capture amino acids before they were recovered by the root (Phillips et al., 2004, 2006). This caveat has limited bearing on the underlying biological fact that medic accessions differ in their net amino acid efflux values and that those values were promoted by elevated CO2 only in the wild, unselected lines (Fig. 6.4).
6.5 Conclusions

This study shows that agronomic selection of medic has resulted in a morphological tradeoff, where C allocation to organs associated with C assimilation compared to organs associated with nutrient uptake is favored in modern cultivars. Ecological theories along with our data suggest that this makes modern cultivars better competitors than wild lines when resource availability is ample. This makes modern cultivars more likely to increase biomass production. However, the stimulated root growth and C efflux in the wild lines under elevated CO₂ might make wild genotypes more likely than cultivated lines to increase soil C sequestration in low N environments under elevated CO₂.
7. Elevated CO₂ increases N rhizodeposition and microbial immobilization of root-derived N.

Abstract

We aimed at determining how elevated CO₂ affects rhizodeposition and the cycling of rhizodeposited N under C₃ and C₄ plants. In addition, we tested how cultivated genotypes of *Triticum turgidum* and *Zea mays* versus their wild relatives responded to elevated CO₂. By constructing an N transfer experiment we could directly assess cycling of the rhizodeposited N and trace the fate of rhizodeposited N in the soil and into receiver plants. Biomass production, rhizodeposition and cycling of root-borne N of maize genotypes were not affected by elevated CO₂. Elevated CO₂ stimulated above- and belowground biomass production of the wheat genotypes on average by 38%, and increased rhizodeposition and immobilization of root derived N on average by 30%. Concurrently, elevated CO₂ reduced mineral ¹⁵N and re-uptake of the root derived N by 50% for wheat. This study shows that elevated CO₂ may enhance N limitation by increasing N rhizodeposition and subsequent immobilization of the root derived N.

7.1 Introduction

During the past two centuries, the atmospheric CO₂ concentration has increased by 31%, and it is expected to continue rising rapidly (Houghton & Ding, 2001). Elevated atmospheric CO₂ directly affects ecosystems by increasing C assimilation in plants. Field studies showed that the greater C uptake results in increased plant production of 20% on average (Ainsworth & Long, 2005; de Graaff et al., 2006). In addition, plant N concentrations are reduced under elevated CO₂ by approximately 7% (Norby et al., 2001). Both the increase in plant growth and decrease in plant N contents change the quantity and quality of plant materials entering the soil. Such CO₂-induced changes in soil organic matter input affect soil microbial activity, thereby potentially altering the soil N cycle (Diaz et al., 1993; Zak et al., 1993). However, no clear pattern in the response of soil N cycling to elevated CO₂ has emerged (Norby & Cotrufo, 1998; Zak et al., 2000). Consequently, predicting how elevated CO₂ will affect soil N cycling throughout various ecosystems remains a challenge.

This challenge is in part due to the problem of understanding soil processes (Rogers et al., 2006). Many studies evaluating the impact of elevated CO₂ on N dynamics have been carried out with soil collected from long-term field experiments and have aimed at determining the impact of changes in litter input and chemistry on soil N cycling in the absence of plants (de Graaff et al., 2006). Even though such experiments are important to evaluate the impact of elevated atmospheric CO₂ on soil N cycling, they do not take into account the living root system, which is essential for mechanistically linking plant production and soil N cycling. A large portion of soil organic matter enters the soil through roots (Lee & Pankhurst, 1992). These root-derived substrates are an important source of labile C and N which is more easily accessible to soil microbes than shoot-derived substrates (Janzen & Bruinsma, 1993; Jensen, 1996; Mayer et al., 2003). Root-derived substrates enter the soil through litter from dead roots, and through living roots transferring C and N to the soil through rhizodeposition, which includes sloughing of living cells and exudation (Lynch & Whipps, 1990, Darrah, 1996). Thus, rhizodeposition is a central process for the coupling of plant production to soil nutrient cycling (Paterson et al., 2003).

The extent to which rhizodeposition affects N cycling under elevated CO₂ depends on species’ specific plant responses to elevated CO₂, which are difficult to predict. To predict the effect of elevated CO₂, plant species have been classified within broad groups. The classifications rely on a broad suite of related plant traits that can generalize how species respond to environmental changes (Eviner & Chapin, 2003). Studies have been conducted using fast and slow growing plant species from different life forms (Paterson et al., 1995; Crookhanks et al., 1998). However, variability within a plant life form was equivalent to variability between plant life forms for most variables, indicating that these groupings provide limited understanding on how soil N cycling will change under elevated CO₂ (Zak et al., 2000). Alternatively, it has been postulated that plants with common functional traits respond similarly to elevated CO₂ (Ainsworth
& Long, 2005). However, in this classification individual plant species within a functional group usually differ in their responses to elevated CO\textsubscript{2} (Hooper & Vitousek, 1997; Tilman \textit{et al.}, 1997; Nowak \textit{et al.}, 2003). Also genotypic variation in response to elevated CO\textsubscript{2} has been reported. For example, Lukac \textit{et al.} (2003) found differences between three \textit{Populus} genotypes in response to elevated CO\textsubscript{2} with regard to root turnover rates. However, like the other classifications, the cause for variation between genotypes in response to elevated CO\textsubscript{2} remains unknown.

Difference in life history between genotypes may be an important reason for variations in CO\textsubscript{2} responsiveness. Modern agriculture has resulted in the development of cultivars that are lacking traits necessary for growth in unpredictable and stressful soil environments (Rengel & Marschner, 2005). As such, selection for high yields under high-input agricultural systems has resulted in cultivars with smaller root systems (Chapin \textit{et al.}, 1989; Siddique \textit{et al.}, 1990; Jackson, 1995) that can differ in their response to nutrient availability (Rengel & Marschner 2005). Thus, differences in life history between selected genotypes and their progenitors may have resulted in what Grime \textit{et al.} (1997) defined as ‘acquisitive’ (competitive) and ‘retentive’ (stress-tolerant) genotypes with respect to growth rate and resource dynamics. As elevated CO\textsubscript{2} is expected to change both growth rates and resource availability (Luo \textit{et al.}, 2004), comparing selected genotypes of plants with their wild progenitors (\textit{i.e.} un-selected for agriculture) allows for another basis to predict the response of plants to elevated CO\textsubscript{2}.

With this study we aimed at testing two hypotheses. Firstly, we expected elevated CO\textsubscript{2} to stimulate rhizodeposition and the incorporation of root-derived materials into the microbial biomass, thereby increasing competition between plants and microbes for rhizodeposited N. Secondly, we hypothesized that the difference in life history between wild and cultivated genotypes is an important reason for variations in CO\textsubscript{2} responsiveness. Therefore, we conducted an N transfer experiment, in which we measured rhizodeposition of \textsuperscript{15}N and cycling of the rhizodeposits between plants and microbes. In addition, we aimed at determining how inter- and intraspecific variation between \textit{C}\textsubscript{3} and \textit{C}\textsubscript{4} plants controlled N cycling of rhizodeposits under elevated CO\textsubscript{2}.
7.2 Materials and Methods

**Plant species and genotypes**

Two wild and one cultivated genotype of both the wheat and the maize species were selected for the experiment. The genotypes were classified as follows:

- *Triticum turgidum, subsp. durum* – *Durelle* (PI 584837) cultivated material, developed in France.
- *Triticum turgidum subsp. dicoccoides* (PI 428031), wild material, collected in Diyarbakir, Turkey (1978).
- *Zea mays subs. mays*, cultivated material, developed in the USA.
- *Zea mays subs. luxurians* (PI 441933), wild material, collected in Jutiapa, Guatemala (1975).
- *Zea mays subsp. parviglumis* (PI 566687), wild material, collected in Mexico, Mexico (1993).

**Experimental design and growing conditions**

Plants were exposed to 425 µmol mol⁻¹ and 850 µmol mol⁻¹ atmospheric CO₂ concentrations in two controlled environment chambers for 4 weeks (six replicates per treatment). The chambers were located in the Controlled Environment Facility at UC-Davis. The chamber conditions used a photosynthetically active (400–700 nm) photon fluence rate of 500 µmol m⁻²s⁻¹, a 12/12 hours (day/night) cycle, and 25°C/20°C. Light was monitored with a Mannix digital light meter on a daily basis to ensure equal distribution throughout the chamber and to ensure equal intensity between the growth chambers. Other climatic factors (CO₂, air, temperature, vapour pressure deficit) were monitored and controlled every minute. To avoid a possible chamber effect, CO₂ treatments along with the plants were rotated weekly between the chambers.

The soil used for the experiment was classified as Yolo series silty loam. Prior to the experiment the soil was air dried and sieved to 2 mm. The pots used were 0.25 L containers (12 cm height and 9 cm diameter at top) and contained 400 g (dry weight) of soil. Water-holding capacity (WHC) was determined by calculating the difference in weight of soils at saturation point and oven-dry weight (100°C). Soils were re-wetted to 60% of WHC prior to seeding and on a daily basis during the experiment.

Experimental sub-treatments were C₃ versus C₄ plant species. Sub-sub treatments were the different genotypes within the plant species. Seeds of the plants were germinated for 5 days in plastic Petri dishes with water agar as the growth medium. Wheat seeds were vernalized at 6°C prior to germination in Petri dishes. Subsequently, two plants of the same genotype were transferred to the containers. Two holes of 3 cm deep were made in the soil to enable planting the seedlings into the soil. The distance
between the seedlings was 4 cm and the distances from the sides of the container for each of the holes were 2.5 cm.

**15N-labeling**

Foliar feeding with 15N-KNO₃ was conducted once during the experiment to determine rhizodeposition of N. Rhizodeposition was defined as the percentage of 15N recovered as total soil N. After 2 weeks of growth, the tips (2 mm) of two leaves were cut off and subsequently the remainders of the leaves were immersed into the labeling solution (3 ml, 20 mM KNO₃, 99 atom % excess ¹⁵N) in two, 2 ml centrifuge vials (Fisher Scientific). Silwet L-77 (0.05%) was added to the solution to facilitate N-uptake. The vials were sealed using Teflon tape. After 24 hours, leaves were removed from the solution, since most of the plants had absorbed the solution. If any solution remained in the vial, the residual ¹⁵N content in the solution was determined by pipetting 100 μl on a 0.5 mm² glass microfibre filter (Whatman GF/C) inserted in a tin cup, in order to precisely quantify the amount of ¹⁵N absorbed by the leaves. The residual ¹⁵N-contents and its isotopic composition were determined by an automated N/C analyzer-isotope ratio mass spectrometer (ANCA-IRMS, Europa Scientific Integra, UK) at the UC-Davis Stable Isotope Facility.

Growing two plants of a single species and genotype simultaneously in one container allowed for determining the impact of elevated CO₂ on cycling of the root-derived N between donor plants (plants receiving the ¹⁵N labeled solution), microbes, and receiver plants (unlabeled plants). To avoid aboveground ¹⁵N contamination care was taken that the leaves of the donor did not touch the leaves of the receiver plant. In addition to the donor and receiver plants, unlabeled control plants were grown to determine the background ¹⁵N levels of the genotypes.

**Plant sampling**

After 4 weeks of growth, both the donor and receiver plants were harvested. To avoid any ¹⁵N contamination, the aboveground biomass donor and receiver plants were harvested and stored separately. The shoots were dried at 70°C for three days and ground in a ball mill for determination of total C and N and ¹⁵N isotopic composition.

The containers with the remaining soil were stored at 4°C for one night prior to harvesting the roots. While harvesting the roots, the roots of the donor and receiver plants were separated as much as possible. The roots that could not be separated were classified as mixed roots and handled separately. These mixed roots were not discarded, rather their dry weight allowed for precisely calculating the total root production per pot. After washing the roots with water, the roots were dried at 70°C for 3 days, ground in a ball mill and their C and N and ¹⁵N isotopic composition determined.
**Soil sampling**  
The soil was mixed thoroughly after which a sub sample of 90 g was collected. Since the roots had occupied the entire volume in the containers, all the soil was considered rhizosphere soil. All roots were removed from the soil by hand picking, these roots were also classified as mixed roots and were use in the calculation of total root biomass per pot. Thirty g of the soil was dried at 40°C for 3 days and ground in a ball mill and its total C and N and 15N isotopic composition was measured.

