

Linking soil C and N dynamics in
managed ecosystems under elevated CO₂

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Linking soil C and N dynamics in
managed ecosystems under elevated CO₂

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Abstract

Rising levels of atmospheric CO₂ are expected to increase C storage in terrestrial ecosystems, thereby possibly slowing climate change. However, given the widespread N limitation of plant growth and soil C input, the potential of this natural feedback system is unclear. Therefore, this dissertation focuses on the interaction between nutrient availability and soil C storage under elevated CO₂. I studied this subject in long-term field studies on managed ecosystems, where atmospheric CO₂ concentrations are increased experimentally.

In a CO₂ enrichment experiment on fertilized Swiss grassland, N additions and atmospheric CO₂ concentrations did not significantly affect total soil C contents. However, isotopic measurements showed that the sequestration of new C and N under elevated CO₂ were highly correlated. These results suggest that new N was used to sequester new C.

In an incubation study with soils from the same field site, I found that plant material grown under elevated CO₂ decomposed more slowly than plant material grown under ambient CO₂. Depressed decomposition rates, combined with an increase in soil C input could lead to soil C storage under elevated CO₂. In theory, a CO₂-induced shift in the soil microbial community could also affect soil C storage. However, an analysis of amino sugars in soils from three different FACE experiments suggests that elevated CO₂ had only minor effects on the composition of the soil microbial population.

I used meta-analyses to synthesize available data from CO₂ enrichment experiments on soil N availability. Averaged over all experiments, elevated CO₂ does not significantly increase net N mineralization. However, it does increase microbial N demand in long-term experiments. Moreover, elevated CO₂ does not stimulate N₂ fixation, the major natural process providing soil N input, unless other essential nutrients (e.g. P, Mo, K) are added. Together, these findings suggest that soil N availability will gradually decrease under elevated CO₂.

To directly test the effect of soil N availability on soil C storage, I also summarized available experimental data on soil C contents. I found that soil C only increases under elevated CO₂ when N is added at rates well above typical atmospheric deposition. These results demonstrate that rapid C accumulations under elevated CO₂ are indeed limited by nutrient availability. Model projections that do not take into account these nutrient constraints are therefore overly optimistic.

Preface

This dissertation is the result of four exciting years of research, done mainly at the University of California, Davis, with a substantial amount of work conducted at Wageningen University as well. It would not have been possible for me to do this without the support and cooperation of a large group of people. I would like to mention some of them.

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1 General introduction

1.1 Relevance

Due to human activities such as fossil fuel burning and deforestation, atmospheric CO₂ concentrations have increased from 260 ppm in pre-industrial times to 382 ppm in February 2006 (Nösberger and Long 2006). The rise in atmospheric CO₂ in 2005 was the largest in its recorded history, and if the current rate of increase continues, atmospheric CO₂ will reach 700 ppm by the end of the 21st century. Carbon dioxide and other gases warm the surface of our planet by trapping solar heat in the atmosphere. This process is essential to keep Earth habitable; without greenhouse gases, our planet would be approximately 30°C cooler. However, mounting scientific evidence suggests that the recent increase in atmospheric CO₂ changes our climate (IPCC 2001). Global surface temperatures have been rising by 0.2°C every decade for the past 30 years (Hansen et al. 2006).

The extent of global change will not only depend on the rate of CO₂ emissions, but also on C uptake by the oceans and the land. Current estimates suggest that the accumulation of CO₂ in the atmosphere and oceans can not fully account for the increase in CO₂ emissions (IPCC 2001). It is generally assumed that the “missing CO₂” is sequestered in soils and vegetation. In other words, plants and soil could act as a C sink to counterbalance rising CO₂ levels. In this context, the soil C pool is of special interest. The Earth’s soils contain approximately $1.5 \cdot 10^{18}$ g of organic C (Batjes 1996). This is twice as much as the total amount of C in the atmosphere, or three times the amount of C in the biosphere (Schimel 1995). As the soil C pool has a relatively long retention time of 10^2 - 10^3 years (Sauerbeck 2001), it might buffer changes in atmospheric CO₂ levels.

The average concentration of CO₂ in soils is between 5 and 100 times higher than in the atmosphere (Brook et al. 1983). As such, rising levels of atmospheric CO₂ will probably not directly affect soil C pools. However, photosynthesis is typically limited by CO₂ availability, and many studies found higher plant growth rates under elevated CO₂ (Ainsworth et al. 2005; De Graaff et al. 2006). An increase in plant growth stimulates soil C input as litter and root exudates, and could in turn raise soil C contents.

By stimulating plant growth, elevated CO₂ alters the flow of C and N through ecosystems. These changes might evoke feedback processes that affect the potential for C storage in both plants and soil on longer time scales. This dissertation aims to provide insight in some of these feedback mechanisms. The next paragraph will briefly describe pools and fluxes of soil C and nutrients, and how they might change

under future CO₂ concentrations. In the last three paragraphs of this chapter, I will formulate my research questions, present research approaches to answer these questions, and outline the content of this dissertation.

1.2 Literature review

1.2.1 Soil organic matter

Soil C is contained in plant residues in various states of decomposition, as microbial and animal biomass and as necromass, in what together is defined as soil organic matter (SOM). Soil C levels are ultimately determined by the balance between the input of plant material, and losses due to decomposition, erosion and leaching. This equilibrium, in turn, depends on physical and chemical soil properties, as well as climatic conditions.

For modeling purposes, the SOM reservoir is often split up in distinct pools, with residence times ranging between a few months for the most labile, to over a thousand years for the most recalcitrant pools. The different SOM pools are mainly characterized by factors that protect them from decomposition. Soil organic matter can be protected by physical, chemical and/or biochemical factors (Christensen 1996; Six et al. 2002). Physical protection occurs through encapsulation of SOM fragments by clay particles or soil aggregates, thereby forming a physical barrier between soil microbes and their substrates. Chemical protection occurs through specific bonds of SOM with colloids or clays, and often involves highly stable organic compounds. Finally, biochemical protection occurs through the formation of recalcitrant SOM compounds during decomposition (Cadisch and Giller 1997).

Following an increase in soil C input, labile SOM fractions often show the strongest increase in C content. However, as these pools are typically small and have high turnover rates, their potential contribution to soil C storage is limited (Hungate et al. 1997a). Thus, a substantial increase in soil C requires that additional C entering the soil is stabilized in long-lived pools.

1.2.2 Soil microbial community

Although the living soil microbial community typically composes less than 3% of the total soil organic C pool (Zak et al. 1994), it forms a crucial link between SOM and plants. By decomposing SOM, microbes provide most of the annual nutrient requirement (i.e. N, P, K, Ca, Mg) of plants (Schlesinger 1997). Moreover, decomposition by microbes is the main process by which C is lost from the soil. The soil microbial community can be divided into two major groups, fungi and bacteria, which together comprise >90% of the total soil microbial biomass, and are

responsible for the majority of SOM decomposition and CO_2 formation (Six et al. 2006). Fungi and bacteria have a distinctly different role in the cycling of soil C and N. Fungi have hyphae that can transport nutrients, allowing the utilization of spatially separated sources of C and N (Frey et al. 2003). In contrast, bacteria rely on sources of C and N in their direct vicinity as their substrate.

Certain fungi called mycorrhizae form symbiotic relationships with plant roots, in which plants supply fungi with carbohydrates, and fungi supply plants with minerals and water. Mycorrhizal fungi increase the nutrient uptake efficiency of plants (Holland and Coleman 1987; Beare et al. 1992) and increase biomass production, in particular under nutrient poor conditions. Some have suggested that fungal biomass is more resistant against decomposition (Nakas and Klein 1979). For these reasons, a soil community dominated by fungi may be favorable to ecosystem C storage, but may also limit N availability.

1.2.3 Decomposition and nutrient availability

Decomposition is a general term that refers to the biochemical breakdown of organic matter. In the specific case of plant material decomposition, most of the biochemical reactions are activated by enzymes, which are released by soil microbes. While decomposing fresh plant material, soil microbes convert organic C to CO_2 , and incorporate N contents in their biomass. The latter process is known as N immobilization. Nitrogen immobilization is not restricted to N that is derived from SOM; soil microbes also accrue nutrients from the soil solution, thereby capturing N that might otherwise have been available to plants.

When the SOM substrate is exhausted, microbial growth slows and there is little further microbial N uptake. Eventually, microbes die or are preyed upon. New substrates are formed, and N contained in microbial tissue may be released as NH_4^+ . The release of nutrients in inorganic form due to decomposition of organic matter, e.g. N as NH_4^+ , is referred to as mineralization. Net mineralization of N typically occurs when C:N ratios of substrates have decreased to 30 or lower. Therefore, mineralization rates are typically low under vegetation producing litter with a high C:N ratio (Wedin and Tilman 1990).

Following mineralization, NH_4^+ is subject to five different fates in the N cycle. Besides microbial immobilization, these processes include plant uptake and fixation in clay minerals. Ammonium ions may also be transformed into NH_3 gas, and lost to the atmosphere by volatilization. Finally, some of the NH_4^+ may be oxidized to NO_2^- and subsequently to NO_3^- by a microbial process called nitrification.

Nitrogen in the NO_3^- form is highly mobile in the soil. Nitrate may be taken up by plants and microbes or may be lost from the ecosystem through leaching, or emissions of nitrogenous gases. Nitrate taken up by soil microbes is reduced to NH_4^+ and used in microbial growth. The concentrations of NH_4^+ and NO_3^- in the soil

solution are the net result of all of these processes occurring simultaneously. Thus, low concentrations of NH_4^+ do not necessarily indicate low mineralization rates; they might also be caused by rapid nitrification. Similarly, low concentrations of NO_3^- do not necessarily indicate slow nitrification; they might also indicate rapid plant uptake.

1.2.4 SOM dynamics under elevated CO_2

A rise in atmospheric CO_2 might induce feedback mechanisms that affect the potential for C storage in both plants and soil. Figure 1.1 summarizes several feedbacks that have been suggested. Elevated CO_2 can affect stabilization of SOM through some of the protection mechanisms discussed in paragraph 1.2.1. Chemical protection is largely determined by soil mineralogy, which is independent of atmospheric CO_2 concentrations. Physical protection on the other hand could play an important role. Several studies found that elevated CO_2 enhances soil aggregation (e.g. Rillig et al. 1999; Prior et al. 2004), presumably as a consequence of increased rhizodeposition, fine root growth and the presence and activity of fungi. The resulting increase in physical protection might stimulate C storage (Six et al. 2001). Elevated CO_2 might also affect the biochemical stabilization of SOM by raising litter lignin contents (Norby et al. 2001), thereby increasing the resistance against decomposition.

Due to their complex nature, some potential feedback mechanisms have not been depicted in Figure 1.1. For instance, some studies suggest that a rise in atmospheric CO_2 stimulates the soil fungal community over the soil bacterial community (Phillips et al. 2002). For reasons mentioned in paragraph 1.2.2, such a shift in the microbial community could stimulate ecosystem C storage. Also, several field studies suggest that an increase in atmospheric CO_2 affect the decomposition of native C (Hoosbeek et al. 2004; Pendall et al. 2003; Cardon et al. 2001). The “preferential substrate utilization hypothesis” (Liljeroth et al. 1994; Cheng 1999) suggests that in fertile soils, an increase in labile C under elevated CO_2 causes a microbial shift towards new C, thereby reducing the decomposition of native SOM. Under low mineral N availability, microbes would preferentially decompose native SOM. The net effect of such a shift on soil C storage remains unclear, though.

Elevated CO_2 might also affect soil C storage through its effect on soil N availability. Residues produced under elevated CO_2 are generally more limited in nutrients but rich in C (Lekkerkerk 1990). When decomposing such substrates, microbes will incorporate N in their biomass and thus decrease the soil available N pool (Diaz et al. 1993). Consequently, plant growth and thus soil C input could become N limited under elevated CO_2 (Luo et al. 2004).

Several mechanisms have been proposed that could release plant growth from N limitation under elevated CO_2 . Some studies showed that an increase in easily available C under elevated CO_2 can increase microbial activity and net N mineralization (Zak et al. 1993). Moreover, plants might grow more roots under elevated CO_2 in an effort to

acquire soil N (Pregitzer et al. 1995). Also, the main natural source of soil N input, N₂ fixation, might increase under elevated atmospheric CO₂ (Zanetti et al. 1996). The relative importance of these processes in determining ecosystem responses to rising atmospheric CO₂ is unclear. Yet, as plant growth in most terrestrial ecosystems is N-limited (Vitousek and Howarth 1991), higher biomass production in a CO₂-rich world can only be sustained if the soil supplies plants with additional N (Zak et al. 2000; Luo et al. 2004).

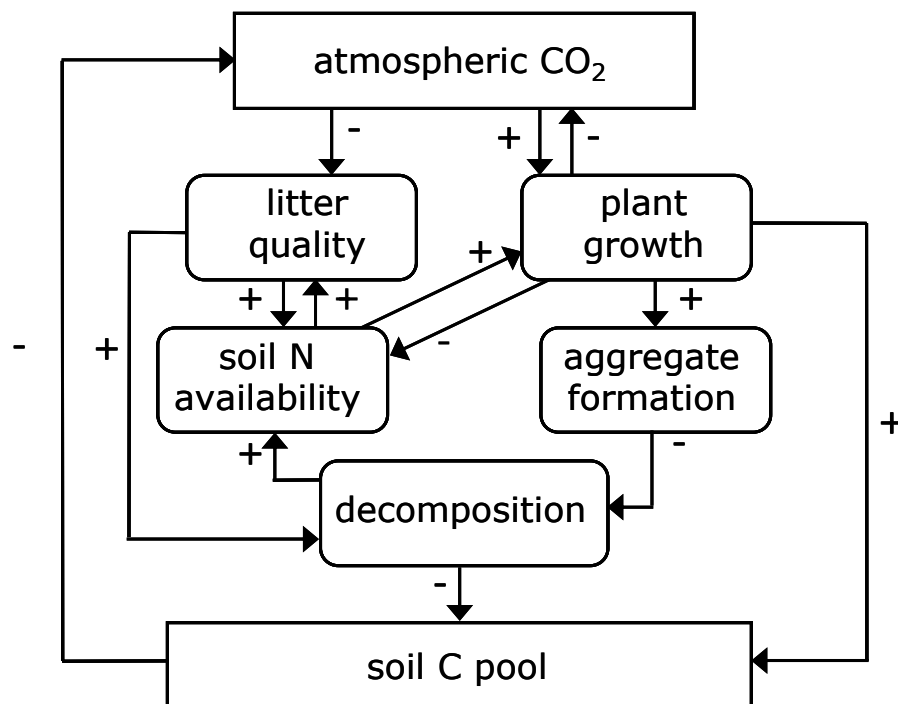


Figure 1.1: A signed diagram depicting feedbacks between atmospheric CO₂ concentrations, plant growth and SOM dynamics. Plus and minus signs indicate positive and negative relationships between pool sizes and process rates. The net effect of a feedback mechanism can be found by multiplying signs of all arrows involved.