A second sub sample (60 g) was used to determine soil microbial biomass N by the fumigation-incubation method (Jenkinson & Powlson, 1976). Thirty g were stored at 4°C for 48 h, while the other 30 g were exposed to chloroform fumigation for 48 h. Subsequently, both the fumigated and non-fumigated soils were incubated for 10 days. Samples were transferred to 120 ml specimen containers and placed in ¼ l Mason jars. Water was added to the samples to obtain 60% of WHC. In addition, 5 ml of water were added to the bottom of the jar to slow soil drying. A septum in the lid allowed gas samples to be removed with a 12 ml syringe. CO2-samples were collected in 10 ml vacutainers (Labco Unlimited, Buckinghamshire, UK). CO2 concentration was determined with an Infra Red Gas Analyzer after 10 days. Both the unfumigated and fumigated samples were extracted instantly. Each sample was mixed with 120 ml of 2 M KCl and shaken for 45 min prior to filtration (Whatman #1). The extracts were analyzed calorimetrically for NH4+ and NO3- content (Forster, 1995). Microbial biomass N was considered to be the total inorganic N flush in the fumigated samples compared to the non-fumigated samples. The 15N determination for both mineral 15N and microbial biomass 15N was performed by diffusing N from the extracts onto acidified disks sealed in Teflon tape (Stark & Hart, 1996). The disks were packed in tin capsules and analyzed for isotopic composition.

Total C, N and the isotopic composition of both soil and plant material was analyzed by an automated N/C analyser-isotope ratio mass spectrometer (ANCA-IRMS, Europa Scientific Integra, UK) at the UC Davis stable Isotope facility.

**15N recovery calculations**  
The percent 15N recovery was calculated as follows:

\[
\text{Percentage recovery} = \left(\frac{15N_{\text{final}}}{15N_{\text{uptake}}}\right) \times 100
\]  

where $15N_{\text{final}}$ is the percentage of 15N recovered in one of the N pools (i.e. total soil N, soil microbial N, soil mineral N, plant shoots and roots) and $15N_{\text{uptake}}$ is the total amount of 15N taken up by the donor plants.
Root-derived N cycling under elevated CO₂

Statistical analyses
The procedure GLM in the SAS system for Windows™ V8 was conducted, with CO₂, species and genotypes as treatments. Means were compared by the Tukey test, if the analysis of variance was significant. The level of significance was P < 0.05.

7.3 Results

Plant biomass production
Elevated CO₂ significantly increased aboveground biomass production of each of the wheat genotypes (Fig 7.1a, Table 7.1). Aboveground biomass production was significantly higher for the cultivated- than for the wild wheat genotypes under both ambient and elevated CO₂ (Fig. 1a, Table 1). Elevated CO₂ significantly stimulated aboveground biomass production of the cultivated wheat genotype by 30 % and that of the two wild genotypes 42% and 30% (P ≤0.01). Elevated CO₂ had no effect on aboveground biomass production of the maize genotypes (Fig 7.1a, Table 7.1).

Root biomass production of the cultivated wheat genotype increased by 32%, whereas it increased by 47% for both of the wild wheat genotypes under elevated CO₂ (Fig. 7.1b, Table 7.1). Elevated CO₂ stimulated root biomass production of the wild wheat genotypes (+1.5) (Elevated CO₂-Ambient CO₂)/ambient CO₂), significantly more than the root production of the cultivated wheat genotypes (+0.83) (P ≤ 0.05). The production of root biomass for the maize genotypes was not significantly affected by elevated CO₂ (Fig. 7.1b, Table 7.1).
Fig. 7.1 (a) Aboveground biomass production of the cultivated and wild wheat and maize genotypes grown under ambient and elevated CO2. (b) Belowground biomass production of the cultivated and wild wheat and maize genotypes grown under ambient and elevated CO2. Values are means with SEM (n=5) indicated by the error bars. Shaded bars represent ambient CO2, open bars represent elevated CO2.

**15N-recovery**

Total 15N recovery, defined as 15N recovered in the donor and receiver shoots and roots plus the recovery of 15N as total soil 15N, ranged from 50% to 72% and was not significantly affected by CO2 concentrations, species or genotypes (data not shown). Total 15N recovery in the maize and wheat donor plants ranged from 39% to 60% and was also not significantly different between species, genotypes and CO2 concentrations (data not shown).

The percentage of 15N recovered as total soil 15N ranged from 6 to 10% (Fig. 7.2a, Table 7.1). The percentage of 15N recovery in the ambient soils was on average 6%, irrespective of the plant species or genotypes grown in the soil (Fig. 7.2a, Table 7.1). In contrast, elevated CO2 increased the amount of 15N recovered in the soil of the wheat genotypes: total soil 15N recovery increased by 33% for the cultivated wheat genotype.
and by 27% and 25% for the two wild wheat genotypes. The difference in the amount of $^{15}$N recovered in the soil between wild and cultivated genotypes was not significant. Recovery of total soil $^{15}$N when maize genotypes were grown was not affected by elevated CO$_2$ (Fig. 7.2a, Table 7.1).

![Graphs showing N recovery in different soil pools and in the receiver plants.](image)

**Fig. 7.2** Percentages N recovery in different soil pools and in the receiver plants. Values are means with SEM (n=5) indicated by the error bars. Percentage $^{15}$N recovery as (a) total soil N, (b) soil mineral N ($^{15}$NO$_3$); and (c) soil microbial N under the cultivated and wild wheat and maize genotypes grown under ambient and elevated CO$_2$. (d) Percentage $^{15}$N recovery in the receiver plants of the cultivated and wild wheat and maize genotypes grown under ambient and elevated CO$_2$. Shaded bars represent ambient CO$_2$, open bars represent elevated CO$_2$.

Elevated CO$_2$ significantly decreased soil mineral N concentrations for one of the wild wheat genotypes, whilst the soil mineral N concentrations under the other two wheat genotypes were reduced, though not significantly, under elevated CO$_2$ (Fig. 7.2b, Table 7.1). The decrease in the percentage of $^{15}$N recovered in the mineral N pool under elevated CO$_2$ was 58% when cultivated wheat genotypes were grown and 60% and 48% for the two wild wheat genotypes (Fig. 7.2b, Table 7.1). In contrast, the $^{15}$N-mineral N content in the soil containing maize plants remained unaffected by elevated CO$_2$ (Fig. 7.2b, Table 7.1).

The percentage recovery of microbial biomass $^{15}$N significantly increased under elevated CO$_2$ for the wheat genotypes (Fig. 7.2c, Table 7.1). Elevated CO$_2$ stimulated the
microbial biomass $^{15}$N recovery in the soil with cultivated wheat genotypes by 43%, while the $^{15}$N recovery increased by 22% and 24% in the soils containing the two wild wheat genotypes. These differences in recoveries were however not significant. No differences in microbial $^{15}$N contents were found between any treatments containing maize genotypes (Fig. 7.2c, Table 7.1).

Table 7.1 Analysis of variation associated with the means and SEM-values represented in graphs 7.1a,b and 7.2a-d.

<table>
<thead>
<tr>
<th>Source of variation ANOVA</th>
<th>Biomass production</th>
<th>Percentage $^{15}$N recovery</th>
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<tbody>
<tr>
<td></td>
<td>Shoots</td>
<td>Roots</td>
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<tr>
<td>wheat</td>
<td></td>
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<tr>
<td>CO$_2$</td>
<td>P≤0.01</td>
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<tr>
<td>Genotypes</td>
<td>P≤0.05</td>
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<tr>
<td>CO$_2$ * Genotypes</td>
<td>ns</td>
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<tr>
<td>maize</td>
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<td>CO$_2$</td>
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<td>CO$_2$ * Genotypes</td>
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<tr>
<td>Species</td>
<td>P≤0.01</td>
<td>P≤0.01</td>
</tr>
<tr>
<td>CO$_2$ * Species</td>
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</table>

The percentage of $^{15}$N recovery in the receiver wheat genotypes significantly decreased under elevated CO$_2$, while elevated CO$_2$ had no effect on the $^{15}$N recovery in the receiver maize genotypes (Fig. 7.2d, Table 7.1). The decrease in $^{15}$N recovery in the donor wheat plant under elevated CO$_2$ amounted up to 40% for the cultivated genotype, while the decrease in $^{15}$N recovery in the two wild wheat genotypes was on average 54%. These recoveries were not significantly different between the wild and cultivated genotypes.

7.4 Discussion

**Rhizodeposition under elevated CO$_2$.**

Foliar application of $^{15}$N-KNO$_3$-solution is a suitable method for estimating rhizodeposition of N and its cycling through various pools (Merbach et al., 2000; Hertenberger & Wanek, 2004). The belowground $^{15}$N recovery ranged from 6 to 10% of the total uptake of $^{15}$N by the plant. These recovery values corroborate results of other $^{15}$N tracer studies estimating rhizodeposition (McNeill et al., 1997). The total amount of $^{15}$N not recovered in any of the N pools was on average 30% and was similar for both species and all genotypes (Table 7.2). Rocco & Mengel (2000) transplanted $^{15}$N-labeled seedlings from a solution to a soil and found that the N unaccounted for was mainly lost through volatilization. In their study, losses reached up to a third of the amount of root
derived $^{15}$N. We expect that in our study volatilization and denitrification during the growing period also led to $^{15}$N losses. The amount recovered belowground, however, was sufficient to trace root derived $^{15}$N in various soil pools and into the receiver plant.

The percentage of total $^{15}$N recovery in the soil was equal for the maize and wheat genotypes under ambient CO$_2$. However, elevated CO$_2$ significantly increased total soil $^{15}$N recovery for the wheat genotypes. This increase in N rhizodeposition was accompanied by a stimulation of root biomass of the wheat genotypes, which did not occur in the maize genotypes. These results confirm the generalization that in contrast to C$_3$ crops, C$_4$ crops do not respond to elevated CO$_2$ with regard to primary production (Ainsworth & Long, 2005) and rhizodeposition (Whipps, 1985).

Even though it has been established that elevated CO$_2$ frequently increases the amount of labile substrates entering the soil through the root system, it is still debated whether elevated CO$_2$ increases input of root-derived materials by stimulating root activity (i.e. stimulating the quantity of rhizodeposition per gram of root), or whether the increase in rhizodeposition is caused by a proportional increase in root growth (Darrah, 1996). When we calculated the inputs of root-derived N per unit fine root biomass in the wheat plants, we found that elevated CO$_2$ did not increase the quantity of rhizodeposits per gram root (data not shown). Instead, the increased input of root-derived N was a result of the stimulated root production. These results corroborate results from both field- (Matamala & Schelsinger, 2000) and laboratory studies (Phillips et al., 2006) and have significant ecological implications. Namely, if root activity was altered by elevated CO$_2$, microbial activity would change irrespective of a change in root production, and increased root production would only amplify this effect on ecosystems. However, when enhanced rhizodeposition follows only from increased root production, rhizosphere processes may not directly be changed, and the impact of elevated CO$_2$ on ecosystem processes may be less profound (Cardon, 1996).

**Cycling of rhizodeposited N under elevated CO$_2$.**

Because of a lack of both a growth and rhizodeposition response to elevated CO$_2$ by maize, an effect of elevated CO$_2$ on soil N cycling processes was not expected. Indeed, the percentages of $^{15}$N recovered in the mineral N pool and microbial biomass were similar under ambient and elevated CO$_2$. Moreover, similar percentages of root derived $^{15}$N were transferred from the donor to the receiver maize genotypes under ambient and elevated CO$_2$. On the contrary, elevated CO$_2$ changed the fate of root derived N released from the wheat genotypes and a greater percentage of the rhizodeposited $^{15}$N was incorporated into the microbial biomass under elevated compared to ambient CO$_2$. In addition, a significantly smaller proportion of the root derived $^{15}$N was recovered in the receiver plants. These results are consistent with the findings of a lower $^{15}$N recovery in the mineral soil N pool when plants were exposed to elevated compared to ambient CO$_2$. These data are in agreement with our hypothesis that elevated CO$_2$ stimulates rhizodeposition and the incorporation of root-derived materials into the microbial biomass, thereby increasing competition between plants and microbes for
rhizodeposited N, but only for C3 plants. Increased incorporation of soil N into the microbial biomass and a concomitant decrease in plant N availability under elevated CO2 has been observed previously (Berntson & Bazzaz, 1997; Williams et al., 2001; Barnard et al., 2004). It is understood that elevated CO2 stimulates the ability of microbes to compete for soil mineral N by an increased supply of labile organic C compounds from plants grown under elevated CO2 (Norby et al., 1987; van Veen et al., 1991; Pregitzer et al., 1995).

There have been some studies evaluating the potential importance of N loss through rhizodeposition in agro-ecosystems. Janzen (1990) showed that the loss of N through rhizodeposition is substantial, since it accounts for 18% or more of the plant assimilated N under nutrient limiting conditions, and these losses are expected to increase as plants mature (Rroco & Mengel, 2000). In addition, rhizodeposited N is important for plant N acquisition under nutrient limiting conditions (Moyer-Henry et al., 2006). The impact of elevated CO2 on the importance of plant N loss through roots and subsequent microbial immobilization for agro-ecosystem functioning, however, has not yet been evaluated. The effects of elevated CO2 on agro-ecosystem functioning could be significant if N rhizodeposition is substantially increased by elevated CO2, and if the lost N would serve as an important pathway for N acquisition. In our study the N loss by wheat genotypes accounted on average for 12% of the plant assimilated N under ambient CO2, whereas the N loss from the wheat genotypes accounted for a significantly (P≤0.01) larger proportion of the plant assimilated N under elevated CO2 (+23%). In addition, the availability of the lost N was significantly smaller due to increased plant-microbial competition under elevated CO2. Such losses of plant available N are likely not important when sufficient N is available. However, when the lost N serves as an important source of N in nutrient limiting systems the impact of elevated CO2 on yield may be more profound.

**Variation in genotypic responses to elevated CO2**

There may be considerable variation in responsiveness to elevated CO2 among individual genotypes of plant species (Lucak et al., 2003). However, we are not aware of any studies which determined the effect of elevated CO2 on N rhizodeposition and N cycling at the genotypic level rather than at the species level. If elevated CO2 decreases soil N availability to plants (van Groenigen et al., 2006, de Graaff et al., 2006), a logical approach for grouping genotypes would be to distinguish between ‘acquisitive’ (competitive) and ‘retentive’ (stress-tolerant) groups of genotypes with respect to growth rate and resource dynamics (Grime et al., 1997). Based on the observations that original plant species are adapted to and function optimally in soils with low levels of available N (Tilman et al., 1987), and that wild progenitors of crop plants tend to have root systems that can exploit more unpredictable and stressful soil environments than their cultivated relatives (Chapin et al., 1989; Jackson, 1995), we hypothesized that the response of a genotype to elevated CO2 is largely determined by its level of domestication.
The response ratio of roots defined as (Root biomass under elevated CO2 – Root biomass under ambient CO2)/Root biomass under ambient CO2) of the wild wheat genotypes exposed to elevated CO2 was on average 1.5 times higher than the root response ratio of cultivated wheat. However, rhizodeposition, or the cycling of rhizodeposits was not different for the maize and the wheat genotypes under both ambient and elevated CO2. These data suggest that the impact of plant selection and breeding appears to have limited effect on genotypic responses to elevated CO2 with regard to N-rhizodeposition. The study therefore fails to accept our hypothesis that the level of domestication significantly influences the response of crop species to elevated CO2.

7.5 Conclusions
Elevated CO2 stimulated shoot and root growth and increased rhizodeposition by 30% in C3 species. No effect of elevated CO2 on shoot, root growth and rhizodeposition was found for maize, a C4 species. For wheat, the increase in plant production and rhizodeposition under elevated CO2 increased competition between plants and microbes for rhizodeposited N. As a result immobilization of root derived N increased on average by 30% under elevated CO2. Concurrently, there was, on average, 50 % less 15N available in the mineral N pool. Consequently, elevated CO2 reduced re-uptake of the root derived N. Since there were no significant genotypic differences in rhizodeposition and N cycling when exposed to elevated CO2, the impact of plant selection and breeding appears to have no effect on genotypic responses to elevated CO2 with regard to N-rhizodeposition. Results showed that elevated CO2 may enhance N limitation by increasing N rhizodeposition in C3 species and subsequent immobilization of the root derived N by microbes.
Abstract

We aimed at determining how CO$_2$-induced increases in rhizodeposition affects microbial N release from recalcitrant SOM, and how contrasting C allocation patterns in wild versus cultivated genotypes of wheat control plant mediated, differential responses in soil N cycling under elevated CO$_2$. To quantify root-derived soil C input plants were exposed to continuous labeling with $^{13}$C under ambient and elevated CO$_2$. To quantify release of N from stable SOM pools, the plants were grown in soil containing $^{15}$N predominantly present in recalcitrant SOM pools. Root-derived soil C input increased by 53% in the active soil C pool, and microbial-$^{13}$C was enhanced by 35% under elevated CO$_2$. Concurrently, plant $^{15}$N-uptake increased under elevated CO$_2$ (+ 24%), while $^{15}$N-contents in the microbial biomass and mineral N pool decreased. Wild genotypes allocated more C to their roots, while cultivated genotypes allocated more C to their shoots under both ambient and elevated CO$_2$. This led to increased N acquisition for the wild, and increased plant production for the cultivated genotypes under elevated CO$_2$. The data suggests that increased rhizodeposition under elevated CO$_2$ stimulates mineralization of N in recalcitrant SOM pools. In addition, contrasting C allocation patterns can explain plant mediated differential responses in soil N cycling to elevated CO$_2$.

8.1 Introduction
The rise in atmospheric CO₂ concentrations stimulates photosynthesis in most plants, leading to an increase in plant production by approximately 20% (Ainsworth & Long, 2005; de Graaff et al., 2006). The stimulation of plant production should enhance soil C input, which in turn may increase soil C sequestration, thereby counterbalancing the rise in atmospheric CO₂ (Gifford, 1994). However, the extent to which elevated CO₂ stimulates soil C storage has proven hard to predict, since it is unclear whether the plant growth response to elevated CO₂ can be sustained in the long-term (de Graaff et al., 2006; Reich et al., 2006; van Groenigen et al., 2006).

Due to increased growth rates under elevated CO₂, plant N demands increase (Luo et al., 2006). In addition, due to greater soil C inputs associated with enhanced plant production, soil microbial N demands increase (de Graaff et al., 2006, 2007). Consequently more N is accumulated in both plant tissues and soil pools under elevated CO₂, which may result in progressive N limitation (PNL) (Luo and et al., 2006). The PNL concept posits that in unfertilized ecosystems N availability progressively decreases under elevated CO₂, since N retention in soil and vegetation is stimulated. This ultimately leads to a decline in plant growth and a concomitant decrease in soil C sequestration (Luo et al., 2004; de Graaff et al., 2006).

In a synthesis of results on plant growth and soil nutrient cycling under elevated CO₂ in long-term field experiments, we showed that under low N availability elevated CO₂ still stimulated plant production by ~10%, even though the data suggested that PNL had developed in these ecosystems (de Graaff et al., 2006). In addition, plant production and soil C contents continue to increase under elevated CO₂ in the Duke FACE experiment, despite there being no evidence of increased net N mineralization, or N uptake rates (Johnson, 2006; Finzi et al., 2006, Pritchard, personal communication). Apparently, PNL can be alleviated by processes that still have to be measured. Hungate and Chapin (1995) postulated that if mineral nutrients are scarce in soils, microbes utilize rhizodeposits as a carbon-source, but break down more SOM in order to mine for nutrients. More nutrients are then moved into the active N pool in soil where, eventually, they may be made available to plants (e.g. "priming").

Prim ing is defined as the stimulation of soil organic matter (SOM) decomposition caused by the addition of labile substrates (Jenkinson et al., 1984; Dalenberg & Jager, 1989). Since elevated CO₂ frequently stimulates rhizodeposition (Cotrufo & Gorissen, 1997; Billes et al., 1993) and increases microbial decomposition of SOM (de Graaff et al., 2006; Carney et al., 2007), priming of more recalcitrant SOM may be responsible for alleviating PNL under elevated CO₂ in low N environments. So far, increased root derived soil C input under elevated CO₂ and increased rates of N mineralization due to decomposition of recalcitrant SOM have never been directly linked.

Plant species differ in mediating changes in soil N cycling in response to global change, but these differences have not been predictable from a knowledge of species'
Rhizodeposition-induced increases in N mineralization under elevated CO₂

biology (Hungate et al., 1996). To predict plant species’ impacts on soil nutrient cycling under elevated CO₂, they have been classified into broad groups relying on a broad suite of related plant traits that may generalize how they respond to environmental changes (Eviner & Chapin, 2003). However, such classifications have yet provided limited understanding on how plant species affect plant and microbial N acquisition under elevated CO₂ (Hungate et al., 1996, Zak et al., 2000). Using plants with genetic similarity, but contrasting C-allocation patterns may provide another approach for elucidating why plants vary in mediating soil N availability under elevated CO₂. Namely, genetic similarity reduces the number of plant traits that can influence a plants’ response to climate change, and C allocation to roots is a key plant trait for explaining differential responses in N cycling as it affects both rhizodeposition and nutrient uptake.

With this study, we aimed at determining how elevated CO₂ affects rhizodeposition of C and how that feeds back to N release from recalcitrant SOM under both wild and cultivated genotypes of *Triticum durum* (wheat). The wild and cultivated genotypes of wheat are expected to have contrasting C allocation patterns, since modern agriculture developed cultivars that function well in favorable soil environments but lack traits necessary for growth in low-resource environments (Rengel & Marschner, 2005). Thus, selection for increased yield under high-input agricultural systems produced cultivars with smaller root systems (Chapin et al., 1989; Siddique et al., 1990; Jackson, 1995) and greater C partitioning to shoots (Gifford et al., 1984). We hypothesized that elevated CO₂ increases the inputs of root derived C thereby stimulating decomposition of recalcitrant SOM and concurrently increasing soil N availability and plant N uptake. In addition, we hypothesized greater C allocation to the roots of the wild wheat genotypes to cause greater rhizodeposition-induced N mineralization from stable SOM pools under elevated CO₂.

### 8.2 Materials and Methods

**Plant species and genotypes**

Three wild and three cultivated genotypes of spring wheat were selected for the experiment. The genotypes were classified as follows:

- *Triticum turgidum subsp. dicoccoides* - (PI 467015) wild material, collected in: Northern Israel (1982).
- *Triticum turgidum subsp. dicoccoides* (PI 467008), wild material, collected in: Northern Israel (1982).
• *Triticum turgidum subsp. durum* (PI 532239), cultivated material, collected in: Oman (1987).

• *Triticum turgidum subsp. durum* (PI 520125), cultivated material, donated from Maryland, United States (1987).

**Experimental design and growing conditions**

Plants were exposed to 400 µmol mol\(^{-1}\) and 800 µmol mol\(^{-1}\) atmospheric CO\(_2\) concentrations in two controlled environment chambers for 32 days (three replicates per treatment). The chambers were located in the Greenhouse Facility at UC-Davis. They were constructed from clear Plexiglas and had a volume of 2000 L. They were entirely sealed for the duration of the experiment, to allow for continuous labelling with \(^{13}\)CO\(_2\)-gas. The chamber conditions used a photosynthetically active (400–700 nm) photon fluence rate of 500 µmol m\(^{-2}\)s\(^{-1}\), a 16/8 hours (day/night) cycle, and 30°C/25°C (day/night) temperature. Humidity and temperature were monitored and controlled continuously. Two fans per chamber allowed for a constant circulation of air. The CO\(_2\) concentration was monitored every 15 minutes.

Continuous labelling with \(^{13}\)CO\(_2\), started after 7 days of growth, and lasted for 25 days. Ten mL of \(^{13}\)C-labeled NaHCO\(_3\) (0.20 g \(^{13}\)C / L at \(\delta^{13}\)C of 10.00‰) was pipetted into a diluted H\(_2\)SO\(_4\) (5 M) solution when the plants had drawn the CO\(_2\) concentration below the threshold of 350 µmol mol\(^{-1}\) and 700 µmol mol\(^{-1}\) for the ambient and elevated CO\(_2\) treatments, respectively. Prior to labelling a CO\(_2\) scrubber (Soda Lime) was used to reduce the CO\(_2\) concentration in both of the chambers to 200 µmol mol\(^{-1}\).

Experimental sub-treatments were wild versus cultivated wheat genotypes. Seeds of the plants were vernalized at 6°C and germinated for 5 days in plastic Petri dishes with water agar as the growth medium. Subsequently, the plants were transferred to containers (deep pot cells, D25L, Stuewe & sons, Corvallis, Oregon; 25 cm height and 4.25 cm diameter at top) containing 350 g (dry weight) of soil. Water-holding capacity (WHC) was determined by calculating the difference in weight of soils at saturation point and oven-dry weight (100°C). Soils were re-wetted to 60% of WHC prior to seeding and on a daily basis during the experiment.