Nitrogen limitation of plant growth can be reduced by adding N fertilizer. Indeed, several studies found that the addition of mineral N increases plant growth, and thus soil C input, under elevated CO₂ (Curtis and Wang 1998; Oren et al. 2001). However, fertilizer N additions can also increase losses of soil C under elevated CO₂ by stimulating decomposition rates (Rice et al. 1994; Niklaus and Körner 1996). Thus, the net effect of N additions on soil C storage under elevated CO₂ remains unclear. As atmospheric depositions of N are likely to double over the next 50 years (Galloway et al. 2004), this knowledge gap causes uncertainty in the projections for future soil C storage (Hungate et al. 2003).

1.3 Motivation and objectives

The current rise in atmospheric CO₂ is generally expected to increase plant growth, thereby altering the flow of C and N through terrestrial ecosystems. In order to predict the effect of elevated CO₂ on the ecosystem level, we need to quantify the various interactions between soil C- and N pools, plants and the soil microbial population. Many efforts have been made in this field, but these were often frustrated by spatial variability and the large size of organic matter pools relative to the fluxes of C and nutrients. Several studies found that plant growth under elevated CO₂ was limited by N availability, suggesting that soil C sinks might be constrained by nutrient availability. Nonetheless, the role of fertilizer N and symbiotically fixed N in sequestering soil C under elevated CO₂ received little attention. Given these research needs, the main objectives of this dissertation are:

- To determine the impact of long-term elevated atmospheric CO₂ concentrations on C sequestration in physically protected SOM pools.
- To determine the quantitative and qualitative effects of prolonged increases in atmospheric CO₂ and N applications on the soil microbial community.
- To determine the effects of prolonged increases in atmospheric CO₂ on soil N availability.
- To determine the role of N application and symbiotically fixed N in sequestering C under elevated CO₂.
- To summarize the effect of elevated CO₂ on SOM dynamics over a wide range of ecosystems.

1.4 Research techniques

1.4.1 CO₂ enrichment studies

Indoor studies

The first CO₂ enrichment studies were conducted indoors, in growth chambers and glass houses. Whereas these studies can provide valuable insight in plant physiological responses to increased CO₂ concentrations, they are less well suited to study questions regarding CO₂ responses of SOM dynamics. First, indoor studies are typically conducted on simplified ecosystems in microcosms or small pots. Yet, the impact of rising CO₂ levels on C and N dynamics in terrestrial ecosystems depends on a set of complex interactions between soil, microbes and plants. Second, indoor studies are typically conducted under optimized climatic conditions, evoking plant CO₂ responses that are probably larger than what can be expected in the real world. Finally, indoor studies typically last for only one or two growing seasons. Yet, the establishment of

equilibrium between SOM input and decomposition can take up to decades or longer. Therefore, only long-term experiments under realistic field situations will allow us to make predictions of changes in ecosystems under future CO₂ concentrations.

Field studies

In the field, the effect of elevated CO₂ on plants and soil is studied in the vicinity of natural CO₂ vents or in controlled CO₂ fumigation experiments. Natural CO₂ vents are often present for several decades, and allow us to study the prolonged effect of elevated CO₂ concentration in relatively undisturbed conditions. However, natural vents often also release SO₂ and other sulfuric gases that may harm plants. Moreover, atmospheric CO₂ concentrations near natural vents show strong temporal and spatial variation, making it difficult to determine average CO₂ concentrations for each sampling point. Studies on natural CO₂ vents will therefore not be considered in this dissertation.

The two methods most often used for controlled CO₂ fumigation in the field are open top chambers (OTC) and Free Air Carbon Enrichment (FACE). Rogers et al. (1983) were the first to use OTC's in an elevated CO₂ field study, thereby creating the most realistic controlled experimental conditions up to that point. However, the small size of these chambers prohibits studies of complex forest systems. Moreover, climatic conditions inside the chambers might differ from outside (Kimball et al. 1997), potentially causing artifacts. Indeed, several studies found that the placement of chambers affects SOM dynamics (Zak et al. 1993; Williams et al. 2000), independent of the CO₂ fumigation.

These limitations do not apply to FACE experiments, as they enable to increase the atmospheric CO₂ concentration over large field plots without using an enclosure. In FACE experiments, ecosystems are surrounded by a ring of pipes venting CO₂. In order to maintain a relative constant CO₂ concentration within the ring, the fumigation by individual pipes is adjusted depending on wind direction and speed. The first FACE-experiment was established in 1989 in Maricopa, AZ, subjecting plots of cotton and wheat to elevated CO₂ and irrigation and fertilizer treatments (Hendrey 1993). Over the last two decades, many OTC and FACE experiments have been conducted, covering a wide range of terrestrial ecosystems.

Most FACE and OTC experiments are conducted on managed ecosystems. In these systems, experimental manipulations of the growing conditions, such as the supply of mineral fertilizers, offer the possibility to detect interactions with other growth factors. Moreover, managed ecosystems typically consist of homogeneous vegetation, planted in field where soil and topography are also relatively uniform. As a result, between-plot variation might be minimized, allowing a higher degree of statistical sensitivity than in natural ecosystems. These systems therefore serve as model, yet real-world systems, where hypotheses of elevated CO₂ effects may be tested.

Soil characteristics related to C and N cycling have been studied in most of these experiments, but no clear pattern has emerged that allows us to generalize about CO₂ enrichment effects on SOM dynamics (Zak et al. 2000). The effects elevated CO₂ are difficult to discern in individual experiments because of spatial variability in soil C and nutrients, and because of the large amount of C in the soil relative to input rates. Several research techniques can tackle these problems, the most important of which will be briefly introduced in the following paragraphs.

1.4.2 Analytical tools

Meta-analyses

Individual experiments that study the effect of elevated CO₂ are often marred by high uncertainty (e.g. Hungate et al. 1996). By combining results from many experiments, one might identify CO₂ effects that go unnoticed in individual studies. One statistical method to summarize research data is called meta-analysis. This technique combines experimental observations from independent studies to calculate average treatment effects (Hedges and Olkin 1985). Observations are weighted, so that studies that are more likely to yield representative CO₂ effects (i.e. well replicated studies of long duration) contribute more strongly to the overall average.

Meta-analytic methods enable placing confidence intervals around effect sizes; therefore they provide a robust statistical test for overall CO₂ effects across multiple studies (Curtis and Wang 1998). Moreover, they enable to test whether categories of studies significantly differ in their mean CO₂ response (Hedges and Olkin 1985). Meta-analyses have recently been used to summarize the effect of elevated CO₂ on plant physiology, crop productivity, litter quality and decomposition rates (Curtis and Wang 1998; Ainsworth et al. 2002; Norby et al. 2001; Long et al. 2006), but not on soil characteristics related to SOM dynamics.

Isotopic measurements

In individual experiments, the sensitivity to identify changes in soil C concentrations might be improved by tracing new C entering the soil. One technique exploits differences in the isotopic signal of C pools. In nature, C can occur as the stable isotopes ¹²C and ¹³C, and as the isotope ¹⁴C. Most of the C (98.8%) occurs as ¹²C, with 1.1% occurring as ¹³C and a tiny amount as ¹⁴C. Using a simple isotopic dilution model, one can calculate the contribution of C sources with different isotopic concentrations to a given C pool. In SOM research, differences in the ¹³C-concentration of C₃ and C₄ plants (Jastrow et al. 1996; Collins et al. 1999), or between plants grown under ambient and elevated, ¹³C depleted CO₂ (Leavitt et al. 1994; Nitschelm et al. 1997; Van Kessel et al. 2000a,b; Schlesinger and Lichter 2001) are used to calculate the contribution of new C to the total soil C pool.

The ^{13}C concentration of a C pool is typically expressed in δ units (‰):

$$\delta^{13}\text{C} = 1000 * \left(\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \quad (1.1)$$

where $R = ^{13}\text{C}/^{12}\text{C}$, using the Vienna-Pee Dee Belemnite (V-PDB) as standard. The fraction of C turnover, f_{C} , is calculated by using the isotope mass balance method (Balesdent et al. 1987):

$$f_{\text{C}} = \frac{\delta_2 - \delta_0}{\delta_1 - \delta_0} \quad (1.2)$$

In the specific case of CO_2 enrichment experiments, δ_2 and δ_0 are $\delta^{13}\text{C}$ values for SOM in the fumigated and ambient plots and δ_1 is the average $\delta^{13}\text{C}$ value of the soil C input in the fumigated plots. The application of ^{13}C depleted CO_2 in field studies does not only allow for tracing newly sequestered C in the whole soil, but can also be applied to trace new C in soil fractions such as aggregate size classes (Six et al. 2001) and amino sugars (Glaser and Gross 2005).

Using a similar mixing model, plant material enriched in ^{14}C can be traced in the soil. As ^{14}C is virtually absent in native SOM, ^{14}C labeled material can be traced with a high accuracy. Several studies used ^{14}C -labeling to assess the effect of elevated CO_2 on C fluxes in plant/soil systems (e.g. Lekkerkerk et al. 1990, Van Ginkel et al. 2000). However, as these studies involve high ^{14}C concentrations, they are restricted to laboratory experiments under artificial conditions. Moreover, the additional precaution required to protect oneself against radiation makes these experiments relatively laborious.

Similar to C, N can be traced into SOM pools using isotopic labeling. Nitrogen occurs as the stable isotopes ^{14}N and ^{15}N . By enriching fertilizer N in ^{15}N , the fraction of fertilizer derived N in the soil sample, f_{N} , can be calculated using the following formula (Warembourg 1993):

$$f_{\text{N}} = \frac{^{15}\text{N atom}\%_{\text{excess SOM}}}{^{15}\text{N atom}\%_{\text{excess fertilizer}}} \quad (1.3)$$

Soil fractionation

Changes in the labile soil C pool can be a precursor for soil C dynamics in more stable pools. Therefore, soil fractionation techniques that separate labile and recalcitrant soil C pools are useful to determine changes in the dynamics of C and N (Hungate et al. 1996).

Several researchers attempted to separate soil into meaningful pools, using chemical and/or physical fractionation techniques. Physical soil fractionation techniques are based on the assumption that the spatial distribution of SOM causes differing degrees of protection from microbial activity and decomposition (see Chapter 1.2). Tisdall and Oades (1982) and Oades (1984) proposed that SOM associated with large aggregates would be less persistent than SOM associated with smaller aggregates. Tiessen and Stewart (1983) reported particulate organic matter (POM) in the sand fraction had the highest turn over rate; slower turnover rates were found in smaller POM fractions. Based on these findings, Six et al. (1998) proposed a SOM model based on aggregate size classes, each class being subjected to different kinds of protection against decomposition. This model predicts increasing turnover rates with increasing iPOM (intra-aggregate particulate organic matter) sizes in all aggregate sizes, and relatively high turnover rates for macro aggregates.

Several studies reported isotopic evidence that macro aggregates are associated with relatively high C contents and fast turnover rates, whereas low SOM contents and more persistent C are associated with microaggregates (Puget et al. 1995; Angers and Giroux 1996; Jastrow et al. 1996). Using ^{13}C labeling, C turnover rates have also been found to increase with POM size classes (Six et al. 2001).

Microbiological analyses

As noted in Chapter 1.2, microbes play a central role in the soil C cycle. Thus, changes in the soil microbial community may affect soil C storage in the long-term and could serve as an early indicator for changing soil C dynamics. Several quantitative and qualitative analyses enable one to characterize the soil microbial community. Quantitative analyses on microbial biomass are usually carried out by one of several techniques involving chloroform fumigation. Microbial activity is typically determined by short-term incubations, or by measurements of CO_2 fluxes in the field.

In addition to these quantitative assessments, the microbial community can also be characterized by qualitative analyses. Most of these analyses focus on living soil microbes. For instance, microbial communities can be described by the range of C sources they can utilize. Other qualitative analyses focus on RNA and DNA, or cell compounds such as phospholipid fatty acids (PLFA) and amino sugars.

Soil microbes adapt quickly to changes in temperature, moisture and C availability, causing seasonal shifts in the soil microbial community composition. Thus, a single sampling of the living microbial population may not fully reveal its response to prolonged elevated atmospheric CO_2 . As microbial cell walls turn over more slowly than living microbial biomass (Guggenberger et al. 1999), their abundance might be a useful indicator of time-averaged soil microbial responses. Fungal and bacterial cell walls contain different types of amino sugars. As such, the

relative abundance of these sugars can be used to determine fungal and bacterial contributions to SOM dynamics (Amelung 2001).

1.5 Outline

Based on further literature research which is presented in Chapters 2 through 7, the objectives stated in Chapter 1.3 resulted in the following hypotheses:

- H1:** Increased soil C input and decreased rates of litter decomposition will cause soil C contents to increase under elevated atmospheric CO₂ concentrations.
- H2:** Increased soil C input under elevated CO₂ decreases the availability of N in soils.
- H3:** Additional N input stimulates plant growth and C sequestration under elevated atmospheric CO₂.
- H4:** Under elevated atmospheric CO₂ more soil C ends up in pools with slow turnover rates.
- H5:** Under elevated atmospheric CO₂ soil aggregation and the ratio fungal:bacterial biomass will increase, making soils more conducive to soil C storage.

These hypotheses are central to the remainder of this dissertation. Chapters 2 through 4 focus on a FACE experiment situated in fertilized Swiss grassland (Figure 1.2). This experimental site is of high scientific value for a number of reasons. Firstly, as



Figure 1.2: One of the CO₂-fumigated rings at the Swiss FACE site. Photo by Manuel Schneider.

temperate grasslands contain approximately 12% of all soil C in the world (IPCC 2001), they represent a large C pool. Secondly, the species grown at this site are all herbaceous perennials, which have a relatively short residence time of assimilated C. Thus, changes in plant growth quickly translate into changes in SOM input. For this reason, the potential effects of elevated CO₂ on SOM dynamics should be relatively easy to detect.

Thirdly, at ten years, the Swiss FACE experiment is the longest running CO₂ enrichment field experiment of its kind. From 1993 to 2002, half of the experimental plots at this site were fumigated with CO₂. Long-term experiments provide more reliable data, as SOM input and output processes have more time to equilibrate following a step increase in atmospheric CO₂ (Hungate et al. 1996). Finally, both the CO₂ used for fumigation and the fertilizer N applied at this site were isotopically labeled, allowing me to trace both new C and new N into the soil.

Chapter 2 focuses on the fate of newly sequestered C and N under *Lolium perenne* grown at this site. I traced new C and N in several aggregate size fractions, which supposedly represent SOM pools with different turnover rates. Chapter 3 reports on similar measurements in plots planted with *Trifolium repens*, an N₂-fixing legume. By comparing SOM dynamics under *T. repens* and *L. perenne*, I investigated the role of N₂ fixation on SOM dynamics and C sequestration under elevated atmospheric CO₂.

Chapter 4 reports on an incubation experiment involving soil from the Swiss FACE experiment. By incubating ¹⁴C labeled plant material grown under two different CO₂ concentrations, I could trace the added C in the CO₂ respired in soils from both the ambient and the fumigated plots. Thus, I could separate between the effect of elevated CO₂ on decomposition through its effect on litter quality, and through its long-term effect on the soil itself.

Chapters 5 through 7 involve multiple CO₂ enrichment experiments, allowing me to test hypotheses over a wider range of ecosystems. Chapter 5 reports on amino sugar contents in soil from three different FACE experiments. I used the relative content of soil amino sugars as an indicator for changes in the microbial community under elevated CO₂. As the Swiss FACE experiment includes N fertilization treatments, I could also investigate the impact of soil N availability on the soil microbial community.

Chapters 6 and 7 apply meta-analytic techniques to summarize results from all available CO₂ enrichment experiments. Chapter 6 limits itself to experiments under field conditions. In this chapter, I summarized the effect of elevated CO₂ on a number of soil characteristics related to soil N availability and microbial activity. Chapter 7 includes available experimental data on soil C contents, to test the effect of soil N availability on soil C storage under elevated CO₂.

This thesis ends with a general discussion, in which I synthesize findings of all previous chapters. I will also discuss the use and limitations of field experiments in making future predictions on terrestrial C sinks.

2 Linking sequestration of ^{13}C and ^{15}N in aggregates in a pasture soil following 8 years of elevated atmospheric CO_2

Abstract

The influence of N availability on C sequestration under prolonged elevated CO_2 in terrestrial ecosystems remains unclear. We studied the relationships between C and N dynamics in a pasture seeded to *Lolium perenne* after 8 years of elevated atmospheric CO_2 concentration (FACE) conditions. Fertilizer- ^{15}N was applied at a rate of 140 and 560 kg N ha $^{-1}$ yr $^{-1}$ and depleted ^{13}C - CO_2 was used to increase the CO_2 concentration to 60 Pa p CO_2 . The ^{13}C - ^{15}N dual isotopic tracer enabled us to study the dynamics of newly sequestered C and N in the soil by aggregate size and fractions of particulate organic matter (POM), made up by intra-aggregate POM (iPOM) and free light fraction (LF). Eight years of elevated CO_2 did not increase total C content in any of the POM fractions at both rates of N application. The fraction of new C in the POM fractions also remained largely unaffected by N fertilization. Changes in the fractions of new C and new N (fertilizer N) under elevated CO_2 were more pronounced between POM classes than between aggregate size classes. Hence, changes in the dynamics of soil C and N cycling are easier to detect in the POM fractions than in the whole aggregates. Within N treatments, fractions of new C and N in POM classes were highly correlated with more new C and N in large POM fractions and less in the smaller POM fractions. Isotopic data show that the microaggregates were derived from the macro-aggregates and that the C and N associated with the microaggregates turned over slower than the C and N associated with the macroaggregates. There was also isotopic evidence that N immobilized by soil microorganisms was an important source of N in the iPOM fractions. In plots receiving 140 kg N ha $^{-1}$ yr $^{-1}$, 3.04 units of new C per unit of fertilizer N were sequestered in the POM fractions. At the highest N fertilization rate, the ratio of new C sequestered per unit of fertilizer N was reduced to 1.47. Elevated and ambient CO_2 concentrations lead to similar ^{15}N enrichments in the iPOM fractions under both low and high N additions, suggesting that the SOM-N dynamics were unaffected by prolonged elevated CO_2 concentrations.

Based on: Van Groenigen KJ, Harris D, Horwath WR, Hartwig UA, Van Kessel C (2002). *Global Change Biology* 11, 1094-1108.

2.1 Introduction

The role of soil N in controlling net C sequestration in terrestrial ecosystems following an increase in atmospheric CO₂ concentration remains a widely debated topic. Increased atmospheric CO₂ concentration is predicted to stimulate primary production, leading to increased C input to the soil (Van Veen et al. 1991). It has been suggested that increased C assimilation by plants and its subsequent sequestration in the soil could offset increases in atmospheric CO₂ (Gifford 1994). However, until recently, limited research has been done on the influence of nutrient availability on soil C sequestration. Low N availability in particular will limit plant growth in many terrestrial ecosystems (Vitousek and Howarth 1991), thereby potentially constraining potential soil C inputs. Therefore, an assessment of future net C sequestration should consider the limitations imposed by available nutrients in the soil.

Several studies have reported that both increased N applications and elevated CO₂ enhance plant growth; however, the long-term combined effect of these treatments on total soil C is unclear. Rice et al. (1994) reported a significant increase in soil organic C (SOC) in tallgrass prairie ecosystems under elevated CO₂ within 3 years after N fertilization. Conversely, Van Kessel et al. (2000a,b) found no effect of either N fertilization or elevated CO₂ on total soil organic matter (SOM) under *Lolium perenne* and *Trifolium repens* after 4 and 6 years of free air carbon enrichment (FACE) conditions.

Positive priming effects of N fertilization on soil C and N or both have been reported (Kuzyakov et al. 2000). However, residues produced under elevated CO₂ are generally more limited in nutrients but rich in C, which can lead to a negative priming effect (Lekkerkerk et al. 1990). In a meta-analysis of data collected from field experiments under elevated CO₂, a decline in leaf litter N concentration was observed but changes in the rate of decomposition were small (Norby et al. 2001). The relationship between N and C sequestration under elevated CO₂ therefore remains uncertain, but is essential to accurately predict soil C sequestration.

Soil fractionation techniques that separate labile from recalcitrant soil C pools are useful to determine the dynamics of C and N (Hungate et al. 1996). Physical soil fractionation is based on the assumption that the spatial distribution of SOM causes differing degrees of protection from microbial activity and decomposition. Differences in physical protection of the various SOM fractions control the dynamics and turnover of C and N in the soil. Tisdall and Oades (1982) and Oades (1984) proposed that the nature, location and dynamics of SOM vary with aggregate size. Soil organic matter associated with large aggregates would be less persistent than SOM associated with smaller aggregates. Six et al. (1998) proposed a conceptual model for aggregate and SOM dynamics that described the position of intra-aggregate particulate organic matter (iPOM) and free light fraction (LF) in the aggregates. The model suggests increasing turnover rates with increasing iPOM sizes in all aggregate sizes.

Isotopic labelling of both C and N allows for tracing newly sequestered C (Balesdent et al. 1987) and N (Warembourg 1993) into SOM pools. Using a simple isotopic dilution model, differences in ¹³C-signature between C3 and C4 plants (Jastrow et al. 1996; Collins et al. 1999), or between plants grown under ambient and elevated, ¹³C depleted CO₂ (Leavitt et al. 1994; Nitschelm et al. 1997; Van Kessel et al. 2000a,b; Schlesinger and Lichter 2001) were used to trace newly sequestered C. Several studies reported isotopic evidence that macroaggregates are associated with relatively high C contents and fast turnover rates, whereas low SOM contents and more persistent C are associated with microaggregates (Puget et al. 1995; Angers and Giroux 1996; Jastrow et al. 1996). Tiessen and Stewart (1983) reported particulate organic matter (POM) in the sand fraction had the highest turnover rate; slower turnover rates were found in smaller POM fractions.

While physical fractionation has been repeatedly used in isotopic research on C dynamics, less attention has been given to the dynamics of added N. From combined isotopic ¹³C¹⁵N studies, it appears that the dynamics of C and N sequestration are only partly linked. Angers et al. (1997) measured incorporation of C and N in water-stable aggregates in a field experiment using ¹³C¹⁵N labeled wheat straw. They reported that a larger proportion of ¹⁵N than ¹³C was recovered in the < 50 µm fraction, which came at the expense of larger fractions (> 50 µm fractions). The high recovery of ¹⁵N in the < 50 µm SOM fraction was partly explained by the presence of soluble N in the straw.

Loiseau and Soussana (2000) studied the effects of elevated CO₂ and temperature on N fluxes in a temperate grassland system. After 28 months, elevated CO₂ concentrations increased immobilization of labeled N in macroaggregates, and decreased N uptake by the grass sward. In contrast, a 3°C temperature increase under elevated CO₂ stimulated N availability and improved N uptake of the sward. However, the relative short duration of the experiment and the absence of a direct link with ¹³C data prohibits predictions of the long-term influence of added N on C sequestration.

The link between C and N dynamics under prolonged elevated CO₂ under field or FACE conditions has not been adequately addressed. Therefore, the main objective of this study was to determine the impact of long-term exposure, i.e. 8 years, of pasture soil to elevated CO₂ on C and N sequestration in physically protected SOM pools. The role of additional N application in sequestering newly fixed C and N under elevated CO₂ was also determined.

2.2 Materials and methods

Site description

The FACE experiment is situated at the Swiss Federal Institute of Technology (ETH) field station in Eschikon, 20 km NE of Zurich. The experiment has been in operation since May 1993. It consists of six 18 m rings: three control rings with ambient air (35 Pa pCO₂), and three rings that receive additional CO₂ (60 Pa pCO₂) during daytime. The $\delta^{13}\text{C}$ signal of the additional CO₂ was approximately -48‰ in the first 2 years and -45‰ in subsequent years. The soil was a clay loam, fertile, eutric cambisol. Experimental plots of *L. perenne* were established using a split plot design with elevated CO₂ as the main plot treatment and N-fertilizer as the subplot treatment, replicated three times. Fertilization rates are 140 kg ha⁻¹ and 560 kg ha⁻¹ N, applied as NH₄NO₃ in four equal splits during the growing season following cutting of swards (Zanetti et al. 1996). The ¹⁵N atom percentage excess was 1.3 for the low N-treatment and 0.3 for the high N-treatment. Subplot treatments are randomized within each ring. Further details about the experimental site are reported elsewhere (Zanetti et al. 1996; Hebeisen et al. 1997).

Sample analysis

Samples were collected in the fall of 2000 at a depth of 0-10 cm. Four 2-cm diameter cores were collected and bulked from each plot. Soil samples were air-dried, after which large roots and stones were removed by hand.

The method for isolation of free light fraction (LF), intra aggregate particulate organic matter (iPOM) and mineral-associated organic matter (mSOM) was adapted from Six et al. (1998). The fractionations are summarized in Table 2.1. Four aggregate sizes were separated using wet sieving through a series of sieves (2000 μm , 250 μm and 53 μm). An air-dried sample of 80 g was evenly spread on top of the 2000 μm sieve and submerged for 5 min in deionized water. The sieve was mechanically shaken up and down 1.5 cm in the water 50 times during a period of 2 min. Afterwards, the intact aggregates were washed off the sieve into an aluminium pan. Floating organic material > 2000 μm was decanted and discarded since, by definition, this is not considered to be SOM (Six et al. 1998). The solution that contained the smaller aggregates was poured onto the 250 μm sieve, and the sieving procedure was repeated. This time, floating material was retained. The procedure was repeated for the 53 μm sieve, so each sample produced four aggregate size classes (> 2000 μm , 250-2000 μm , 53-250 μm and < 53 μm). All fractions were dried at 50°C and weighed.

Free LF and iPOM classes were isolated by density flotation and sieving of the aggregates, respectively. A 5-g subsample of each aggregate size was weighed in a 50-mL conical centrifuge tube and suspended in 35 mL of a 1.85-g cm⁻³ NaI solution. The suspended subsample was mixed without disrupting the aggregates by gently

tilting the tube by hand (10 strokes). Centrifuge tubes were placed under vacuum in a dessicator for 10 min to evacuate air entrapped within the aggregates. Samples were centrifuged (1250 g) at 20°C for 60 min. Floating material defined as free LF was aspirated onto a preweighed 1.2-micron nylon filter, mounted onto a millipore glass filter. The filter with LF was rinsed with demineralized water to remove excess NaI, transferred into a preweighed small aluminium pan, and dried at 50°C. After 72 h, each filter was weighed. Glass filters with LF were cut in half and folded in two separate 12 × 5 mm tin capsules. The remaining heavy fraction in the centrifuge tube

Table 2.1: The physical soil fractions obtained from a pasture soil exposed to elevated CO₂ for 8 years.