**Pre-treatment of the soil**

The soil used for the experiment was derived from the Swiss Free Air Carbon dioxide Experiment (FACE) in March of 2003. This soil had received \(^{15}\)N fertilization treatments for 10 consecutive years, which has lead to incorporation of a significant amount of the \(^{15}\)N into the more stable SOM pools (de Graaff, unpublished data). The samples were taken to a depth of 25 cm from both ambient and elevated CO\(_2\) field plots consisting of *L. perenne* that had received low rates of N fertilization (140 kg N ha\(^{-1}\) y\(^{-1}\), with an atom%\(^{15}\)Nexcess 0.3841 in 1995 and 1.0602 from 1996-2000. No differences in total N, C or fertilizer derived N were observed between the ambient and elevated CO\(_2\) treatments after 10 years (van Kessel *et al.*, 2006).
Rhizodeposition-induced increases in N mineralization under elevated CO₂

The soil of the ambient and elevated CO₂ treatments was composited, air dried and sieved to 2 mm. Next, sterile sand (30% to dry weight) was added, to increase its volume and to facilitate root extraction at termination of the experiment. Subsequently, the soils were incubated for 145 days in ten plastic filters (Nalgene Filter model 7111; Becton Dickenson Labware, Lincoln Park, NJ, USA) at 35°C (Kaye et al., 2002). A glass fiber filter (Whatman GF/A, Whatman Inc., Ann Arbor, MI, USA), and an “extra thick” glass fiber prefilter (Gelman Sciences, Ann Arbor, MI, USA) were used to replace the filter originally in the filter unit. Water was added to obtain 60% of water holding capacity.

To deplete the soil from labile N and 15N, the soils were incubated at 35°C and leached at days: 1, 8, 25, 43, 58, 86, 100, 120 and 145, with a leaching solution containing all essential nutrients except for N (Stanford and Smith, 1972; Nadelhoffer, 1990; Kaye et al., 2002)). At each leaching, 1 L of the N-free leaching solution was added to the top of the filter, allowed to equilibrate with the soil for 45 min. and then drawn through the filter with a weak vacuum until all the leachate was collected (Kaye et al., 2002). The leachates were collected in 120 mL specimen cups and frozen until further analyses for labile N. Leaching was terminated when the rate of labile 15N efflux from the soil was near zero (6.8*10⁻⁵ mg⁻¹ g⁻¹ day⁻¹) for the last 3 leaching events. The intensive leaching of the soils ensured that the 15N in the soil was predominantly present in the stable SOM fractions, namely the SOM decomposition rate in the control soil was low at 6.8*10⁻⁵ mg 15N g⁻¹ d⁻¹. This indicates that if the plants predominantly take up mineral N, the 15N had to pass the microbial loop before being available to plants. The soil was removed from the filters and composited, after which it was transferred to the containers.

**Plant sampling**

After 32 days of growth, the plants were harvested. The shoots were cut off at the soil, and dried at 70°C for three days. The containers with the remaining soil were stored at 4°C for one night prior to harvesting the roots. After washing the roots with water, the roots were dried at 70°C for three days. Both the dry root and shoot material were ground in a ball mill and their C and N, and 15N and 13C isotopic composition was determined. Total C, N and the isotopic composition of the plants were analyzed by an automated N/C analyser-isotope ratio mass spectrometer (ANCA-IRMS, Europa Scientific Integra, UK) at the UC Davis stable Isotope facility.

**Soil sampling and analyses**

The soil was mixed thoroughly after which a sub sample of 110 g was collected. Since the roots had occupied the entire volume in the containers, all the soil was considered rhizosphere soil. The roots were removed from the soil by hand picking. Twenty g of the soil was dried at 40°C for 1 day and ground in a ball mill after which its total C and N and 15N isotopic composition was measured.
A second sub sample (30 g) was used to determine mineral N in the soil. Soil N mineralization was measured by extracting 30 g of each soil with 120 mL 2 M KCL. The solution was shaken for 45 min, extracted and analyzed calorimetrically for NH4+ and NO3- content (Forster, 1995). The amounts of 15N were derived by diffusing N according to Stark and Hart (1996), after which the 15N isotopic composition was measured.

The third and fourth sub samples (30 g each) were used to determine C in the active soil C pool and soil microbial biomass C and N by the fumigation-incubation method (Jenkinson & Powlson, 1976). Thirty g were stored at 4°C for 48 h, while the other 30 g were exposed to chloroform fumigation for 48 h. Subsequently, both the fumigated and non-fumigated soils were incubated for 10 days. Samples were transferred to 120 mL specimen containers and placed in ¼ L Mason jars. Water was added to the samples to obtain 60% of WHC. In addition, 5 mL of water were added to the bottom of the jar to slow soil drying.

A septum in the lid allowed gas samples to be removed with a 12 mL syringe. CO2 samples were collected in 10 ml vacutainers (Labco Unlimited, Buckinghamshire, UK). The concentration of CO2 and its PDB-13C signature were determined at the University of California-Davis Stable Isotope Facility using a continuous flow, isotope mass spectrometer (PDZ Europa TGII trace gas analyzer and Geo 20–20 isotope ratio mass spectrometer, Cheshire UK). Carbon mineralization data were expressed on the basis of oven-dry (40 °C) weight of soil. Active soil C was defined as the CO2-C efflux from the nonfumigated soil. Microbial C was defined as CO2-C efflux from the fumigated soil minus CO2-C efflux from the nonfumigated soil (Ec), using an efficiency coefficient, k, of 0.35 (Vance et al., 1987).

Both the unfumigated and fumigated samples were extracted instantly after gas-sampling. Each sample was mixed with 120 mL of 2 M KCl and shaken for 45 min prior to filtration (Whatman #42). The extracts were analyzed colorimetrically for NH4+ and NO3- content (Forster, 1995). Microbial biomass N was considered to be the total inorganic N flush in the fumigated samples compared to the non-fumigated samples. The 15N determination for both mineral 15N and microbial biomass 15N was performed by diffusing N from the extracts onto acidified disks sealed in Teflon tape (Stark & Hart, 1996). The disks were packed in tin capsules and analyzed for isotopic composition.

15N and 13C recovery calculations

Recovery of 15N in the soil mineral N pool, the microbial biomass N pool and the plant tissues was calculated as the proportion of 15N incorporated in the pools relative to the total amount of 15N incorporated in the stable SOM pools after the leaching events and prior to plant growth. Recovery of 13C in the plant and soil pools was calculated as a percentage of total 13C recovery.
Statistical analyses
The procedure GLM in the SAS system for Windows™ V8 was conducted, with CO₂ and genotypes as treatments. Means were compared by the Tukey test, if the analysis of variance was significant. The level of significance was \( P < 0.01 \) and \( P < 0.05 \).

8.3 Results

Plant biomass production
Elevated CO₂ significantly stimulated aboveground biomass production of both the wild and cultivated genotypes, by 24.6% and 40.0%, respectively (Fig. 8.1a, Table 8.1). Root biomass production was significantly stimulated by elevated CO₂ for the wild and cultivated genotypes, 36.0% and 42.7%, respectively (Fig. 8.1a, Table 8.1). Due to the significantly greater root biomass of the wild compared to cultivated genotypes, root:shoot (R:S) ratios were significantly greater for the wild than the cultivated genotypes under both ambient and elevated CO₂ (+48.1%, on average). Elevated CO₂ had not altered R:S ratios for either one of the genotypes (Fig. 8.1b, Table 8.1).

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Fig. 8.1. Organ masses (a) and Root: Shoot ratios (b) of the 3 wild and 3 cultivated wheat genotypes, under ambient and elevated CO₂. Unshaded and shaded bars report ambient and elevated CO₂ concentrations, respectively. Values are means ± SE (n=3). Statistics are presented in table 8.2.
Table 8.1 Analysis of variation associated with the means and SEM-values represented in figures 1a-b, 2a-b, 3a-c

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Biomass production (Fig. 1)</th>
<th>13C recovery (%) (Fig. 2)</th>
<th>15N recovery (%) (Fig. 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Shoots</td>
<td>Roots</td>
<td>R:S ratio</td>
</tr>
<tr>
<td>Wild genotypes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CO2</td>
<td>P&lt;0.05</td>
<td>P&lt;0.01</td>
<td>ns</td>
</tr>
<tr>
<td>Cultivated genotypes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CO2</td>
<td>P&lt;0.01</td>
<td>P&lt;0.05</td>
<td>ns</td>
</tr>
<tr>
<td>Genotypes</td>
<td>P&lt;0.05</td>
<td>P&lt;0.05</td>
<td>P&lt;0.01</td>
</tr>
<tr>
<td>CO2 * Genotypes</td>
<td>P&lt;0.05</td>
<td>P&lt;0.05</td>
<td>P&lt;0.01</td>
</tr>
</tbody>
</table>

13C recovery

The allocation of 13C to the shoots and roots consistently followed the results on biomass production. Overall significantly more 13C was recovered in the shoots of the cultivated than the wild genotypes under both ambient and elevated CO2 (+11.9% on average), while 13C recovery in the roots was greater in the wild compared to the cultivated genotypes under ambient and elevated CO2 (+40.0% on average) (Table 8.2). Elevated CO2 significantly increased 13C recovery in the shoots (+13.9%), but not in the roots of the cultivated genotypes. On the contrary, 13C recovery was significantly enhanced in the roots (+8.1%), but not in the shoots of the wild genotypes under elevated CO2 (Table 8.2).

Due to the fast turnover of root derived C, the incorporation of 13C in the total soil C pool was minor compared to the 12C background values, which made it impossible to accurately determine recovery of total soil 13C. However, 13C recovery in the active soil C pool could be determined and was significantly greater under elevated than ambient CO2, by 50.9% for the wild and 54.3% for the cultivated genotypes (Fig 8.2a, Table 8.1). The recovery of 13C was significantly greater for the cultivated compared to the wild genotypes under ambient CO2, but was equal under elevated CO2 (Fig 8.2a, Table 8.1).

Under elevated CO2 significantly more of the root-derived 13C was incorporated in the microbial biomass compared to ambient CO2, by 40.4% for the wild and 28.7% for the cultivated genotypes (Fig 8.2b).
Fig. 8.2. Carbon-13 recovery in the active soil C pool as a percentage of total $^{13}$C recovery (a) and $^{13}$C recovery in the microbial biomass as a percentage of total $^{13}$C recovery (b) in the soils of the 3 wild and 3 cultivated wheat genotypes under ambient and elevated CO$_2$. Shading conventions as in Fig. 8.1. Values are means ± SEM (n=3). Statistics are presented in table 8.2.

$^{15}$N recovery

The total release of $^{15}$N from stable SOM pools as measured in the mineral N pool and in the plants was significantly greater when plants had grown in the soils, compared to decomposition rates in soils without plants, by 63% and 70% under ambient and elevated CO$_2$, respectively (data not shown). The $^{15}$N recovery as a percentage of the total amount of $^{15}$N incorporated in the stable SOM pools was 5.0 % and 6.3% for the soils under ambient and elevated CO$_2$, respectively. The total recovery (sum of recovery in plants, microbial biomass and mineral N pool) of $^{15}$N under elevated CO$_2$ was significantly greater than recovery under ambient CO$_2$.

Total plant N concentrations were equal for all genotypes, and were significantly greater under ambient than elevated CO$_2$ (Table 8.2). Consequently, the tissue C:N ratios of the roots (±9.1%, on average) and shoots (±27.1%, on average) were significantly enhanced under elevated CO$_2$ for both wild and cultivated genotypes (Table 8.2). In contrast, the percentage $^{15}$N recovery in the total plant biomass was significantly enhanced by elevated CO$_2$ in both the wild and cultivated genotypes (±24.0% on average) (Fig. 8.3a). In addition, the wild genotypes had acquired more $^{15}$N
under both ambient and elevated CO\textsubscript{2} than the cultivated genotypes (+33.9% on average) (Fig. 8.3a).

**Table 8.2** Total plant N concentrations, C:N ratios and relative \textsuperscript{13}C recovery. Values are means ± SEM (n=3).