Aggregate size	Density fraction (1.85 g cm ⁻³)	Sieving of broken-down aggregates
1) Macroaggregates (> 2000 µm)	1.1) free LF	
	1.2) heavy fraction	1.2.1) mSOM (< 53 µm) 1.2.2) fine iPOM + sand (53-250 µm) 1.2.3) coarse iPOM + sand (>250 µm)
2) Macroaggregates (250-2000 µm)	2.1) free LF	
	2.2) heavy fraction	2.2.1) mSOM (< 53 µm) 2.2.2) fine iPOM + sand (53-250 µm) 2.2.3) coarse iPOM + sand (>250 µm)
3) Microaggregates (53-250 µm)	2.1) free LF	
	2.2) heavy fraction	3.2.1) mSOM (< 53 µm) 3.2.2) fine iPOM + sand (53-250 µm)
4) Microaggregates (< 53 µm)		

*Free LF = free light fraction ; POM = Particulate organic matter ; iPOM = intra-aggregate POM ;
SOM = Soil organic matter ; mSOM = mineral associated soil organic matter*

was rinsed two times with deionized water and dispersed by shaking in 0.5% hexametaphosphate on a reciprocal shaker for 18 h. After dispersal, the heavy fraction was passed through 2000 µm, 250 µm and 53 µm sieves, depending on the aggregate size being analysed. The material on the sieve, defined as iPOM was rinsed with 500 mL of demineralized water. The iPOM was back washed into an aluminium pan, dried at 50°C and weighed. The iPOM in the size classes 250-2000 µm and > 2000 µm that were derived from the > 2000 µm aggregates were pooled for C and N measurements.

The C and N contents and δ¹³C and atom%¹⁵N were measured for all aggregate size classes, mSOM, iPOM classes and LF. Except for the LF, sample preparation

prior to isotopic analyses was similar for all samples. Three grams of each sample was ballmilled and sub-samples of 30 mg were weighed into Ag capsules. Soil carbonates were removed using HCl fumigation as described by Harris et al. (2001). Total C, total N, $\delta^{13}\text{C}$ and atom% ^{15}N were determined at the UC Davis Stable Isotope Facility using a continuous flow, isotope ratio mass spectrometer (CF-IRMS, PDZ-Europa Scientific, Sandbach, UK) interfaced with a CN sample converter.

Data analyses

Results of the C isotope analysis are expressed in δ units (‰):

$$\delta^{13}\text{C} = 1000 * \left(\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \quad (2.1)$$

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

The $\delta^{13}\text{C}$ values were determined in relation to Vienna-Pee Dee Belemnite (V-PDB). The fraction of C turnover, f_C , is calculated by using the isotope mass balance method (Balesdent et al. 1987):

$$f_C = \frac{\delta_2 - \delta_0}{\delta_1 - \delta_0} \quad (2.2)$$

where δ_2 and δ_0 are $\delta^{13}\text{C}$ values for SOM pools in the fumigated and ambient plots and δ_1 is the average $\delta^{13}\text{C}$ value of *L. perenne* roots and litter grown in the fumigated plots, as reported by Van Kessel et al. (2000b).

The fraction of new N (N from fertilizer, that is) in the sample, f_N , was calculated according to Warembourg (1993):

$$f_N = \frac{{}^{15}\text{N atom}\%_{\text{excess SOM}}}{{}^{15}\text{N atom}\%_{\text{excess fertilizer}}} \quad (2.3)$$

Statistical analysis

The results of the experiment were analysed as a split-plot design (multiple splits), with CO_2 as the main plot treatment and fertilizer N as subplot treatment. An ANOVA was conducted with blocks and fractions as random effects, and treatments as fixed effects. Differences between means were tested using the Least Significant Difference (LSD); significance was determined at a level of $P < 0.05$.

2.3 Results

Whole soil

Across all treatments, the mean total soil C content ranged from 3114 to 3834 g C m⁻². No significant differences between treatments were found (Table 2.2). Eight years of elevated CO₂ significantly decreased the ¹³C signature for both N treatments. On average, the fumigated soils were 3.05‰ more depleted in ¹³C than soils under ambient CO₂. Based on these data, the fraction of newly sequestered C equals 0.23 for both N treatments (Table 2.2). The mean total soil N content was not significantly

Table 2.2: Total soil C content, ¹³C-signatures, and the fraction of new C (f_C) of a pasture soil (0-10 cm depth) as affected by elevated CO₂ and N availability (n=3). The f_C values for elevated CO₂ plots were calculated according to Formula 2.2, using ¹³C-signatures of SOM in the ambient CO₂ plots with the same N treatment as a reference.

Treatments				
CO ₂	N	C content (g C m ⁻²)	δC ¹³ (‰)	f_C
Ambient	High	3433 ± 636†	-27.17 ± 0.31	-
	Low	3733 ± 463	-27.18 ± 0.31	-
Elevated	High	3114 ± 517	-30.25 ± 0.36	0.23 ± 0.04
	Low	3834 ± 254	-30.21 ± 0.38	0.23 ± 0.03
ANOVA				
CO ₂		NS	**	-
N		NS	NS	NS
CO ₂ *N		NS	NS	-

† Mean ± SE; *,** Significant at the 0.05 and 0.01 probability level, respectively.

Table 2.3: Total soil N content, ¹⁵N-signatures, the fraction of new N (f_N) and the C:N ratio of a pasture soil (0-10 cm depth) as affected by elevated CO₂ and N availability (n=3).

Treatments					
CO ₂	N	N content (g N m ⁻²)	Atom% ¹⁵ N excess	f_N	C:N
Ambient	High	329 ± 58	0.03 ± 0.00	0.11 ± 0.01	10.4 ± 1.2
	Low	362 ± 44	0.06 ± 0.00	0.04 ± 0.00	10.3 ± 1.2
Elevated	High	331 ± 44	0.06 ± 0.00	0.18 ± 0.01	9.5 ± 0.6
	Low	377 ± 16	0.09 ± 0.01	0.07 ± 0.01	10.3 ± 0.6
ANOVA					
CO ₂		NS	*	**	NS
N		NS	*	**	NS
CO ₂ *N		NS	NS	NS	NS

† Mean ± SE; *,** Significant at the 0.05 and 0.01 probability level, respectively.

different among N treatments, but the fraction of fertilizer N was significantly increased by high N additions and by elevated CO₂. Likewise, the atom percentage excess of ¹⁵N increased for elevated CO₂ treatments. The C:N ratio ranged from 9.5 to 10.4, and was not significantly affected by CO₂ or N treatments (Table 2.3).

Aggregate size distribution

For each combination of subtreatments, at least 96% of the soil remained aggregated upon wet sieving. For all treatments, the aggregate distribution was dominated by macroaggregates (80-88%). The weight distribution among macroaggregates was significantly affected by CO₂ treatment and N additions. Both for fumigated and ambient treatments, high N additions caused a decrease in macroaggregates > 2000 µm accompanied by an increase in macroaggregates 250-2000 µm compared to the low N plots (Figure 2.1). High N caused a significant decrease in microaggregates 53-250 µm under ambient conditions. Within both N treatments, elevated CO₂ caused a similar shift in distribution among macroaggregates compared to ambient CO₂.

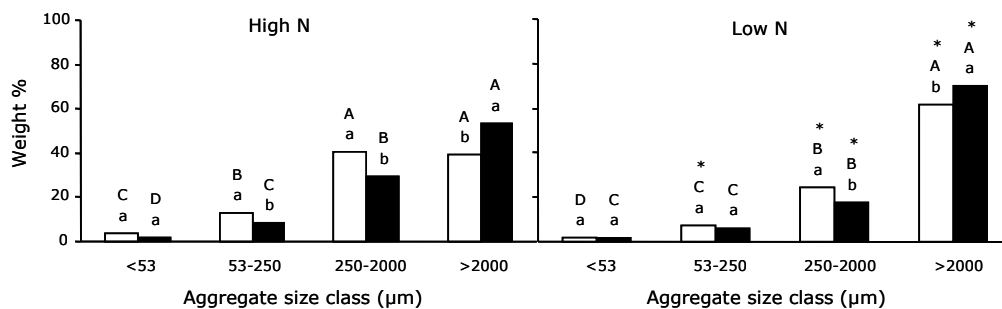


Figure 2.1: Weight distribution among aggregate size classes under high and low N additions for both ambient (light bars) and elevated CO₂ (dark bars) treatments. Values followed by a different lowercase letter are significantly different within aggregate size and among CO₂ treatment. Values followed by a different capital are significantly different within CO₂ treatment and among aggregate size. Values followed by * are significantly different within aggregate size and among N treatment.

Aggregates: C and N contents

In all treatments, C and N contents of microaggregates < 53 µm were significantly lower than in larger aggregates (Figures 2.2 and 2.3). In the low N treatments, C and N contents did not show significant differences within aggregate size and among CO₂ treatments. Only within treatments of elevated CO₂ and high N, macroaggregates contained significantly more C and N than microaggregates.

Among the treatments receiving high additional N, elevated CO₂ significantly increased the C content in aggregates > 2000 μm and the N content in aggregates 250-2000 and > 2000 μm (Figures 2.2 and 2.3). Among elevated CO₂ treatments, an increase in additional N caused a significant decrease in C content of aggregates in the 53-250 μm class compared to the low N treatment (Figure 2.2). For all treatments, C:N ratios of the microaggregates < 53 μm material were significantly lower than for all other aggregates size classes. No differences were found among aggregate classes within any treatment, and no differences within aggregate size among treatments were observed (Table 2.4).

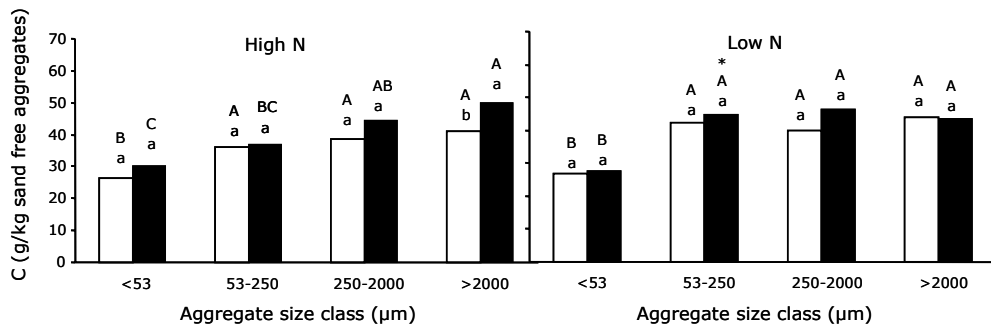


Figure 2.2: Carbon concentrations of aggregate size classes under high and low N additions for both ambient (light bars) and elevated CO₂ (dark bars) treatments. See Figure 2.1 for the explanation of the symbols.

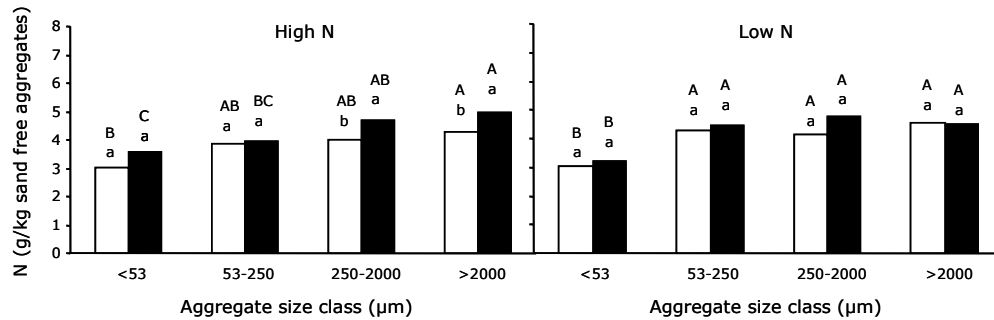


Figure 2.3: Nitrogen concentrations of aggregate size classes under high and low N additions for both ambient (light bars) and elevated CO₂ (dark bars) treatments. See Figure 2.1 for the explanation of the symbols.

Table 2.4: Total soil C and N content, ^{13}C - and ^{15}N -signatures and C:N ratio of soil fractions as affected by elevated CO_2 and N treatments (n=3).

Nitrogen Treatment	Aggregate size (μm)	SOM class (μm)	C (g/kg sand free aggregates)		$\delta\text{C}^{13}(\text{‰})$		N (g/kg sand free aggregates)		Atom% ^{15}N excess		C:N	
			Ambient	+ CO_2	Ambient	+ CO_2	Ambient	+ CO_2	Ambient	+ CO_2	Ambient	+ CO_2
High	>2000		40.7 \pm 5.5	49.1 \pm 3.6	-26.96 \pm 0.32	-31.13 \pm 1.08	4.27 \pm 0.65	4.96 \pm 0.41	0.04 \pm 0.01	0.05 \pm 0.01	9.5 \pm 0.2	9.9 \pm 0.4
		mSOM	34.4 \pm 6.0	34.9 \pm 4.6	-26.40 \pm 0.40	-29.17 \pm 0.24	3.70 \pm 0.54	4.05 \pm 0.57	0.03 \pm 0.00	0.03 \pm 0.00	8.5 \pm 0.2	8.4 \pm 0.1
		53-250	5.9 \pm 1.3	6.9 \pm 1.5	-27.73 \pm 0.13	-33.35 \pm 0.78	0.52 \pm 0.14	0.62 \pm 0.19	0.06 \pm 0.01	0.07 \pm 0.01	11.5 \pm 0.8	12.0 \pm 1.0
		>250	3.2 \pm 0.3	4.6 \pm 0.9	-28.36 \pm 0.18	-36.11 \pm 0.84	0.21 \pm 0.04	0.27 \pm 0.04	0.09 \pm 0.01	0.12 \pm 0.01	15.5 \pm 1.3	16.5 \pm 1.3
		free LF	0.2 \pm 0.1	0.4 \pm 0.2	-28.37 \pm 0.10	-35.46 \pm 1.10	0.01 \pm 0.00	0.02 \pm 0.01	0.11 \pm 0.01	0.12 \pm 0.01	16.4 \pm 1.1	16.4 \pm 0.8
	250-2000		38.6 \pm 6.9	44.3 \pm 6.2	-27.04 \pm 0.22	-30.21 \pm 0.35	3.99 \pm 0.73	4.72 \pm 0.76	0.03 \pm 0.00	0.04 \pm 0.00	9.7 \pm 0.2	9.4 \pm 0.2
		mSOM	28.2 \pm 4.6	34.0 \pm 5.1	-26.84 \pm 0.09	-28.41 \pm 0.10	3.18 \pm 0.52	3.89 \pm 0.58	0.02 \pm 0.00	0.02 \pm 0.00	8.8 \pm 0.1	8.8 \pm 0.0
		53-250	4.5 \pm 1.3	5.9 \pm 1.3	-27.76 \pm 0.06	-32.50 \pm 0.23	0.39 \pm 0.13	0.52 \pm 0.14	0.05 \pm 0.01	0.06 \pm 0.00	12.2 \pm 1.3	11.8 \pm 0.8
		>250	2.0 \pm 0.5	3.5 \pm 0.1	-28.05 \pm 0.13	-34.47 \pm 0.78	0.13 \pm 0.02	0.25 \pm 0.02	0.08 \pm 0.01	0.09 \pm 0.01	15.3 \pm 1.8	12.7 \pm 0.8
		free LF	0.4 \pm 0.0	0.7 \pm 0.1	-28.61 \pm 0.09	-35.70 \pm 0.85	0.03 \pm 0.00	0.03 \pm 0.00	0.11 \pm 0.02	0.12 \pm 0.01	15.6 \pm 1.4	17.6 \pm 0.6
	53-250		36.2 \pm 8.4	36.9 \pm 7.1	-27.32 \pm 0.20	-29.38 \pm 0.38	3.87 \pm 0.73	3.94 \pm 0.74	0.03 \pm 0.00	0.03 \pm 0.00	9.4 \pm 0.0	9.3 \pm 0.2
		mSOM	30.0 \pm 4.7	30.7 \pm 4.5	-26.70 \pm 0.15	-27.87 \pm 0.37	3.28 \pm 0.52	3.30 \pm 0.50	0.02 \pm 0.00	0.02 \pm 0.00	9.3 \pm 0.3	9.3 \pm 0.3
		53-250	9.6 \pm 4.2	8.8 \pm 2.8	-27.72 \pm 0.21	-32.01 \pm 0.19	0.89 \pm 0.13	0.78 \pm 0.28	0.04 \pm 0.01	0.05 \pm 0.01	12.6 \pm 2.0	11.8 \pm 0.6
		free LF	0.3 \pm 0.1	0.4 \pm 0.2	-27.62 \pm 0.16	-32.89 \pm 0.31	0.02 \pm 0.02	0.03 \pm 0.01	0.07 \pm 0.02	0.08 \pm 0.00	14.4 \pm 1.4	13.7 \pm 0.9
	<53		26.6 \pm 1.9	30.3 \pm 4.4	-26.72 \pm 0.05	-28.19 \pm 0.05	3.01 \pm 0.26	3.60 \pm 0.53	0.03 \pm 0.00	0.04 \pm 0.00	8.9 \pm 0.2	8.4 \pm 0.2
Low	>2000		43.3 \pm 7.8	43.1 \pm 5.1	-27.55 \pm 0.18	-30.28 \pm 0.85	4.57 \pm 0.83	4.50 \pm 0.65	0.07 \pm 0.01	0.08 \pm 0.02	9.5 \pm 0.0	9.6 \pm 0.3
		mSOM	35.1 \pm 7.7	33.2 \pm 6.2	-26.33 \pm 0.59	-28.26 \pm 0.12	3.86 \pm 0.67	3.78 \pm 0.72	0.05 \pm 0.01	0.04 \pm 0.01	9.0 \pm 0.6	8.8 \pm 0.1
		53-250	7.6 \pm 3.4	6.1 \pm 2.0	-28.24 \pm 0.58	-31.90 \pm 0.49	0.70 \pm 0.33	0.56 \pm 0.22	0.10 \pm 0.01	0.10 \pm 0.01	11.7 \pm 1.0	11.9 \pm 1.2
		>250	4.0 \pm 1.4	2.9 \pm 0.7	-28.76 \pm 0.22	-34.45 \pm 0.85	0.24 \pm 0.09	0.20 \pm 0.07	0.20 \pm 0.03	0.16 \pm 0.02	17.5 \pm 2.0	15.4 \pm 2.2
		free LF	0.1 \pm 0.0	0.3 \pm 0.1	-28.42 \pm 0.23	-33.15 \pm 1.16	0.00 \pm 0.00	0.02 \pm 0.00	0.22 \pm 0.02	0.16 \pm 0.01	18.6 \pm 0.6	16.7 \pm 1.5
	250-2000		40.0 \pm 5.8	46.4 \pm 9.0	-27.63 \pm 0.16	-30.22 \pm 0.82	4.14 \pm 0.60	4.79 \pm 1.08	0.07 \pm 0.01	0.09 \pm 0.01	9.7 \pm 0.1	9.7 \pm 0.3
		mSOM	29.5 \pm 4.6	32.7 \pm 4.7	-26.96 \pm 0.21	-28.83 \pm 0.17	3.23 \pm 0.52	3.64 \pm 0.58	0.05 \pm 0.01	0.06 \pm 0.01	9.1 \pm 0.1	9.0 \pm 0.2
		53-250	5.6 \pm 1.7	6.9 \pm 1.8	-28.07 \pm 0.05	-32.96 \pm 0.79	0.46 \pm 0.15	0.59 \pm 0.20	0.12 \pm 0.01	0.13 \pm 0.02	12.6 \pm 0.8	12.3 \pm 1.1
		>250	3.1 \pm 0.5	5.7 \pm 1.6	-28.41 \pm 0.28	-34.54 \pm 0.78	0.21 \pm 0.04	0.44 \pm 0.18	0.15 \pm 0.03	0.20 \pm 0.02	15.6 \pm 1.7	14.0 \pm 0.8
		free LF	0.9 \pm 0.3	0.9 \pm 0.2	-29.98 \pm 0.55	-36.91 \pm 0.73	0.05 \pm 0.02	0.05 \pm 0.01	0.21 \pm 0.01	0.25 \pm 0.03	17.6 \pm 0.9	18.6 \pm 0.9
	53-250		42.5 \pm 8.4	44.7 \pm 6.9	-27.63 \pm 0.22	-29.92 \pm 0.65	4.28 \pm 0.76	4.48 \pm 0.93	0.06 \pm 0.01	0.08 \pm 0.01	9.9 \pm 0.2	10.0 \pm 0.7
		mSOM	30.3 \pm 4.5	29.0 \pm 6.1	-26.83 \pm 0.16	-28.93 \pm 0.14	3.07 \pm 0.47	2.93 \pm 0.75	0.04 \pm 0.01	0.06 \pm 0.01	9.9 \pm 0.1	9.9 \pm 0.4
		53-250	12.7 \pm 4.4	12.4 \pm 2.6	-27.90 \pm 0.36	-32.86 \pm 0.36	1.15 \pm 0.45	1.06 \pm 0.29	0.10 \pm 0.02	0.13 \pm 0.02	11.8 \pm 1.0	12.0 \pm 0.6
		free LF	0.5 \pm 0.3	0.3 \pm 0.1	-28.38 \pm 0.37	-34.19 \pm 0.43	0.03 \pm 0.02	0.02 \pm 0.01	0.18 \pm 0.03	0.21 \pm 0.03	17.1 \pm 1.4	17.0 \pm 1.2
	<53		26.8 \pm 1.9	27.6 \pm 3.7	-26.64 \pm 0.10	-28.86 \pm 0.36	3.04 \pm 0.19	3.24 \pm 0.51	0.07 \pm 0.01	0.08 \pm 0.01	8.8 \pm 0.0	8.5 \pm 0.3

\dagger Mean \pm SE

Table 2.4. continued: ANOVA

		C (g/kg sand free aggregates)	$\delta C^{13}(\text{‰})$	N (g/kg sand free aggregates)	Atom% ^{15}N excess	C:N
CO ₂		NS	**	NS	NS	NS
N		NS	NS	NS	**	**
Fraction		**	**	**	**	**
CO ₂ *N		NS	NS	NS	NS	NS
CO ₂ *Fraction		NS	*	NS	NS	NS
N*Fraction		NS	*	NS	NS	NS
CO ₂ *N*Fraction		NS	NS	NS	NS	NS
Between aggregates	< 53 / 53-250	**	**	**	NS	NS
	53-250 / 250-2000	NS	NS	NS	NS	NS
	250-2000 / > 2000	NS	NS	NS	NS	NS
Within aggregates	53-250	**	**	**	**	**
between POM	250-2000	**	**	**	**	**
	> 2000	**	**	**	**	**

*,** Significant at the 0.05 and 0.01 probability level, respectively.

Aggregates: ^{13}C and ^{15}N

For both N treatments, $\delta^{13}\text{C}$ values of all aggregate size classes decreased under elevated CO_2 compared to ambient conditions, indicating the overall presence of newly sequestered C (Table 2.4). Within ambient CO_2 treatments, microaggregates $< 53 \mu\text{m}$ had significantly higher $\delta^{13}\text{C}$ values than all other aggregate size classes, with no significant differences in $\delta^{13}\text{C}$ values between aggregate size classes $> 53 \mu\text{m}$. Yet, in CO_2 -fumigated plots that received high N additions, $\delta^{13}\text{C}$ values decreased with increased aggregate size. As a consequence, N fertilizer application significantly affected the f_c values of the different aggregate size classes (Figure 2.4). Whereas macroaggregates had significantly higher f_c values than microaggregates under high N

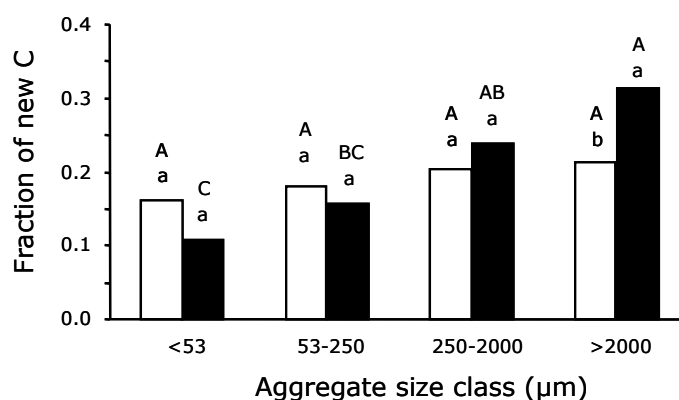


Figure 2.4: Fraction of new carbon (f_c) of aggregate size classes under high (dark bars) and low (light bars) N additions. Values followed by a different lowercase letter are significantly different within aggregate size and among N treatment. Values followed by a different capital letter are significantly different within N treatment and among aggregate size.

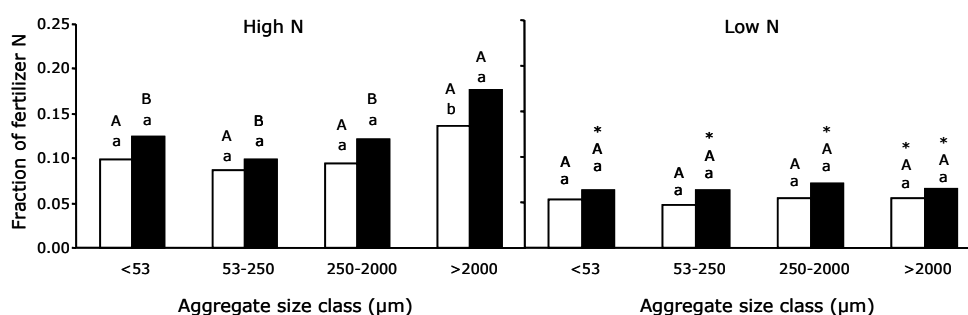


Figure 2.5: Fraction of new nitrogen (f_N) of aggregate size classes under high and low N additions for both ambient (light bars) and elevated CO_2 (dark bars) treatments. See Figure 2.1 for the explanation of the symbols.

additions, no differences among aggregate size classes were found for low N treatments. In aggregates > 2000 µm, high N additions caused a significant increase in f_C values compared to the low N treatment.

The atom percentage excess of ¹⁵N showed little variation among aggregate size classes. As a consequence, f_N values showed less variation among aggregate sizes than f_C values. No differences in f_N values between microaggregates < 53 µm and all other aggregate sizes were found for low N treatments (Figure 2.5). The f_N values increased significantly with increased N additions in all aggregate sizes under elevated CO₂, and in the macroaggregates under ambient CO₂. Although elevated CO₂ increased the

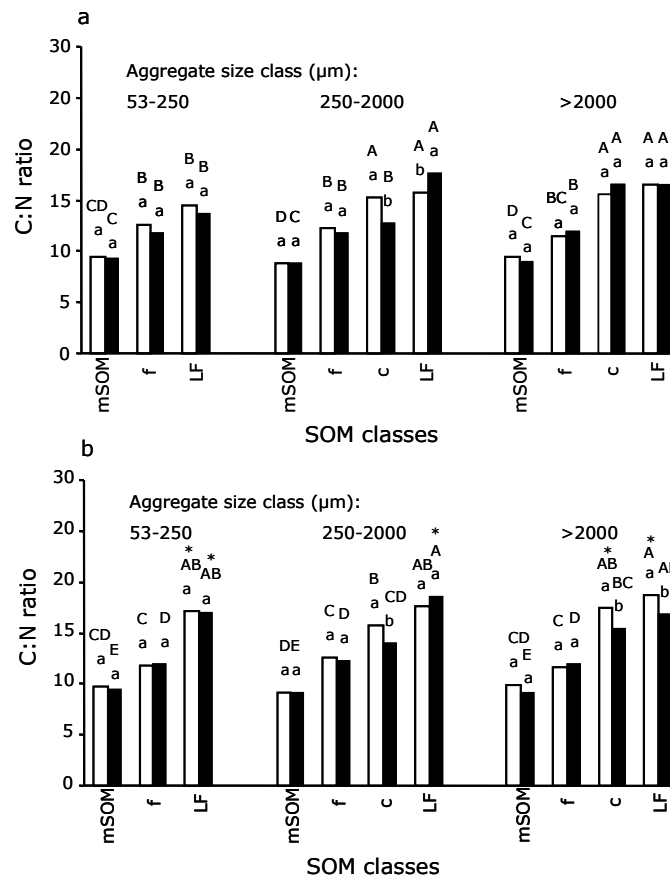


Figure 2.6 a,b: C:N ratio of free LF, iPOM 53-250 µm (f), iPOM > 250 µm (c) and mSOM of aggregate size classes under high (a) and low (b) N additions for both ambient (light bars) and elevated CO₂ (dark bars). Values followed by a different lowercase letter are significantly different within SOM class and among CO₂ treatment. Values followed by a different capital letter are significantly different within CO₂ treatment and among SOM class. Values followed by * are significantly different within SOM class and CO₂ treatment, and among N treatment.

average f_N in all aggregate sizes for both N rates, a significant difference was only found for aggregates $> 2000 \mu\text{m}$ in plots receiving high additions of N (Figure 2.5).