<table>
<thead>
<tr>
<th>Genotype</th>
<th>CO\textsubscript{2} treatment</th>
<th>Shoot N concentration (mg g\textsuperscript{-1})</th>
<th>Root N concentration (mg g\textsuperscript{-1})</th>
<th>C:N ratio Shoot</th>
<th>C:N ratio Root</th>
<th>Percentage \textsuperscript{13}C recovery Shoot</th>
<th>Percentage \textsuperscript{13}C recovery Root</th>
</tr>
</thead>
<tbody>
<tr>
<td>wild</td>
<td>Ambient CO\textsubscript{2}</td>
<td>15.93 ± 0.67</td>
<td>10.28 ± 0.29</td>
<td>26.98 ± 1.09</td>
<td>40.23 ± 0.50</td>
<td>54.78 ± 1.63</td>
<td>27.55 ± 1.89</td>
</tr>
<tr>
<td></td>
<td>Elevated CO\textsubscript{2}</td>
<td>12.46 ± 0.93</td>
<td>8.64 ± 0.22</td>
<td>35.26 ± 1.21</td>
<td>45.40 ± 0.44</td>
<td>58.98 ± 2.06</td>
<td>29.99 ± 1.68</td>
</tr>
<tr>
<td>cultivated</td>
<td>Ambient CO\textsubscript{2}</td>
<td>16.94 ± 0.42</td>
<td>10.80 ± 0.50</td>
<td>25.73 ± 0.77</td>
<td>39.33 ± 0.87</td>
<td>59.94 ± 2.84</td>
<td>17.34 ± 1.95</td>
</tr>
<tr>
<td></td>
<td>Elevated CO\textsubscript{2}</td>
<td>11.72 ± 0.25</td>
<td>9.56 ± 0.26</td>
<td>37.19 ± 0.47</td>
<td>42.18 ± 0.47</td>
<td>69.70 ± 2.92</td>
<td>17.10 ± 2.27</td>
</tr>
</tbody>
</table>

**Source of variation ANOVA**

<table>
<thead>
<tr>
<th>Wild genotypes</th>
<th>CO\textsubscript{2}</th>
<th>P ≤ 0.05</th>
<th>ns</th>
<th>P ≤ 0.01</th>
<th>P ≤ 0.05</th>
<th>ns</th>
<th>P ≤ 0.05</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cultivated genotypes</td>
<td>CO\textsubscript{2}</td>
<td>P ≤ 0.05</td>
<td>ns</td>
<td>P ≤ 0.01</td>
<td>P ≤ 0.05</td>
<td>ns</td>
<td>P ≤ 0.05</td>
</tr>
<tr>
<td>Genotypes</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>P ≤ 0.05</td>
<td>P ≤ 0.05</td>
<td>ns</td>
</tr>
<tr>
<td>CO\textsubscript{2} * Genotypes</td>
<td>P ≤ 0.05</td>
<td>ns</td>
<td>P ≤ 0.01</td>
<td>P ≤ 0.05</td>
<td>P ≤ 0.05</td>
<td>P ≤ 0.05</td>
<td>P ≤ 0.05</td>
</tr>
</tbody>
</table>

Fig. 8.3. Nitrogen-15 recovery in the plant tissues of the 3 wild and 3 cultivated wheat genotypes as a percentage of total \textsuperscript{15}N in the recalcitrant SOM pools (a), \textsuperscript{15}N recovery in the mineral soil N pool of the 3 wild and 3 cultivated wheat genotypes as a percentage of total \textsuperscript{15}N recovery (b) and \textsuperscript{15}N recovery in the soil microbial biomass of the 3 wild and cultivated genotypes (c).
3 cultivated wheat genotypes as a percentage of total $^{15}$N recovery (c) under ambient and elevated CO$_2$. Shading conventions as in Fig. 8.1. Values are means ± SEM (n=3). Statistics are presented in table 8.2.

Total soil N contents and the percentage total soil $^{15}$N recovery were not different between the soils exposed to ambient and elevated CO$_2$ and wild and cultivated genotypes. Total mineral soil N contents were also equivalent under ambient and elevated CO$_2$ treatments and for all the genotypes, but mineral $^{15}$N contents were significantly smaller under elevated compared to ambient CO$_2$ treatments for the wild genotypes only (-30.8%) (Fig. 8.3b).

Microbial biomass $^{15}$N recovery was smaller under elevated compared to ambient CO$_2$ for the wild genotypes (-12%) (Fig. 8.3c). There were no differences in microbial biomass N or $^{15}$N contents between the different genotypes (Fig. 8.3c).

### 8.4 Discussion

**Linking rhizodeposition to N released from recalcitrant SOM under elevated CO$_2$**

Elevated CO$_2$ significantly stimulated plant production and the amount of root-derived C incorporated in the active soil C pool. In addition, the amount of root-derived C incorporated in the microbial biomass was significantly enhanced by elevated CO$_2$. These data suggest that elevated CO$_2$ increased rhizodeposition. A CO$_2$ induced increase in C rhizodeposition has been reported by numerous studies (Cotrufo & Gorissen, 1997; van Ginkel et al., 2000). It is still debated, however, whether elevated CO$_2$ increases the input of root-derived materials by stimulating the quantity of rhizodeposition per gram of root, or whether the increase in rhizodeposition is caused by a proportional increase in root growth (Darrah, 1996). In this study, the increase in root-derived soil C under elevated CO$_2$ was accompanied by a proportional increase in root biomass for each of the wheat cultivars (data not shown). This corroborates other studies showing that increased root production under elevated CO$_2$ was responsible for enhanced C rhizodeposition (Billes et al., 1993; Cotrufo & Gorissen, 1997). These results suggest that elevated CO$_2$ is not likely to affect rhizosphere processes unless belowground biomass production is stimulated.

The presence of plants had stimulated $^{15}$N release by approximately 70%, compared to the N that would have been released when the soils were incubated without plants, suggesting plant-induced N mineralization had occurred. A similar positive effect of plant growth on N mineralization was shown by Herman et al., (2006). More importantly, the recovery of $^{15}$N in the plants exposed to elevated CO$_2$ was significantly greater than $^{15}$N recovery in the plants exposed to ambient CO$_2$. This suggests that increased root-derived soil C inputs under elevated CO$_2$ enhanced mineralization of N from recalcitrant SOM pools. The stimulating effect of increased rhizodeposition on N
mineralization has only been shown previously in a grassland where clipping of the grass promoted root exudation of C, which was then linked to increased soil N availability (Hamilton & Frank, 2001). However, so far no other study has directly linked increased rhizodeposition under elevated CO₂ to increased release of N from recalcitrant SOM pools.

The correlation between root derived C in the active C pool and ¹⁵N uptake however, was insignificant (data not shown). This insignificance, may have been caused by the fact that measured root derived soil C input in our study was an approximation of rhizodeposition. Namely, similar to what Cotrufo & Gorissen (1997) found, most of the additional root derived C entering the soil under elevated CO₂ was prone to high turnover rates, which is confirmed by the large increase in microbial biomass ¹³C under elevated CO₂. Therefore, a proportionally small amount of C was incorporated in more stable SOM pools. Consequently, the large background ¹²C values of the soil prevented us from accurately measuring ¹³C incorporated in the total soil C pool. In addition, our experimental set-up did not allow for measuring soil respiration rates during the experiment, which has probably led to an underestimation of rhizodeposition.

Increased efficiency of nutrient uptake could have also been responsible for the greater ¹⁵N recovery under elevated CO₂. Yet, if SOM decomposition did not increase mineral N availability, increased fine root growth could not benefit N uptake. Many plants though, can also take up organic N when faced with low N availability (Chapin et al., 1993), and the presence of mycorrhizal fungi can stimulate this process (Hawkins et al., 2000). The magnitude of organic N uptake on the other hand is estimated to be very low (Chapin et al., 1993). We are not sure how much DON was available in the soils during the experiment, nor did we quantify mycorrhizal colonization of the roots.

The large turnover rates of ¹³C and significantly greater incorporation of ¹³C in the microbial biomass under elevated CO₂ (+35%, on average), however, strongly indicate that increased rhizodeposition-induced microbial activity under elevated CO₂ was responsible for the greater ¹⁵N uptake rates under elevated compared to ambient CO₂. In addition, since the microbial N pool reflects the balance of N mineralization and immobilization (Hart et al. 1994), the reduction of microbial biomass ¹⁵N contents under elevated CO₂, suggests that microbial ¹⁵N mineralization had increased. The smaller mineral ¹⁵N pool under elevated compared to ambient CO₂ further indicates that the plants had taken up the mineral ¹⁵N made available from the stable SOM pools by microbes. Moreover, the stimulation of ¹⁵N uptake was significantly greater under elevated compared to ambient CO₂ (+7%). The increase in C rhizodeposition rates, incorporation of root derived C in the microbial biomass, along with the simultaneous decrease of ¹⁵N contents in the soil microbial biomass and mineral N pools and the concurrent increase of ¹⁵N uptake by the plants under elevated CO₂ strongly support the conclusion that CO₂-induced increases in rhizodeposition can increase soil N availability.
This conclusion has important implications for our understanding of ecosystem functioning under elevated CO\textsubscript{2}. Namely this mechanism may be responsible for sustained increases in plant production and soil C sequestration under elevated CO\textsubscript{2} in unfertilized ecosystems. Nonetheless, total plant N concentrations were still reduced by elevated CO\textsubscript{2} in our experiment. This suggests that, despite this mechanism, the plant growth response to elevated CO\textsubscript{2} in the long-term may still decrease. A similar conclusion was drawn by Dijkstra \textit{et al.} (2007) who found that CO\textsubscript{2}-induced increases in water availability in a semi-arid grassland had increased N mineralization over a five year period. However N concentrations in the plant tissues were also significantly lower under elevated CO\textsubscript{2}, therefore it remains unclear whether the enhanced N mineralization rates can be sustained for longer than five years in this system.

\textbf{Genotypic variation in response to elevated CO\textsubscript{2}}

In this study, we hypothesized that contrasting C allocation patterns between wild and cultivated genotypes of wheat may be an important parameter for explaining plant mediated variations in soil N cycling under elevated CO\textsubscript{2}. Under ambient CO\textsubscript{2} concentrations, more C was partitioned to the shoot compared to the root for the cultivated genotypes. On the contrary, the wild genotypes allocated more C to their roots. These data indicate that agronomic selection of wheat has resulted in a morphological tradeoff, where C allocation to organs associated with C assimilation compared to organs associated with nutrient uptake is favored in modern cultivars (Gifford \textit{et al.}, 1984).

It has been argued that a high R:S ratio is a plant characteristic representing an 'adaptive strategy' that enables plants to survive in stressful (\textit{e.g.} low-resource) environments (Grime, 1977; Tilman 1988). On the contrary, the ability to allocate soil resources to stimulate shoot production is a characteristic of competitive species usually dominating fertile environments (Grime, 1979; Tillman, 1988; Ryser & Notz, 1996). Based on these ecological theories we hypothesized that cultivated genotypes are more likely to increase aboveground plant production in response to the CO\textsubscript{2} fertilization effect, while wild genotypes are more likely to efficiently acquire nutrients in low resource environments.

Elevated CO\textsubscript{2} increased aboveground biomass production of both wild and cultivated genotypes, but the growth response of the cultivated genotypes was significantly greater than the growth response of the wild genotypes. By contrast, belowground biomass production was significantly greater for the wild compared to the cultivated genotypes under both ambient and elevated CO\textsubscript{2}. In addition, the wild genotypes had acquired more 15N under elevated CO\textsubscript{2} than the cultivated genotypes. Concurrently, there was significantly less 15N under elevated CO\textsubscript{2} in the mineral N pool and the microbial biomass for the wild genotypes only. This corroborates the hypothesis that wild genotypes representing an "adaptive life strategy" making them more likely to sustain nutrient acquisition when elevated CO\textsubscript{2} reduces nutrient availability in the long-term. This suggests that agricultural systems that do not rely
heavily on mineral nutrient fertilizer inputs might benefit from using plants with similar traits under elevated CO₂, since the ability of the wild genotypes to effectively compete with microbes for N makes them more likely to sustain growth and C sequestration in low N input systems.

8.5 Conclusions

Based on the increase in microbial activity due to enhanced rhizodeposition under elevated CO₂, the concurrent stimulation of ¹⁵N availability by the microbial biomass from the stable SOM pools, and the subsequent increased mineral ¹⁵N uptake, we suggest that elevated CO₂ stimulates rhizodeposition-induced priming which benefits plant production. The significantly lower tissue N concentrations under elevated CO₂, however, indicate that plant N limitation was not prevented, which suggests that the plant growth response to elevated CO₂ and associated N uptake mechanisms may not be sustained in the long-term.

In addition, our data suggest that agronomic selection of wheat has resulted in a morphological tradeoff, where C allocation to organs associated with C assimilation compared to organs associated with nutrient uptake is favored in modern cultivars. Initially, this makes them more likely to increase biomass production under elevated CO₂ than their wild relatives. However, when elevated CO₂ reduces nutrient availability in the long-term, the ability of the wild genotypes to effectively compete with microbes for N makes them more likely to sustain growth and C sequestration in low N input systems. Thus, contrasting C allocation patterns in this study could explain plant mediated differential responses in soil N cycling to elevated CO₂.
9. General discussion

9.1 The long-term impact of elevated CO₂ on soil C decomposition, input and sequestration

Accumulation of SOC requires a positive imbalance between inputs to and outputs from SOM stocks. Thus, enhanced soil C sequestration under elevated CO₂ can only occur if the rate of soil C decomposition lags behind the CO₂ induced increase in soil C input (Raich & Schlesinger, 1992). This part of the dissertation has explored how long-term exposure of ecosystems to elevated CO₂ has affected soil C inputs versus SOM decomposition, and how these changes ultimately feedback to soil C sequestration.

It has been postulated that elevated CO₂ can reduce SOM decomposition by decreasing litter quality, which may stimulate soil C sequestration (Lambers, 1993; Norby et al., 1986). At the Swiss FACE experiment, elevated CO₂ did not significantly affect litter quality or litter decomposition of L. perenne and T. repens (de Graaff et al., 2004). This is in contrast to studies showing that both litter quality and decomposition decreased under elevated CO₂ (Cotrufo et al., 1994; Cotrufo & Ineson 1996; van Groenigen et al., 2005). Litter for these studies, however, was derived from plants grown under artificial conditions, which amplifies the impact of elevated CO₂ on litter quality (Norby et al., 2001). It is also contradictory to a study showing that in situ root decomposition of L. perenne and T. repens was reduced in the Swiss FACE experiment (Jongen et al., 1995). Nonetheless, even though decomposition of root litter had decreased in the Swiss FACE experiment, exposure of the swards to ten years of elevated CO₂ had not significantly increased soil C sequestration. This indicates that the reduction in root litter quality and decomposition was of little importance for long-term soil C sequestration. The limited significance of litter quality for long-term C stabilization is corroborated by Latter et al. (1998), who showed that the quantity of litter input affects soil C sequestration to a greater extent than the quality of litter input. In addition, several reviews revealed that the impact of elevated CO₂ on litter quality in most field studies was minor (Norby et al., 2001; Luo et al., 2006, de Graaff et al., 2006), and that the impact of elevated CO₂ on litter decomposition was inconsistent (Norby et al., 2001). These results indicate that elevated CO₂ will not significantly affect soil C concentrations through its impact on litter quality (van Groenigen, 2007).

Alternatively, elevated CO₂ could increase C losses from ecosystems by stimulating the decomposition of SOM, thereby reducing the potential for soil C sequestration (Cheng, 1999). Namely, the increased influx of labile C to the soil may stimulate microbial mining of SOM for nutrients (Carney et al., 2007). This process is referred to as “priming” (Cheng, 1999). Soil C decomposition did not increase in the Swiss FACE experiment under elevated CO₂ (de Graaff et al., 2004, Chapter 4). In contrast, the Meta analysis showed that increased labile soil C inputs under elevated CO₂ (van Groenigen et al., 2006) stimulated soil microbial activity (de Graaff et al., 2006). It is unclear why
soil microbial activity in the Swiss FACE experiment was not enhanced by elevated CO2. Perhaps priming of SOM was responsible for increased respiration rates in some of the relatively short-term experiments included in the Meta analysis (e.g. Hoosbeek et al., 2004; Pendall et al., 2003; Cardon et al., 2001), but not in the relatively long-term Swiss FACE experiment, since priming is expected to be a transient process (Hungate et al., 2003). Increased microbial respiration may be caused by a CO2- induced change in the soil microbial community (Carney et al., 2007). There was no evidence of a change in the microbial community in the Swiss FACE soils under elevated CO2 (Marilley et al., 1998, 1999; van Groenigen et al., 2007), which may explain the lack of a response of microbial respiration in the Swiss FACE experiment.

Enhanced soil C respiration under elevated CO2 decreases the potential for net soil C sequestration, but the Meta analysis showed that the increase in soil C respiration was not sufficient to offset soil C input, leading to a small increase in soil C storage under elevated CO2. These results are in agreement with the review of Jastrow et al. (2005), who also reported an increase in soil C concentrations under elevated CO2. This suggests that elevated CO2 will affect soil C concentrations mostly through its effect on input, rather than output of SOM. However, when classifying ecosystem responses to elevated CO2 by low and high N availability treatments, I found that soil C sequestration under elevated CO2 was strongly constrained by the availability of nutrients. Chapter 5 and 4 show that elevated CO2 increased microbial N immobilization and retention of N in more stable SOM pools. Consequently, the stimulating effect of elevated CO2 on plant production was reduced in unfertilized systems. The importance of N availability for sustained increases in plant production under elevated CO2 was confirmed by a recent study to the impact of long-term elevated CO2 on biomass production in a perennial grassland (Reich et al., 2006). In addition, fixation of N2 could not alleviate N limitation, since accelerated depletion of P and Mb under elevated CO2 reduced rates of N2 fixation. Consequently, under low N availability soil C respiration rates exceeded soil C input rates, and soil C contents were unaltered under elevated CO2. In contrast, when nutrient limitation was alleviated by additional nutrient inputs through fertilization, soil C input exceeded soil C respiration rates, leading to net soil C sequestration.

These results corroborate the Progressive Nitrogen Limitation (PNL) conceptual framework which postulates that the increased C assimilation and concurrent soil C inputs under elevated CO2 will progressively cause N retention in both plant and soil pools (Luo et al., 2004). This is expected to cause enhanced competition for N between plants and microbes, leading to a decrease in plant growth and soil C sequestration under elevated CO2. Hence, only when soil nutrient availability is ample, leading to sustained increases in plant growth under elevated CO2, there is potential for net soil C sequestration regardless of SOC decomposition rates (de Graaff et al., 2006; van Groenigen et al., 2006).

Even though the Meta analysis suggests that additional nutrient fertilizer inputs are needed for sustained increases in plant growth and soil C sequestration under elevated
results from some individual FACE experiments indicate that elevated CO\textsubscript{2} can actually affect some processes that prevent N limitation in these systems. At Duke FACE, 10 years of elevated CO\textsubscript{2} continues to increase tree growth and soil C sequestration, despite there being no evidence of increased net N mineralization rates (Johnson, 2006; Finzi et al., 2006). Researchers at this site are still uncertain about the processes leading to these sustained responses in tree production under elevated CO\textsubscript{2} (Pritchard, personal communication). In addition, Dijkstra et al. (2007) showed that 5 years of elevated CO\textsubscript{2} enhanced N availability in an unfertilized semi-arid grassland. They attributed the increase in N mineralization rates to CO\textsubscript{2}-induced increases in water availability, which is particularly important in dry ecosystems.

Conversely, at the Swiss FACE experiment, soil C sequestration was not significantly stimulated by elevated CO\textsubscript{2} despite the high fertilization rates, concurrent increases in plant growth, and relatively low decomposition rates. Possibly the C pool was saturated, thereby prohibiting stable soil C formation (Kool et al., 2007). However, if this is the cause, the labile C pool as determined by biological fractionation in chapter 4 should have been larger, or CO\textsubscript{2} respiration rates should have increased under elevated compared to ambient CO\textsubscript{2}. Perhaps litter input was not sufficient to affect C sequestration due to the frequent cuttings and removal of the biomass. However, root biomass had increased significantly under elevated CO\textsubscript{2}, which is a major source of soil C input. Nonetheless, the ratio of root- over shoot-derived C decreases with time: from 6.1 after 5 months to 2.9 after 12 months and to 1.8 and 1.2 after 18 months of addition of labelled plant material (Six et al., 2002). Thus, lower shoot-derived soil C inputs could have been responsible for the lack of soil C storage in the long term.

This shows that even though integrating experimental results across multiple studies by using Meta analysis allowed me to determine the driving force behind soil C sequestration under elevated CO\textsubscript{2} (i.e. nutrient availability), the predictive power of this analysis for soil C sequestration in individual ecosystems appears to be ambiguous. Namely, (1) management practices in fertilized agro ecosystems can reduce soil C input or increase decomposition rates under elevated CO\textsubscript{2}, thereby counter balancing the stimulating effect of fertilizer applications on soil C sequestration, and (2) the impact of elevated CO\textsubscript{2} on nutrient availability in unfertilized ecosystems is still uncertain, since N limitation can apparently be offset by –still partially undefined- processes occurring at the plant-soil interface.

9.2 Plant-soil interactions under elevated CO\textsubscript{2} from a rhizo-centric point of view

Long-term field experiments have only partially advanced our understanding of interactions between plants and soil nutrient cycling under elevated CO\textsubscript{2}. Chapter 9.1 of this dissertation showed that we are still particularly uncertain about the impact of elevated CO\textsubscript{2} on microbial regulation of soil N availability in unfertilized ecosystems. Since, the potential for soil C sequestration under elevated CO\textsubscript{2} in unfertilized
ecosystems is largely dependent on microbial regulation of soil N availability we do need to understand the mechanisms leading to the varied response of microbial N transformation processes.

Our inability to explain the impact of elevated CO$_2$ on soil N availability may be attributed to our lack of understanding of processes occurring in the rhizosphere. Nutrient availability in the rhizosphere can be stimulated under elevated CO$_2$ by a number of mechanisms, e.g. increased water availability, increased fine root growth, increased mycorrhizal growth or rhizodeposition-induced decomposition (Phillips, 2007). These processes may alleviate progressive N limitation in unfertilized ecosystems. For this thesis I focused on rhizodeposition-induced microbial regulation of soil N cycling.

9.2.1 Rhizodeposition under elevated CO$_2$

A change in root-derived substrates entering the soil under elevated CO$_2$ can consist of a change in the quantity, and/ or quality of rhizodeposits. The impact of elevated CO$_2$ on compound specific exudation was measured in sterile hydroponic systems. Due to rapid turnover of root exudates by microbes in soil, it is very difficult to obtain meaningful data about the quality of root exudates in the soil. This set-up allowed us to measure net efflux of 12 amino acids from roots of seedlings, which reflects both influx and efflux components. In contrast to the exudation of organic acids (Hoffland et al., 1992) the input of amino acids and sugars by roots into the soil happens through passive leaching (Jones & Darrah, 1993), while their re-uptake is mediated by an active transport process (Mühling, 1993; Jones & Darrah, 1996). This enables the plant to regulate the net N-loss to the soil (Jones & Darrah, 1994). Elevated CO$_2$ increased the total exudation of amino acids, but did not change the net exudation rates of individual amino acids. This suggests that elevated CO$_2$ changed the quantity, rather than the quality of C and N compounds released from the roots. This result is partially corroborated by Phillips et al., 2006. In their study, elevated CO$_2$ had not affected total net efflux of amino acids, since amino acid influx rates exceeded amino acid efflux rates. However the efflux of six amino acids (arginine, alanine, proline, tyrosine, lysine and leucine) was promoted by elevated CO$_2$. This indicates that my experiment could have not detected some changes in the efflux of individual amino acids, since I did not measure efflux and influx separately. If efflux increases under elevated CO$_2$ when plants are grown in soil rather than nutrient solution, amino acids may be captured by soil particles and microorganisms before they are recovered by the root (Phillips et al., 2004, 2006). The impact of elevated CO$_2$ on efflux of individual amino acids remains to be determined in further experiments.

Elevated CO$_2$ significantly increased the amounts of root derived C and N entering the soil under C$_3$, but not under C$_4$ plants. It is still debated whether elevated CO$_2$ increases input of root-derived materials by stimulating the quantity of rhizodeposition per gram of root, or whether the increase in rhizodeposition is caused by a proportional increase in root growth (Darrah, 1996). When enhanced rhizodeposition follows only
from increased root production, rhizosphere processes may not directly be changed, on the other hand if rhizodeposition per unit of root is altered by elevated CO$_2$, microbial activity could change irrespective of a change in root production, and increased root production would amplify this effect on ecosystems (Cardon, 1996). Increased rhizodeposition in my studies only occurred as a result of increased root production under elevated CO$_2$. Therefore, I submit that a change in microbial regulation of N availability in the rhizosphere is only expected when root production is stimulated by elevated CO$_2$.

A CO$_2$ induced increase in C rhizodeposition has been reported by several studies (Cotrufo & Gorissen, 1997; van Ginkel et al., 2000), however, to our knowledge, no other studies have investigated the impact of elevated CO$_2$ on N rhizodeposition. The increase in N rhizodeposition under elevated CO$_2$ confirms that elevated CO$_2$ stimulated amino acid exudation, and reaffirms the potential importance of elucidating how elevated CO$_2$ alters exudation of specific amino acids. The increased loss of N-rich compounds from plants to low-N soils under elevated CO$_2$ leads to the question whether this N-loss impairs plant performance, or whether total rhizodeposition stimulates microbial activity and microbial nutrient mobilization in the soil and therefore leads to enhanced growth of the plants.

9.2.2 Rhizosphere processes under elevated CO$_2$

When plant production was stimulated under elevated CO$_2$, enhanced rhizodeposition led to increased competition between plants and microbes for root derived N. This suggests that elevated CO$_2$ may enhance N limitation by increasing N loss from the roots and subsequent immobilization of the root derived N by microbes. These processes, however, will only enhance N limitation if the fraction of N lost from the roots is significant relative to the N assimilated by the plant. Our study showed that this fraction was indeed substantial and significantly larger than the fraction lost under ambient CO$_2$; 12% and 23%, respectively. Such losses of plant available N are likely not important when sufficient N is available. However, when the lost N serves as an important source of N in nutrient limiting systems, the impact of elevated CO$_2$ on yield may be more profound.