POM: C, N and C:N ratios

For all treatments, the relative contribution of the mSOM to total soil C and N is the highest in microaggregates (Table 2.4). Within each aggregate size class of each treatment, the C:N ratio significantly increased for larger iPOM size classes (Figures 2.6a,b). Among free LF, the C:N ratio in microaggregates was lower than in macroaggregates for high N treatments (Figure 2.6a). Within iPOM and among aggregate sizes no significant differences in the C:N ratio were found for low N treatments (Figure 2.6b).

Significant differences in C:N within iPOM size class and among treatments were confined to coarse iPOM of macroaggregates. Within plots treatment that received high additional N, elevated CO_2 caused a significant decrease of the C:N ratio in the largest iPOM class of the $250\text{--}2000 \mu\text{m}$ aggregates (Figure 2.6a). Within low N treatments, elevated CO_2 decreased the C:N ratio of coarse iPOM in both macroaggregate classes.

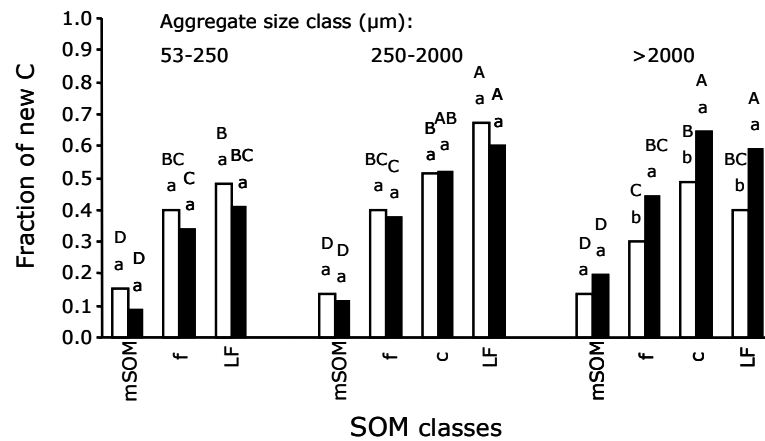


Figure 2.7: Fraction of new C (f_c) in free LF, iPOM $53\text{--}250 \mu\text{m}$ (f), iPOM $> 250 \mu\text{m}$ (c) and mSOM of aggregate size classes under high (dark bars) low (light bars) N additions. Values followed by a different lowercase letter are significantly different within SOM class and among N treatment. Values followed by a different capital letter are significantly different within N treatment and among SOM class.

POM and mSOM: ^{13}C and ^{15}N

Under ambient CO_2 , $\delta^{13}\text{C}$ values of mSOM were significantly lower than iPOM within each aggregate size (Table 2.4). No significant differences within iPOM classes in any aggregate size class are found for the ambient CO_2 treatments. Elevated CO_2

decreased $\delta^{13}\text{C}$ values of iPOM and LF in all aggregate sizes for both N treatments (Table 2.4). For all elevated CO₂ treatments, $\delta^{13}\text{C}$ values increased for smaller iPOM classes within each aggregate size class, resulting in significantly smaller f_c values for fine iPOM (Figure 2.7). In all aggregate sizes, f_c values were the lowest for mSOM.

The distribution of fertilizer N within POM and mSOM classes largely resembles the distribution of newly sequestered C. Within the high N treatments, the atom percentage ^{15}N excess of mSOM was significantly lower than POM, and fine iPOM had lower values than coarse iPOM within all aggregate classes (Figure 2.8a). Under low N additions, the same pattern emerges, though differences between fine and coarse iPOM are significant only within aggregates > 2000 μm . Compared to the low N fertilizer application, the high rate of N fertilizer application increased the fraction of new N in all SOM fractions with the exception of mSOM of the microaggregates.

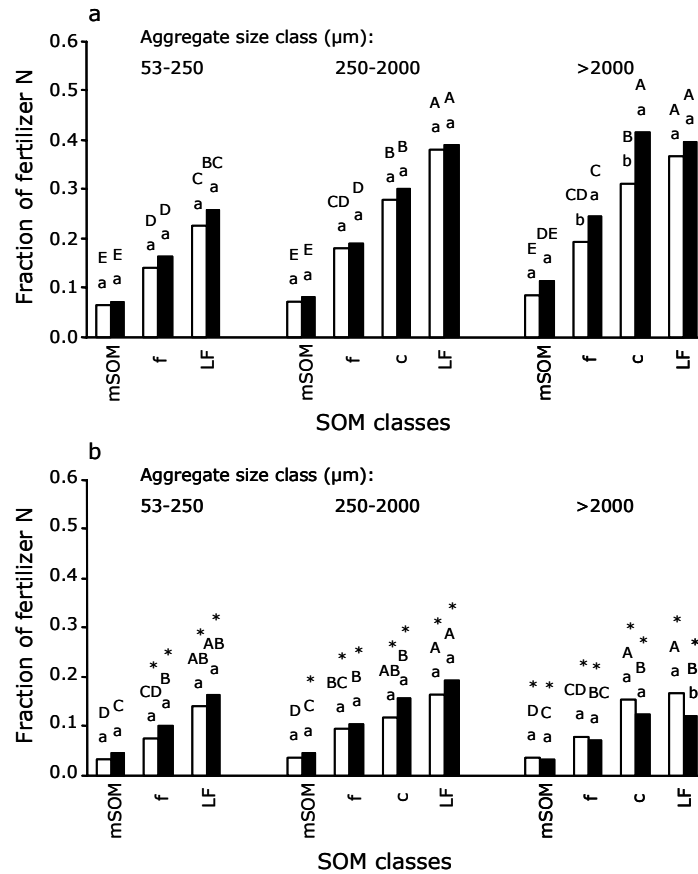


Figure 2.8 a,b: Fraction of fertilizer N (f_N) in free LF, iPOM 53-250 μm (f), iPOM > 250 μm (c) and mSOM of aggregate size classes under high (a) and low (b) N additions for both ambient (light bars) and elevated CO₂ (dark bars). See Figure 2.6 for the explanation of the symbols.

In all aggregate sizes, free LF has f_C and f_N values that are slightly higher or similar to coarse iPOM (Figures 2.7, 2.8a,b). In contrast to iPOM, free LF shows significant differences among aggregate size classes; f_C and f_N values of free LF are lowest in the aggregates 53-250 μm .

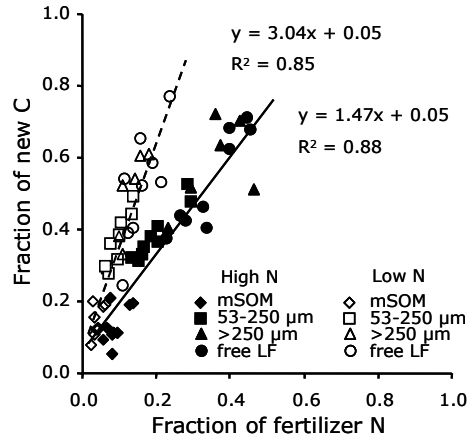


Figure 2.9: Fraction of new carbon against fraction of fertilizer N in free LF, iPOM 53-250 μm (f), iPOM > 250 μm (c) and mSOM of all aggregate size classes, for elevated CO_2 treatments under low and high N additions.

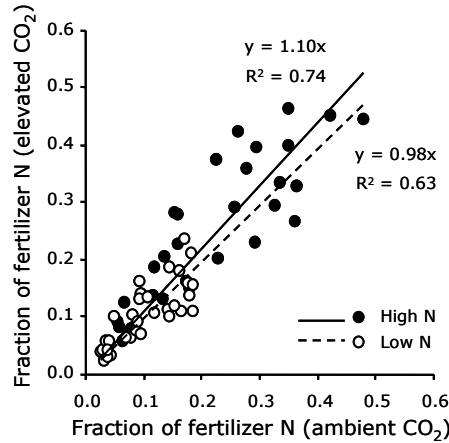


Figure 2.10: Fraction of fertilizer N (f_N) under ambient CO_2 against f_N under elevated CO_2 in free LF, iPOM and mSOM of all aggregate size classes, under low and high N additions.

After 8 years of elevated CO_2 , the $f_C : f_N$ ratio was 1.47 under high N availability and increased to 3.04 under low N availability (Figure 2.9). There was a highly significant correlation between the amount of new C and N in the iPOM fractions which was independent of the amount of N fertilizer applied (Figure 2.9). Eight years of elevated

CO₂ did not significantly affect the fraction of old N that was replaced by fertilizer N, regardless of N treatment (Figure 2.10).

2.4 Discussion

Whole soil

There was no significant effect of 8 years of elevated CO₂ on total soil C content at either level of N additions. Within both N treatments, an average of 23% of total soil C was new C. From the same FACE experiment, Van Kessel et al. (2000b) reported that after 6 years 24% of the total soil C pool was new C. It appears that new C sequestration had reached equilibrium within 6 years of elevated atmospheric CO₂. This observation corroborates recent results of Hungate et al. (1996) and Niklaus et al. (1998) that showed no significant changes in total soil C content when grasslands were exposed to elevated CO₂ for 3 years. A similar conclusion was reached for unfertilized forest soils where the potential to sequester additional C was considered to be limited (Schlesinger and Lichter 2001). Others, however, have found increased net C sequestration following the exposure of grasses and crops to elevated CO₂ (Leavitt et al. 1994; Rice et al. 1994). Possible differences in soil mineralogy and biomass production between the various studies may have caused these observed differences. Higher clay activity may protect C from microbial activity leading to an increase in total soil C content (Hassink 1997).

However, caution is warranted interpreting net C sequestration under different treatments by comparing total SOC. An absolute increase in the total soil C is difficult to detect because the size of the original C pool is often large (Hungate et al. 1996). Furthermore, spatial variation in total soil C content lowers the sensitivity of the experiment to detect a change in total C content. In addition, small changes in soil bulk density may also interfere with detecting SOC changes. For these reasons, validating changes in SOC storage remains problematic.

Aggregates - weight distribution

Both elevated CO₂ and low N additions significantly increased the level of soil aggregation in our experiment. These results are in agreement with Six et al. (2001), who reported an increase in large macroaggregates after 6 years of elevated CO₂ in *L. perenne* plots in the same experiment. The data cannot be compared quantitatively on the aggregate level, because of differences in the pretreatment of the samples. Dry sieving before wet sieving was eliminated in the current study in order to avoid the possible breakdown of macroaggregates into microaggregates and overall aggregate destruction.

Rillig et al. (1999) reported an increase of aggregate sizes in two Mediterranean grassland systems exposed to elevated CO₂. They suggested that the shift in aggregate size distribution was caused by increased mycorrhizal production of glomalin, which promotes soil aggregation. The shift in weight distribution among macro aggregate size classes (250-2000 µm and > 2000 µm) between N and CO₂ treatments in our experiment might be explained by changes in root growth. Macroaggregates are mainly associated with roots (Tisdall and Oades 1982) and root growth is known to respond to N availability and atmospheric CO₂ concentrations. A 92% increase in root growth for *L. perenne* after 2 years of elevated CO₂ was observed (Van Ginkel et al. 1996). In our FACE experiment, Hebeisen et al. (1997) and Hartwig et al. (2000) reported that the ratio of root/shoot yield for *L. perenne* increased under elevated CO₂ which was related to an increase in N limitation promoted by elevated CO₂ (Zanetti et al. 1997).

Aggregates - C and N dynamics

The f_c values significantly decreased for smaller aggregate sizes within high N plots (Figure 2.4). Although average f_c values also decreased at low N treatments, this trend was not significant. Between high and low N additions, aggregates > 2000 µm showed a significant increase in f_c values.

Compared to newly sequestered C, new N was distributed more evenly over the aggregate sizes. Elevated CO₂ increased f_N in whole soil samples, but this increase was only found in large macroaggregates under high N treatments. Differences between whole soil and aggregate size classes can be explained by the discarding of floating organic matter during the physical fractionation. This floating material consisted of relatively fresh plant material, which has high f_N values. At high N additions, f_c decreases for smaller aggregate size classes whereas f_N did not change significantly. Apparently, the additional N was distributed relatively fast to the microaggregates, which are associated with stable C. These results corroborate with earlier findings of a fast sequestering of new N in micro aggregate sizes (Balabane 1996). Angers et al. (1997) also reported that a greater proportion of ¹⁵N than ¹³C was recovered in the < 50 µm fraction after the incorporation of ¹³C¹⁵N labeled wheat straw. Since microbial biomass is mainly associated with microaggregates (Tisdall and Oades 1982), the greater proportion of new N relative to new C within the microaggregates might be caused by microbial immobilization of the new N.

Other studies report differences in C contents and turnover rates between aggregate size classes, and significantly more macroaggregates (Puget et al. 1995; Angers and Giroux 1996; Jastrow et al. 1996). The high level of aggregation in our experiment might explain the absence of significant differences in f_c values between aggregates. The effect of increased aggregation on the distribution of f_c values under low N additions did not cause differences in f_c values between micro- and macroaggregates (Figure 2.4). An increase in aggregation in low N treatment

compared to high N treatment (Figure 2.1) is related to the incorporation of microaggregates into macroaggregates, thereby increasing the relative contribution of old C to its SOM pool. Organic matter that would end up in the smaller aggregates if a more destructive physical fractionation method was used, remains in macroaggregates, thereby reducing differences in the f_C values between different aggregates. This implies that one should be cautious when studying SOM dynamics by solely comparing characteristics of aggregate size classes between treatments. Changes in SOM content and turnover rates at the level of aggregate size classes might well be caused by differences in aggregation instead of a true change in SOM dynamics.