An alternative hypothesis would be that retention of root derived N prevents it from leaching, and serves as a benefit in conserving N lost from roots (Griffiths & Robinson, 1992; Jimenez et al., 2001). Much of the N would then be available for subsequent uptake due to turnover of the rhizosphere microbial biomass in the longer-term (Hodge et al., 2000; Phillips, 2007). Microbial turnover could increase under elevated CO$_2$ because of a stimulation of faunal grazing of the microbes (Yeates et al., 2003). However, no consistent results have been generated considering the response of microbial grazers under elevated CO$_2$ (Sonneman & Wolters, 2005). Increased efficiency of nutrient uptake under elevated CO$_2$ by increased production of fine roots and/or an increased occurrence of fungal hyphae, could also alleviate N limitation in the long-term (Hu et al., 2004). In addition, organic N uptake may be stimulated in
systems with low N availability (Chapin et al., 1993), and the presence of mycorrhizal fungae can stimulate this process (Hawkins et al., 2000). Another hypothesis could be that the root derived losses of N are minor compared to potential N gains from root induced stimulation of SOM decomposition, i.e. priming (Phillips, 2007). However, so far no study has directly demonstrated how increased C rhizodeposition under elevated CO₂ feeds back to microbial N release from stable SOM. This dissertation shows that elevated CO₂ increases root derived soil C input and simultaneously enhances plant N uptake from the more stable SOM pools. This mechanism may have been responsible for sustained plant growth in low nutrient input FACE systems, and the importance of this process for ecosystem functioning should be explored further.

In conclusion, this dissertation indicates that the rhizosphere plays an important role in mediating plant-soil interactions under elevated CO₂. Sustained increases in plant growth in unfertilized systems exposed to FACE conditions, is probably the result of a variety of benefits resulting from CO₂-induced increased root growth. The benefits of increased C allocation to roots under elevated CO₂, however, are expected to be transient in low N systems. Namely, these mechanisms did not increase plant tissue N concentrations and do not contribute to a net gain of ecosystem N, but rather cause redistribution of available N. Redistribution of N under elevated CO₂ is expected to have a limited effect on N availability and soil C sequestration in the future (i.e. decades to centuries) (Hungate et al., 2003).

9.3 Predicting plant responses to elevated CO₂

Plant species differ in mediating changes in soil C and N cycling in response to global change, but these differences have not been predictable from a knowledge of species’ biology (Hungate et al., 1996). To predict plant species’ impacts on soil nutrient cycling under elevated CO₂, plants have been classified into broad groups (e.g. fast- and slow-growing plant species and species with different functional types), relying on a broad suite of related plant traits that may generalize how species respond to environmental changes (Eviner & Chapin, 2003). However, differences within a group frequently were equivalent to differences between groups for most parameters (Zak et al., 2000). Hence, such classifications have provided limited understanding on how plant species affect plant and microbial N acquisition under elevated CO₂ (Hungate et al., 1996; Zak et al., 2000). Using plants with genetic similarity, but contrasting C-allocation patterns may provide another approach for elucidating why plants vary in mediating nutrient cycling under elevated CO₂. Namely, genetic similarity reduces the number of plant traits that can influence a plant’s response to climate change, and C allocation to roots is a key plant trait for explaining differential responses in C and N cycling as it affects both rhizodeposition and nutrient uptake.

The amount of C that a plant allocates to its root versus shoot is a trait that relates to the habitat occupied by a species: generally, species from fertile sites have inherently low R:S ratios, while species that develop in less favorable conditions have high R:S
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ratios (Chapin, 1980; Grime 1977, 1979; Grime et al., 1986). In line with this theory some studies have shown that cultivars developed for modern agriculture function well in favorable soil environments, but lack traits necessary for growth in low-resource environments (Chapin et al., 1989; Siddique et al., 1990; Jackson, 1995; Gifford et al., 1984; Rengel & Marschner, 2005). In this dissertation a number of crop plants have been used to test the hypothesis that crop genotypes used for modern agriculture allocate less C to their roots than wild genotypes. The number of genotypes used to test this hypothesis exceeds by far the amount of genotypes used to test similar hypotheses previously. This dissertation indicates that agronomic selection of wheat and Medicago truncatula (medic) has resulted in a morphological tradeoff, where C allocation to organs associated with C assimilation compared to organs associated with nutrient uptake is favored in modern cultivars (Gifford et al., 1984).

Such intrinsic differences in R:S ratios may be linked to specific adaptation strategies to environmental change, as differences in the habitats in which cultivated versus wild genotypes have developed may have resulted in ‘acquisitive’ (competitive, with low R:S ratios) versus ‘retentive’ (stress-tolerant, with high R:S ratios) genotypes with respect to growth rate and resource dynamics (Grime et al., 1997). The results showed that if the genotypes had contrasting C allocation patterns under ambient CO₂ concentrations, they responded to elevated CO₂ according to this ecological theory. Namely, cultivated genotypes showed their competitive nature by allocating more C to aboveground biomass production under elevated CO₂ than wild types. By contrast, the wild genotypes increased C allocation to the roots in response to elevated CO₂. Concurrently, the wild genotypes were able to acquire more N from stable SOM pools under elevated CO₂ than the cultivated genotypes. Thus, when elevated CO₂ reduces nutrient availability in the long-term, the ability of wild genotypes to effectively compete with microbes for N makes them more likely to sustain growth and C sequestration in low N input systems. This result corroborates the hypothesis that sink strength is an important plant trait for controlling the ecosystem responses to elevated CO₂.

9.4 Concluding remarks

Elevated CO₂ is expected to increase soil C sequestration, but only when soil C input continues to be stimulated by elevated CO₂. Enhanced plant production under elevated CO₂ can only be sustained when ample nutrients are available, which can be achieved by adding mineral nutrient fertilizer in agro ecosystems. However, increasing nutrient fertilization rates frequently enhances N₂O emissions from soils (Smith & Conen, 2004). The global warming potential of this greenhouse gas is approximately 300 times greater than that of CO₂ (Smith & Conen, 2004). Therefore the increased assimilation of atmospheric CO₂ due to fertilization may be offset by increased N₂O output. In addition, other commonly used management practices in agro ecosystems, such as tillage will enhance SOM decomposition, thereby counterbalancing the increased soil C
inputs. Moreover, removing the harvestable biomass will reduce soil C input under elevated CO₂, thus reducing soil C sequestration. Because of these reasons it remains to be determined how much agro ecosystems can actually contribute to mitigating global warming.

In low nutrient input, or natural ecosystems CO₂-induced nitrogen limitation is expected to diminish the impact of elevated CO₂ on plant production and soil C sequestration in the future (i.e. decades to centuries). This is an important observation when considering that natural ecosystems cover approximately 70% of the global land area (Mock, 2001). However, not many natural biomes have been represented in the FACE studies. This makes the global impact of elevated CO₂ on natural ecosystems hard to predict. In addition, not many studies have evaluated the possibility of succession and migration to climate change. Increased N immobilization and depletion under elevated CO₂ likely causes a negative plant-soil feedback (Berendse, 1994), which is expected to contribute to species replacement (Reynolds et al., 2003). In addition, palaeoecological data suggest that migration is a common response of plants to climate change (Huntley, 1991). This dissertation shows that plant species and even genotypes are profoundly different in their response to elevated CO₂, and that their roots play a major role in regulating the soil C and N cycles. Understanding how elevated CO₂ alters plant species compositions, how plant species influence rhizosphere processes, and how other plant species respond to these changes, will greatly enhance our predictive capacity on the impact of elevated CO₂ on natural ecosystems.

So far, few FACE studies have taken into account changes in other environmental variables. For example, the rise in global temperatures will increase SOM decomposition rates (Davidson & Janssens, 2006), which could counterbalance any increase in soil C sequestration or decrease in nutrient availability to elevated CO₂. In addition, increased ozone levels could inhibit photosynthesis, thereby countering the increase in plant growth to elevated CO₂ (Broadmeadow & Jackson, 2000). I believe that future research questions should consider how the interactive effects of elevated CO₂ and other environmental perturbations, such as ozone concentrations, temperature and water availability, affect species compositions in some of the most prevalent natural ecosystems. Within this research the rhizosphere should be viewed as an integral component of plant-soil interactions. This requires that plant and soil scientists integrate their knowledge to advance science conducted at the plant-soil interface. Developing methods such as compound specific mass spectrometry, soil microbial and plant genetics and microbial indicators for specific root exudates can greatly help advance this field.
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Summary

Human activities such as fossil fuel burning and deforestation may cause global warming by increasing the atmospheric concentration of the greenhouse gas CO₂. Elevated levels of atmospheric CO₂ frequently stimulate photosynthesis, resulting in greater plant production and greater soil C input. If the increased assimilation of CO₂ by plants stimulates long-term soil C sequestration, global warming may naturally be mitigated. However, whether soils will serve as CO₂ sinks under elevated CO₂ is still widely debated.

Sequestration of atmospheric CO₂ depends on complex interactions between plants and soil nutrient cycling. Accumulation of soil C requires a positive imbalance between inputs and outputs of soil organic matter (SOM). Thus, enhanced soil C sequestration under elevated CO₂, can only occur if the rate of soil C decomposition lags behind the increase in soil C input caused by elevated CO₂. However, it is unclear how elevated CO₂ will affect soil C input and decomposition. Namely, the interaction between soil C input and decomposition is not linear, since the processes are inter-reliant: i.e. soil C input affects decomposition and decomposition affects soil nutrient availability, which feeds back to the plant growth response to elevated CO₂.

In the first part of this dissertation, I explored how long-term elevated CO₂ affects soil C inputs versus SOM decomposition, and how these changes ultimately feedback to soil C sequestration. This research was carried out in a Free Air Carbon dioxide Experiment (FACE) in Switzerland that had been exposed to elevated CO₂ and N fertilization treatments for 10 years. The isotopic label of the applied CO₂ and N allowed for tracing new C and N dynamics in the system. In addition, I summarized available data related to plant growth and soil nutrient cycling from long-term CO₂-enrichment experiments using the statistical tool Meta analysis.

On the one hand, soil C sequestration might increase under elevated CO₂ due to a CO₂ induced increase in litter C:N ratios, which would reduce litter decomposition rates. By incubating litter and soil derived from Swiss FACE, I concluded that the impact of elevated CO₂ on litter quality and litter decomposition rates was minor. Therefore, elevated CO₂ is not expected to affect soil C contents through its impact on litter quality and decomposition. On the other hand, increased soil C inputs under elevated CO₂ may stimulate microbial activity and hence decomposition rates. It appeared that elevated CO₂ frequently increases SOM decomposition in long-term field experiments, suggesting that elevated CO₂ might not stimulate net soil C sequestration. The Meta analysis, however, showed that the main driver of soil C sequestration is not SOC decomposition, but soil C input through plant growth, which is strongly controlled by nutrient availability. If soil nutrient availability was high, soil C input outweighed C decomposition leading to net C sequestration. However, if soil nutrient availability was low, soil C input rates lagged behind soil C decomposition rates due to
CO2-induced nutrient immobilization, which had reduced plant growth. Thus ample nutrient availability is required for additional soil C storage under elevated CO2. In the Swiss FACE experiment however, soil C sequestration did not increase under elevated CO2, despite the high fertilization rates, concurrent increases in plant growth, and relatively low decomposition rates. This may be due to frequent harvests and shows that the potential for soil C sequestration in individual agro-ecosystems is still uncertain, due to management practices that can affect soil C input and/or soil C decomposition.

The potential for soil C sequestration in individual unfertilized/natural ecosystems is also unclear, since frequently unexplained processes appear to prevent N limitation in some of these FACE systems. These processes may occur in the rhizosphere, which is often overlooked, but plays a vital role in mechanistically coupling plant production and soil nutrient cycling. In the second part of this dissertation I focused on how rhizodeposition affects microbial regulation of soil N availability. Elevated CO2 stimulated the amounts of root-derived C and N substrates entering the soil, but without specific exudation of amino acids. Enhanced rhizodeposition was accompanied by a proportional increase in root production, suggesting that rhizodeposition under elevated CO2 only increases when root biomass production is stimulated. The increase in rhizodeposition of N under elevated CO2 comprised a significant portion of the plant assimilated N, and was quickly immobilized by microbes upon entering the soil. This shows another pathway by which elevated CO2 may enhance nutrient limitation in low N-input systems. Alternatively, elevated CO2 may alleviate N limitation by stimulating rhizodeposition induced decomposition, leading to the release of N retained in stable SOM pools. This dissertation shows that increased rhizodeposition of C under elevated CO2 may be responsible for sustained plant growth in low nutrient input FACE systems. Since this mechanism did not increase plant tissue N concentrations, and does not contribute to a net gain of ecosystem N, however, it is not expected to offset nutrient limitation under elevated CO2 in the future (i.e. decades to centuries).