Free LF and iPOM

Within both N fertilizer treatments, the f_C and f_N values and the C:N ratios among iPOM size classes showed clear differences within each aggregate size class. For both ambient and elevated CO₂ treatments, coarse iPOM tend to have higher C:N ratios and f_C and f_N values than fine iPOM in all aggregate classes. This trend probably reflects the degree to which SOM has been processed by microorganisms (Feigl et al. 1995). Differing degrees of decomposition were also reflected by $\delta^{13}C$ values of microaggregates < 53 μm and mSOM under ambient CO₂ concentrations, which were 1-1.5‰ enriched to the coarser fractions (Table 2.4). The enrichment in ^{13}C in the mSOM fraction can be explained by isotopic fractionation during decomposition (Dzurec et al. 1985).

Differences in C:N ratio, f_C and f_N values between mSOM and POM classes within each aggregate size are more pronounced than differences between total aggregate size classes (Table 2.4). The procedure of POM fractionation is less elaborate and easier to standardize compared to fractionation of soil into macro- and microaggregates. This makes the physical fractionation of POM an important and easy tool for studying SOM dynamics. Our results are in agreement with Sohi et al. (2001), who analysed SOM pools yielded by density-size and size-only fractionations using ^{13}C nuclear magnetic resonance. They concluded that a density-size fractionation yielding free and intra-aggregate fractions provide a sound basis for a model of SOM turnover based on measurable pools.

The absence of significant differences in f_C values within POM and mSOM classes among aggregate sizes (Table 2.4), suggest that differences in f_C between aggregate size classes are merely caused by differences in the contribution of the POM and mSOM to the total soil C of each aggregate size. Indeed, the contribution of mSOM derived C to the total C content of aggregates increased for smaller size classes whereas the contribution of coarse iPOM increases for macroaggregates (Table 2.4). These results are in accordance with results of Puget et al. (1995), who proposed a simple model in which macroaggregates contain additional organic C with a faster turnover compared to microaggregates.

A significant impact of elevated CO_2 on f_N values in iPOM and LF was only found in aggregates $> 2000 \mu\text{m}$ (Figures 2.8a and 2.8b). Similarly, the impact of N addition on f_C values was mainly found for iPOM and LF in aggregates $> 2000 \mu\text{m}$. These observations emphasize the additional benefit of using both aggregate size fractionation and POM fractionation. Isotope tracer studies showed that over time labeled C is redistributed from macroaggregates to microaggregates (Angers et al. 1997) suggesting that microaggregates are formed within macroaggregates (Oades 1984). The binding agents in macroaggregates are made of labile organic matter which will degrade relatively fast, resulting in a loss of macroaggregate stability and the release of stable microaggregates. These microaggregates will then become the building blocks for new macroaggregates (Tisdall and Oades 1982; Six et al. 2000). As macroaggregates turn over faster than microaggregates (Jastrow et al. 1996), the impact of elevated CO_2 and N application on litter would appear first in the largest aggregate size class.

The f_C and f_N values of free LF are higher than for coarse iPOM of aggregate sizes $< 2000 \mu\text{m}$, and similar to coarse iPOM for aggregates $> 2000 \mu\text{m}$. These results agree with the aggregation model of Six et al. (1998) who proposed that free LF is incorporated first into macroaggregates as coarse iPOM, which is then further decomposed and fragmented into fine iPOM.

The f_C and f_N values of all POM and mSOM clearly show the dynamics of new C and N entering SOM pools (Figure 2.9). There was a strong relationship with R^2 values of 0.85 and 0.88 for low and high N additions, respectively, between new C and fertilizer N entering the POM and mSOM fractions. After 8 years, the highest fraction of new C and fertilizer N was present in the largest iPOM fractions and in the LF. The lowest fractions of new C and fertilizer N were found in the fractions $< 53 \mu\text{m}$.

Sequestration of new C and N

Whereas all C in stable SOM fractions is ultimately derived from plants, N can be derived from two sources; N that is associated with C in the plant litter, or the mineral N that is immobilized by soil microbes and is incorporated into cell wall material. It remains largely unclear, which of these two pathways of the incorporation of N in iPOM is the dominant one.

The increase in the absolute f_C and f_N values in the iPOM fractions following the application of N is likely caused by the increase in new soil C and fertilizer N. In this study, the growth of *L. perenne* did not respond strongly to elevated CO_2 , while N fertilization increased above ground dry matter production by 100% (Daepp et al. 2000). A similar response to elevated CO_2 and N fertilization on aboveground biomass production by *L. perenne* was also found in a related study conducted at the same site (Van Kessel et al. 2000a). In addition, a major portion of the new C and N in the soil is derived from root input (Puget and Drinkwater 2001). In this FACE

experiment, root biomass production by *L. perenne* increased significantly under elevated CO₂ (Hebeisen et al. 1997). It should be pointed out that the determination of root biomass at a particular point in time indicates only whether the total root biomass has changed under higher N input. However, it is the rate of root turnover (and other forms of below ground C deposition) that determine the amount of new C and N that enters the soil through below ground processes.

The ratio of the fractions of new C and fertilizer N (i.e., the $f_C : f_N$ ratio) in iPOM was 1.47 and 3.04 under the high and low rate of N application, respectively (Figure 2.9). However, 8 years of high N fertilizer applications did not change the C:N ratios of any of the iPOM fractions under elevated CO₂ (Figure 2.6a,b). With that, the total C and N content of the iPOM fractions remained unchanged following the addition of more N (Table 2.4). Apparently, the observed decline in the $f_C : f_N$ ratio under high N applications was not accompanied by changes in the net sequestration of C or N.

One possible mechanism that would cause an increase in the ratio of $f_C : f_N$ is dilution of the ¹⁵N-enrichment of the soil mineral N pool by ¹⁴N from mineralization of unlabeled pool of SOM. If all the new C and N had been obtained from plant input and the size of the iPOM fractions remained constant, the $f_C : f_N$ ratio would be unity. However, if N released from unlabeled sources by mineralization was captured in the POM fractions, the ratio of $f_C : f_N$ would increase. The magnitude of this effect would be larger when less ¹⁵N fertilizer was applied. When the relative contribution of fertilizer N to the soil mineral N was low, more of the N entering iPOM either via plant uptake or microbial immobilization would come from other sources, primarily via mineralization of older SOM. This dilution of the fertilizer N will increase the $f_C : f_N$ ratio. Conversely, when the input of fertilizer N was high this dilution was minimized and $f_C : f_N$ ratio remained low.

No additional sequestration of C occurred under high N treatments (Table 2.4 and Figure 2.9). If high N had increased both the input and the additional sequestration of new C, higher f_N values would be accompanied by higher f_C values. The f_C and f_N values of high the N treatments of POM and mSOM would be higher than the f_C and f_N values for the low N treatment. However, under high N, less new C was sequestered per unit of fertilizer N and the f_C value did not increase. The absence of an increase in the f_C values under high N input implies that additional N did not lead to more new C or any increase was offset by increased decomposition.

There is no consensus regarding the influence of elevated CO₂ concentrations on N dynamics. Diaz et al. (1993) proposed a feedback mechanism at the ecosystem scale, in which increased competition between microbial biomass and plants for inorganic N accounts for a decline in soil N availability. The resulting nutritional limitation on plant growth would prohibit increased C storage. Increased N fertilization might offset these effects. For example, Oren et al. (2001) reported a strong increase of growth in maturing pines after 3 years of FACE conditions, with additional N fertilization. Without fertilization, elevated CO₂ had no effect on plant

growth at sites with low soil fertility, and a marginal transient effect at sites with moderate soil fertility.

Conversely, Zak et al. (1993) reported a significant increase in N availability in the bulk soil of *Populus grandidentata* grown under elevated CO₂. This increase was explained by greater below ground C inputs, causing an increase in soil microbial biomass and enhanced rates of organic matter turnover and N availability.

In the current study, 8 years of elevated CO₂ did not significantly affect the fraction of old N that was replaced with fertilizer N in the POM fractions under both N treatments (Figure 2.10). Since total soil N in any of the SOM pools was also unaffected, our results provide isotopic evidence that prolonged elevated CO₂ does not affect N dynamics in grassland soil. While our study deals with a relatively long-term elevated CO₂ experiment, initial changes in N dynamics might have occurred. However, our results are in agreement with Randlett et al. (1996) who reported that leaf litter produced under elevated CO₂ did not alter N cycling after a 32-week incubation period. Even when the N dynamics were affected in the current study, no increase in total soil C was found in any POM fraction for both N treatments. With that, the strong decrease of the $f_C : f_N$ ratio for highly fertilized *L. perenne* (Figure 2.9) suggests that the effect of N additions on total soil C in grassland soils under elevated CO₂ will be limited.

2.5 Conclusions

The combined use of ¹³C-depleted CO₂ and ¹⁵N labeled fertilizer provided a unique opportunity to study C and N dynamics in a grassland ecosystem. After 8 years of CO₂ elevation and N additions, the dynamics of C and N were strongly related among POM and mSOM classes. Increases in f_C were consistently accompanied by increased f_N values. The differences in C:N ratio, f_C and f_N values between POM classes within each aggregate size class were more pronounced than differences between total aggregate size classes. This suggests that the physical fractionation of POM is an important tool for studying SOM dynamics. After 8 years of elevated CO₂ no net change in total C content of the soil was detected. Elevated and ambient CO₂ concentrations lead to similar ¹⁵N enrichments in the POM fractions at both low and high N additions, suggesting that the SOM-N dynamics were largely unaffected by prolonged elevated CO₂ concentrations. An increase in N additions did increase f_N values, but it did not affect total soil C contents, or the rate at which old C was replaced by new C under elevated CO₂.

3 Soil ^{13}C - ^{15}N dynamics in an N_2 -fixing clover system under long-term exposure to elevated atmospheric CO_2

Abstract

Reduced soil N availability under elevated CO_2 may limit the plant's capacity to increase photosynthesis and thus the potential for increased soil C input. Plant productivity and soil C input should be less constrained by available soil N in an N_2 -fixing system. We studied the effects of *Trifolium repens* (an N_2 -fixing legume) and *Lolium perenne* on soil N and C sequestration in response to 9 years of elevated CO_2 under FACE conditions. ^{15}N -labeled fertilizer was applied at a rate of 140 and 560 kg N ha $^{-1}$ yr $^{-1}$ and the CO_2 concentration was increased to 60 Pa p CO_2 using ^{13}C -depleted CO_2 . The total soil C content was unaffected by elevated CO_2 , species, and rate of ^{15}N -fertilization. However, under elevated CO_2 the total amount of newly sequestered soil C was significantly higher under *T. repens* than under *L. perenne*. The fraction of fertilizer N (f_N) of the total soil N pool was significantly lower under *T. repens* than under *L. perenne*. Rate of N fertilization, but not elevated CO_2 , had a significant effect on f_N values of the total soil N pool. The fractions of newly sequestered C (f_C) differed strongly among intra-aggregate SOM fractions, but were unaffected by plant species and rate of N fertilization. Under elevated CO_2 , the ratio of the fractions of new C and fertilizer N increased under *T. repens* compared to *L. perenne*. The *L. perenne* system sequestered more ^{15}N -fertilizer than *T. repens*: 179 vs. 101 kg N ha $^{-1}$ for the low rate of N fertilization and 393 vs. 319 kg N ha $^{-1}$ for the high N fertilization rate. As the loss of fertilizer- ^{15}N contributed to the ^{15}N -isotope dilution under *T. repens*, the input of fixed N into the soil could only be estimated semi-quantitatively. Although N_2 fixation was an important source of N in the *T. repens* system, there was no significant increase in total soil C compared to a non- N_2 -fixing *L. perenne* system. This suggests that N_2 fixation and the availability of N are not the main factors controlling soil C sequestration in a *T. repens* system.

Based on: Van Groenigen KJ, Six J, Harris D, Blum H, Van Kessel C (2003). *Global Change Biology* 9, 1751-1762.

3.1 Introduction

Following an increase in the atmospheric CO₂ concentration, a stimulation of primary production is predicted, leading to an increased C input to the soil (Van Veen et al. 1991). Enhanced plant growth under elevated CO₂, however, may decrease nutrient availability (Diaz et al. 1993). Low N availability has been reported to limit plant growth in many terrestrial ecosystems (Vitousek and Howarth 1991), thereby constraining potential soil C inputs. Indeed, a lack of response to CO₂ in loblolly pine has been attributed to soil nutrient limitation (Oren et al. 2001). However, others (Zak et al. 1993) reported an increase in N availability under elevated CO₂. Clearly, studies on the potential of C sequestration under elevated CO₂ should take into consideration soil fertility factors.

Symbiotic N₂ fixation is one of the main processes contributing to the input of N in terrestrial ecosystems. Worldwide, legumes are grown on approximately 250 Mha fixing about 20 Tg of N₂ per year compared to 80 Tg of N applied as fertilizer (Smil 1999). Increases in N₂ fixation in legumes under elevated atmospheric CO₂ have been reported (Soussana and Hartwig 1996; Zanetti et al. 1996).

Differences in ¹³C-signature between C₃ and C₄ plants (Balesdent et al. 1987) or between plants grown under ambient and elevated ¹³C depleted CO₂ (Leavitt et al. 1994; Nitschelm et al. 1997; Van Kessel et al. 2000a,b; Schlesinger and Lichter 2001) have been used to trace newly sequestered C. Using ¹³C labeling, C turnover rates have been found to increase with aggregate size (Puget et al. 1995; Angers and Giroux 1996; Jastrow et al. 1996). These results are in line with Tisdall and Oades (1982), who proposed that soil organic matter (SOM) associated with large aggregates would be less persistent than SOM associated with smaller aggregates. Six et al. (2001) and Van Groenigen et al. (2002) reported a higher turnover for larger particulate organic matter (POM) using ¹³C labeling. These results corroborate Tiessen and Stewart (1983), who reported that POM in the sand fraction had the highest turn over rate, with decreasing rates for smaller sizes of POM.