A third aim of this dissertation was to increase the understanding of plant specific responses to elevated CO2. Therefore, I compared the responses of plants with genetic similarity but contrasting C allocation patterns, so reducing the number of plant traits that can explain a plants' response to elevated CO2. In addition, C allocation to roots is a key plant trait for explaining differential responses in C and N cycling as it affects both rhizodeposition and nutrient uptake. I showed that agronomic selection has resulted in a morphological tradeoff, where C allocation to organs associated with C assimilation compared to organs associated with nutrient uptake is favoured in modern cultivars. As a result modern cultivars are more likely to increase shoot biomass production under elevated CO2 than their wild relatives in fertilized ecosystems. On the other hand, greater root production and N uptake rates indicate a greater potential for sustained plant growth and soil C sequestration under elevated CO2 for the wild compared to the cultivated genotypes in low N-input systems. These data showed that sink strength is an important trait for controlling plant responses to elevated CO2.
In conclusion, elevated CO$_2$ can increase soil C sequestration when sufficient nutrients are available. The extent of the increase however is still unclear in agro-ecosystems, due to a set of management practices that affect soil C decomposition and soil C input. In unfertilized ecosystems, simultaneous increases in N demands of microbes and plants reduce nutrient availability. Increased C allocation to roots under elevated CO$_2$ will benefit nutrient acquisition and C sequestration in low N systems but this mechanism is expected to be transient. Therefore, in natural ecosystems soil C sequestration is likely to be constrained in the future (i.e. decades to centuries) by progressive nutrient limitation.
Samenvatting

Door menselijke activiteiten, zoals het verbranden van fossiele brandstof en het kappen van bossen stijgt de atmosferische concentratie van het broeikasgas koolstofdioxide (CO₂). Over het algemeen stimuleert de verhoogde CO₂ concentratie fotosynthese waardoor planten meer biomassa produceren. Het gevolg is dat de toevoer van koolstof (C) afkomstig van planten naar de bodem toeneemt. Als de extra CO₂ die via deze weg in de bodem komt, voor een lange tijd wordt vastgelegd, kan de atmosferische CO₂ concentratie op een natuurlijke manier gereduceerd worden. Op dit moment is het echter onduidelijk of de bodem als CO₂ reservoir kan dienen.

Complexe interacties tussen planten en nutrienten kringlopen bepalen of de bodem op de lange termijn CO₂ kan ophalen. De mate van C opslag in de bodem is afhankelijk van de balans tussen plantengroei en organische stof afbraak. Koolstof opslag vindt alleen plaats onder hogere CO₂ concentraties als de toevoer van C naar de bodem via plantengroei groter is dan de hoeveelheid C die weer vrijkomt na afbraak van organische stof. Op dit moment is het onduidelijk hoe de verhoogde CO₂ concentratie plantengroei en organische stof-afbraak op de lange termijn verandert. De interactie tussen plantengroei en decompositie is namelijk niet lineair, omdat de processen onderling afhankelijk zijn; m.a.w. C beschikbaarheid in de bodem beïnvloedt strooiselafbraak, strooiselafbraak beïnvloedt de beschikbaarheid van nutrienten, en de nutrienten beschikbaarheid bepaalt de groeisnelheid van planten onder hogere CO₂ concentraties.

In deel 1 van dit proefschrift heb ik onderzocht hoe plantengroei en organische stof afbraak beïnvloed worden door langdurige blootstelling aan verhoogde CO₂ concentraties en hoe veranderingen in deze processen uiteindelijk terug koppelen naar C opslag in de bodem. Dit onderzoek is deels uitgevoerd in een FACE (Free Air Carbon dioxide Enrichment) experiment in Zwitserland, waar proefvelden bestaande uit klaver en gras 10 jaar lang blootgesteld waren aan CO₂- en bemestings behandelingen. De stikstof (N) en de CO₂, die toegebracht werd door middel van beroking, waren isotopisch gelabeled, waardoor we met precieze konden meten hoe de kringlopen van nieuwe C en N waren beïnvloed door verhoogde CO₂ concentraties. Daarnaast heb ik met behulp van een statistisch programma “Meta analyse”, data betreffende plantengroei en nutrienten kringlopen afkomstig van andere CO₂ verrijkingsexperimenten geïntegreerd, om een gemiddeld effect van CO₂ op ecosystemen te berekenen.

Aan de ene kant kan de verhoogde CO₂ concentratie de strooisel afbraakshendheid verkleinen omdat strooisel afkomstig van planten gekweekt onder hoge CO₂ concentraties een hogere C:N ratio kan hebben, wat kan leiden tot CO₂ vastlegging in de bodem. In mijn studie echter, was de invloed van CO₂ op de kwaliteit en de afbraaksnelheid van strooisel minimaal. Daarbij was C vastlegging niet beïnvloed door
een reductie in strooisel kwaliteit. Dit suggereert dat de verhoogde CO₂ concentratie C vastlegging in de bodem niet zal beïnvloeden via haar effect op strooise kwaliteit. Aan de andere kant kan de afbraaksnelheid van organische stof toenemen onder hogere CO₂ concentraties, omdat een grotere toevoer van C naar de bodem de activiteit van microben kan vergroten. De afbraaksnelheid van organische stof was inderdaad groter onder verhoogde CO₂ concentraties. Desalniettemin, bleef de balans tussen invoer en uitstoot van C positief, omdat de plantengroei ook hoger was onder de verhoogde CO₂ concentratie. Deze positive balans werd echter alleen bereikt als voldoende nutrienten in de bodem beschikbaar waren om plantengroei blijvend te stimuleren. Dit komt omdat de vraag naar nutrienten van zowel planten als microben groter wordt onder hogere CO₂ concentraties, waardoor de nutrienten beschikbaarheid op de lange termijn afneemt. In bodems met een lage nutrienten beschikbaarheid kon C niet accumuleren, omdat de plantengroei achterbleef bij de afbraaksnelheid van organische stof. Voor verhoogde koolstof opslag onder verhoogde CO₂ concentraties zijn dus voldoende nutrienten nodig. Koolstof accumuleerde echter niet in het Zwitserse FACE experiment, ondanks de bemestings behandelingen, de verhoogde plantengroei en de relatief lage afbraaksnelheid van organische stof. Dit zou veroorzaakt kunnen zijn door maaien en afvoeren van de biomassa en toont aan dat zelfs onder hoger nutrienten beschikbaarheid koolstof accumulatie niet zonder meer plaats vindt.

Het is ook onduidelijk hoeveel koolstof vastgelegd kan worden in individuele natuurlijke/onbemeste ecosystemen, omdat nutrienten limitatie wordt tegen gegaan in een aantal CO₂ verrijkings experimenten door nog onbekende processes. In veel CO₂ verrijkings experimenten is het wortelstelsel van planten genegeerd, hoewel wortels een grote invloed hebben op organische stof omzetting en op nutrienten beschikbaarheid. In het tweede deel van dit proefschrift heb ik daarom de invloed van hogere CO₂ concentraties op wortel exudatie en microbiële regulatie van N bepaald. De verhoogde CO₂ concentratie vergrootte wortel exudatie van C en N, maar had geen invloed op specifieke exudatie van amino zuren. Daarbij was wortel exudatie alleen hoger als de wortel biomassa ook verhoogd was. Dit geeft aan dat hogere CO₂ concentraties wortel exudatie alleen vergroten als de totale wortel massa toeneemt. Grotere plantengroei onder hogere CO₂ concentraties verhoogde de competitie tussen bodem microben en de plant voor N. Het gevolg was dat de ge-exudeerde N vastgelegd werd door microben en dat de planten een relatief grote hoeveelheid van hun geassimileerde N verloren onder de verhoogde CO₂ concentratie. Dit suggereert dat verhoogde exudatie onder hogere CO₂ concentraties kan bijdragen aan de nutrienten limitatie. Een alternatieve hypothese is dat het N verlies via exudatie gering is vergeleken met het stimulerende effect van exudatie op organische stof afbraak. Dit proefschrift toont aan dat C-exudatie microbiële omzetting van stabiele organische stof en daarmee N beschikbaarheid stimuleert. De hoeveelheid N die op deze manier wordt vrijgemaakt echter, was niet voldoende om een reductie van de N concentratie in het planten materiaal tegen te gaan, en draagt niet bij aan een netto toename van N in het
systeem. Daarom lijkt het niet dat dit mechanisme nutrienten limitatie onder hogere CO₂ concentraties kan vermijden de toekomst (d.w.z. tientallen jaren en eeuwen).

In het derde deel van dit proefschrift heb ik onderzocht hoe we de variabiliteit in de reacties van planten op hogere CO₂ concentraties kunnen verklaren. Daarvoor heb ik planten met elkaar vergeleken die genetisch vergelijkbaar zijn maar contrasterende C distributie patronen hebben. Op die manier heb ik het aantal planten eigenschappen die de reactie van een plant op verhoogde CO₂ concentraties kan verklaren verkleind. Daarnaast, is C distributie naar het wortelstelsel een belangrijke eigenschap om te verklaren hoe planten nutrienten kringlopen beïnvloeden, omdat het wortelstelsel exudatie en nutrienten opname beïnvloedt. Ik heb aangetoond dat wilde en gecultiveerde genotypes binnen een gewas gewas contrasterende C distributie patronen hebben: wilde genotypes distribueren meer C naar hun wortels, hoewel gecultiveerde genotypes meer C naar hun bovengrondse biomassa distribueren. Het gevolg is dat de verhoogde CO₂ concentratie de bovengrondse biomassa van gecultiveerde genotypes stimuleerde, hoewel het wortelstelsel van de wilde genotypes meer gestimuleerd werd. De grotere distributie van C naar het wortelstelsel in wilde genotypes kan leiden tot hogere CO₂ opslag in de bodem vergeleken met de gecultiveerde genotypes. Dit onderzoek toont aan dat sink-strength de reactie van een plant op verhoogd CO₂ kan bepalen.

In conclusie: de bodem kan alleen C accumuleren onder hoge CO₂ concentraties als de nutrienten beschikbaarheid in de bodem voldoende is om plantengroei blijvend te stimuleren. Koolstof accumulatie is dan nog afhankelijk van menselijke activiteiten zoals bodem en gewas bewerking, die de invoer en afbraak van organische stof beïnvloeden. In natuurlijke/ onbemeste ecosystemen wordt plantengroei gelimiteerd onder een hoge CO₂ concentratie wegens voortgaande verlaging van nutrienten beschikbaarheid in de bodem. Een groter wortelstelsel kan de nutrienten limitatie niet verhelpen. Daarom is C accumulatie in onbemeste ecosystemen in de toekomst (d.w.z. tientallen jaren en eeuwen) gelimiteerd door geringe nutrientenbeschikbaarheid.
Marie-Anne de Graaff was born on the 2nd of January 1978 in Gorinchem, the Netherlands. From September 1990 to June 1996 she followed secondary education at CSG de Oude-Hoven in Gorinchem. In September 1996, Marie-Anne started her study in Forestry and Nature Management at Wageningen University. During her study she specialized in Forest and Nature Development. In 2001 and 2002, she worked on two M. Sc. Projects: the first project was conducted at the departments of Plant Ecology and Soil Quality at Wageningen University, she investigated how differential nutrient uptake mechanisms in plants control plant species coexistence in species rich grasslands. For her second project, Marie-Anne stayed at the University of California, Davis, where she studied the effect of elevated CO2 on SOM decomposition in fertilized grasslands. She received her M. Sc. degree in June 2003. Soon after that, Marie-Anne started her Ph. D. research, which was conducted at the Department of Plant Sciences at the University of California in Davis. This research started in September 2003 and was finished in July 2007.
The SENSE research school declares that Mrs. Marie-Anne de Graaff has successfully fulfilled all requirements of the educational PhD Programme of SENSE with a work load of 33 ECTS, including the following activities:

**SENSE PhD courses:**
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- Rhizosphere Ecology
- Revising Scientific Prose
- Journal Club on the N cycle
- Advanced Soil Microbiology

**Activities:**
- Soil and plant sampling at Experimental Station of the Institute of Plant Sciences ETH.
- Site specific training for use of HPLC
- Safety coordinator for 2 laboratories
- Workshop on minirhizotron techniques

**Oral presentations:**
- Decomposition of soil and plant carbon from pasture systems after 9 years of exposure to elevated CO₂. December 2003, San Francisco, USA.
- Soil C and N dynamics in natural and managed ecosystems under elevated atmospheric CO₂. November 2004. Seattle, USA.

**Poster presentations:**
- Decomposition of soil and plant carbon from pasture systems after 9 years of exposure to elevated CO₂. April 2004, Ascona, Switzerland.
- The impact of elevated atmospheric CO₂ on rhizodeposition and N cycling under wheat and corn. November 2004. Seattle, USA.
- Interactions between plant growth and soil nutrient cycling under elevated CO₂: a meta-analysis. December 2005, San Francisco, USA.

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