The dilution of ¹⁵N in N₂-fixing legumes compared to non N₂-fixing reference crops has been used to determine the amount of fixed N in plant material (Van Kessel and Hartley 2000). As the precursor of SOM, plant-N and -C enter the SOM pool mainly through dead plant material or root exudation. Once large quantities of ¹⁵N-diluted legume material have entered the soil, a dilution of ¹⁵N in SOM in an N₂-fixing system compared to a non-N₂-fixing system would become apparent. Because of the large amount of N in the total SOM pool, large quantities of legume material will be needed before a change in the ¹⁵N signature of SOM can be detected. However, changes in the ¹⁵N isotopic signature in SOM fractions due to input of ¹⁵N-diluted plant material from N₂-fixing legumes may be easier to detect in large aggregates or POM associated with sand, due to their high turnover rates (Van Groenigen et al. 2002).

The intricacies of soil C and N dynamics in an N₂-fixing system, exposed to prolonged elevated CO₂ have rarely been investigated. Here we report on the use of a long-term, double ¹⁵N - ¹³C labeling, elevated CO₂ study to study the impact of an N₂-fixing perennial legume on SOM dynamics. The atmospheric CO₂ concentration was increased using ¹³C-depleted CO₂ and ¹⁵N enriched fertilizer N was repeatedly applied over a period of 9 years. In a previous related publication, we reported on the dynamics of ¹³C and ¹⁵N in the soil of a *L. perenne* system (Van Groenigen et al. 2002). We found that the values of the fraction of new C (f_c) and fertilizer N (f_N) values in the SOM fractions were highly correlated and that the C and N associated with the microaggregates turned over more slowly than the C and N associated with the macroaggregates. There was isotopic evidence that the overall SOM-N dynamics were unaffected by prolonged elevated CO₂ concentrations. The main objective of the present study was to compare the dynamics of C and N under *T. repens*, an N₂-fixing legume, with that under *L. perenne*, a non-N₂-fixing plant. In particular, we investigated the role of N₂ fixation as affected by elevated atmospheric CO₂ concentration on soil N dynamics and C sequestration.

3.2 Materials and methods

Site description

The experimental plots were established at the FACE experiment at the Swiss Federal Institute of Technology (ETH) field station in Eschikon, 20 km NE of Zurich. The FACE experimental setup consists of six 18 m diameter rings: three control rings with ambient air (35 Pa pCO₂), and three rings that receive additional CO₂ (60 Pa pCO₂) during daytime. The additional CO₂ was ¹³C depleted, leading to a $\delta^{13}\text{C}$ signal of approximately -21‰ for CO₂ in the fumigated rings against -8‰ for ambient CO₂. The soil was a clay loam, fertile, eutric cambisol. Experimental plots of *L. perenne* and *T. repens* were established using a split plot design with CO₂ concentrations as the main plot treatment and N-fertilizer as the subplot treatment. Fertilization rates are 140 kg ha⁻¹ (low N) and 560 kg N ha⁻¹ (high N) per year, applied as NH₄NO₃ in 5 splits during the growing season following cutting of swards. The ¹⁵N content for the double ¹⁵N-labeled NH₄NO₃ was 1.3 atom % excess for the low N-treatment and 0.3 atom % excess for the high N-treatment. Treatments are randomized within each ring. Further details about the experimental site are reported elsewhere (Zanetti et al. 1996).

Sampling

We collected soil samples in the fall of 2000 at a depth of 0-10 cm, 9 growing seasons after the initiation of the experiment. From each plot, four 2-cm diameter soil cores

were collected and bulked. Samples were air-dried, and large roots and stones were removed by hand.

Fractionation

Three water-stable aggregate sizes were obtained from 80 g air-dried samples, using wet sieving through a series of sieves (2000 μm , 250 μm and 53 μm). All fractions were dried at 50°C and weighed. Free Light fraction (LF), intra-aggregate particulate organic matter (iPOM) and mineral associated organic matter (mSOM) were isolated from aggregate size classes according to Van Groenigen et al. (2002). In short, free LF was isolated from the aggregates using density flotation in a 1.85 g cm⁻³ NaI solution. The remaining heavy fraction was rinsed with deionized water and dispersed by shaking in 5 g l⁻¹ hexametaphosphate. The dispersed fraction was passed through 2000 μm , 250 μm and 53 μm sieves, yielding the three iPOM size classes and mSOM. The iPOM of 250-2000 μm and >2000 μm that was derived from aggregates >2000 μm was pooled for C and N measurements. Total C, total N, $\delta^{13}\text{C}$ and atom%¹⁵N were determined at the UC Davis Stable Isotope Facility using a continuous flow, isotope ratio mass spectrometer (CF-IRMS, PDZ-Europa Scientific, Sandbach, UK) interfaced with a CN sample converter.

Calculations

Results of the C isotope analysis are expressed in δ units (‰):

$$\delta^{13}\text{C} = 1000 * \left(\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \quad (3.1)$$

where $R = {}^{13}\text{C}/{}^{12}\text{C}$, using the Vienna-Pee Dee Belemnite (V-PDB) as standard. The fraction of C turnover, f_C , is calculated by using the isotope mass balance method (Balesdent et al. 1987):

$$f_C = \frac{\delta_2 - \delta_0}{\delta_1 - \delta_0} \quad (3.2)$$

where δ_2 and δ_0 are $\delta^{13}\text{C}$ values for SOM pools in the CO₂ fumigated and ambient plots and δ_1 is the average $\delta^{13}\text{C}$ value of *L. perenne* and *T. repens* roots and litter grown in the fumigated plots, as reported by Van Kessel et al. (2000b).

The fraction of fertilizer derived N in the soil sample, f_N , was calculated according to Warembourg (1993):

$$f_N = \frac{{}^{15}\text{N atom}\% \text{excess SOM}}{{}^{15}\text{N atom}\% \text{excess fertilizer}} \quad (3.3)$$

Statistical analysis

The results of the experiment were analyzed as a Randomized Complete Block Design, with CO₂ as the main plot treatment and N fertilization and species as sub-treatments. An ANOVA was conducted with blocks and fractions as random effects, and treatments as fixed effects. Significance was determined at a level of $P < 0.05$.

3.3 Results

Whole soil - C and N

After 9 years of elevated CO₂, the mean total soil C content ranged from 30.5 to 35.9 Mg C ha⁻¹ across all treatments (Table 3.1). Total soil C content was unaffected by N treatment, plant species or CO₂ concentration. On average, soils exposed to elevated CO₂ for 9 years were 2.93‰ and 2.84‰ more depleted in ¹³C than soils at ambient CO₂ for *T. repens* and *L. perenne*, respectively. Based on these isotope data, the f_C values for *T. repens* were 0.32 and 0.22 for the low and high N treatment, respectively. For *L. perenne* the f_C values were 0.21 and 0.22 for the low N and high N treatment, respectively. The total input of new C ranged from 7.17 to 11.40 Mg C ha⁻¹ across all treatments. The amount of new C was significantly higher under *T. repens* than *L. perenne* but N-fertilizer rate had no effect.

Across all treatments, the mean total soil N content ranged from 3.35 to 3.87 Mg N ha⁻¹ with no significant differences between any of the treatments (Table 3.2). Nine years of elevated CO₂ did not significantly affect the soil ¹⁵N signature under either *L. perenne* or *T. repens*. The fraction of total soil N derived from ¹⁵N fertilizer, (f_N) ranged between 0.02 and 0.11. Higher rates of ¹⁵N fertilizer increased the f_N values while lower f_N values were found for *T. repens* compared to *L. perenne* (Table 3.2). The amount of fertilizer N recovered in the soil ranged from 82 to 192 kg ha⁻¹ across all low N treatments. For the high N treatments, the amount of fertilizer N recovered ranged from 280 to 405 kg ha⁻¹. The total amount of fertilizer N recovered in the upper 10 cm of the soil profile was higher in the *L. perenne* than the *T. repens* soil (Table 3.2). The percent of fertilizer N recovered in SOM compared to the total amount of fertilizer N applied over 9 years in the high N treatment ranged between 5.5% for *T. repens* under ambient CO₂ and 8.0 % for *L. perenne* under elevated CO₂. For the low N treatment, fertilizer N recovery ranged between 6.5 % for *T. repens* under ambient CO₂ concentration and 15.3 % for *L. perenne* under elevated CO₂ concentration.

Table 3.1: Total soil C content, ^{13}C -signatures, fraction of new C (f_c), and total new and old C content of soil under *L. perenne* and *T. repens* (0-10 cm depth) as affected by CO_2 and N fertilizer application (n=3). The f_c values for elevated CO_2 plots were calculated according to Formula 3.2, using ^{13}C -signatures of SOM in the ambient CO_2 plots with the same N treatment and plant species as a reference.

Species	N	C content (kg C ha ⁻¹)	$\delta\text{C}^{13}(\text{‰})$	f_c	New C (kg C ha ⁻¹)	Old C (kg C ha ⁻¹)
<i>T. repens</i>						
Ambient	High	32090 ± 6373	-26.42 ± 0.41	-	-	-
	Low	30530 ± 4370	-26.41 ± 0.24	-	-	-
+CO ₂	High	34561 ± 6375	-28.84 ± 0.23	0.22 ± 0.01	7668 ± 1289	26893 ± 5101
	Low	35906 ± 6983	-29.86 ± 0.39	0.32 ± 0.02	11403 ± 2004	24503 ± 5274
<i>L. perenne</i>						
Ambient	High	33020 ± 5403	-26.78 ± 0.21	-	-	-
	Low	33504 ± 4024	-27.65 ± 0.46	-	-	-
+CO ₂	High	34386 ± 6797	-29.62 ± 0.25	0.21 ± 0.01	7172 ± 1393	27215 ± 5419
	Low	34983 ± 6368	-30.49 ± 0.28	0.22 ± 0.01	7803 ± 1494	27197 ± 4921
ANOVA						
CO ₂		NS	**	.	.	.
N		NS	NS	NS	NS	NS
Species		NS	NS	*	**	**
CO ₂ *N		NS	NS	.	.	.
CO ₂ *Species		NS	NS	.	.	.
N*Species		NS	NS	NS	NS	NS
CO ₂ *N*Species		NS	*	.	.	.

† Mean ± SE; *,** Significant at the 0.05 and 0.01 probability level, respectively.

Aggregates and POM - ^{15}N

There were no significant effects of CO_2 , N and species on aggregate size class distribution (data not shown). Averaged over all treatments, 52% of the soil remained in aggregates >2000 μm , 34% in aggregates 250-2000 μm , 11% in aggregates 53-250 μm and 3% in the <53 μm size class after wet sieving. The ^{15}N enrichment of aggregates varied little with aggregate size class under *L. perenne* (data not shown). Under *T. repens*, the ^{15}N enrichment of macroaggregates > 2000 μm was higher than for smaller aggregates within all treatments, except under elevated CO_2 with high N (data not shown).

For both species, differences in ^{15}N enrichment were more pronounced between intra-aggregate SOM classes than between aggregate size classes. Within each aggregate size class of each treatment, the atom% ^{15}N excess of intra-aggregate SOM increased with increasing size of SOM particles. Independent of CO_2 concentration

and N treatment, a linear relationship was found between the atom% ¹⁵N excess of intra-aggregate SOM classes under *T. repens* and *L. perenne* (Figures 3.1 and 3.2).

Table 3.2: Total soil N content, ¹⁵N-signatures, fraction of fertilizer N (f_N), total fertilizer N, and the C:N ratio of soil under *L. perenne* and *T. repens* (0-10 cm depth) as affected by CO₂ and N fertilizer application (n=3).

Species	N	N content (kg N ha ⁻¹)	Atom% ¹⁵ N excess	f_N	Fertilizer N (kg N ha ⁻¹)	C:N
<i>T. repens</i>						
Ambient	High	3435 ± 620†	0.02 ± 0.00	0.08 ± 0.01	280 ± 62	9.3 ± 0.2
	Low	3352 ± 454	0.03 ± 0.00	0.02 ± 0.00	82 ± 6	9.1 ± 0.1
+CO ₂	High	3712 ± 631	0.03 ± 0.01	0.10 ± 0.02	359 ± 31	9.3 ± 0.2
	Low	3868 ± 726	0.04 ± 0.01	0.03 ± 0.01	119 ± 30	9.3 ± 0.1
<i>L. perenne</i>						
Ambient	High	3493 ± 584	0.03 ± 0.00	0.11 ± 0.01	381 ± 57	9.5 ± 0.1
	Low	3532 ± 406	0.06 ± 0.01	0.05 ± 0.00	165 ± 25	9.5 ± 0.1
+CO ₂	High	3676 ± 695	0.03 ± 0.00	0.11 ± 0.01	405 ± 50	9.3 ± 0.1
	Low	3620 ± 721	0.07 ± 0.01	0.06 ± 0.01	192 ± 7	9.7 ± 0.2
ANOVA						
CO ₂		NS	NS	NS	NS	NS
N		NS	**	*	**	NS
Species		NS	*	**	*	NS
CO ₂ *N		NS	NS	NS	NS	NS
CO ₂ *Species		NS	NS	NS	NS	NS
N*Species		NS	NS	NS	NS	NS
CO ₂ *N*Species		NS	NS	NS	NS	NS

† Mean ± SE; *,** Significant at the 0.05 and 0.01 probability level, respectively.

The ¹⁵N enrichment of the SOM classes under *T. repens* were always lower than the corresponding atom%¹⁵N excess values under *L. perenne*. For low N treatments, the slope of the relationship ranged from 0.49 to 0.58 under elevated and ambient CO₂, respectively (Figure 3.1). For high N treatments, these ratios increased to 0.77 and 0.80 under elevated and ambient CO₂, respectively (Figure 3.2).

When all the intra-aggregate SOM classes were combined, mSOM contained the highest amount of fertilizer N, independent of CO₂ concentration, rate of fertilizer N application and species (Figures 3.3 and 3.4). However, the mSOM under *T. repens* contained significantly smaller amounts of ¹⁵N-fertilizer than *L. perenne*, independent of N treatment or CO₂ concentration. *L. perenne* retained up to 40 and 74 kg ha⁻¹ more fertilizer N in the mSOM pool than *T. repens* under low and high N treatments, respectively. Elevated CO₂ increased the amount of fertilizer N in the mSOM class and iPOM >250 μm under both *L. perenne* and *T. repens* receiving high N treatments (Figure 3.4).

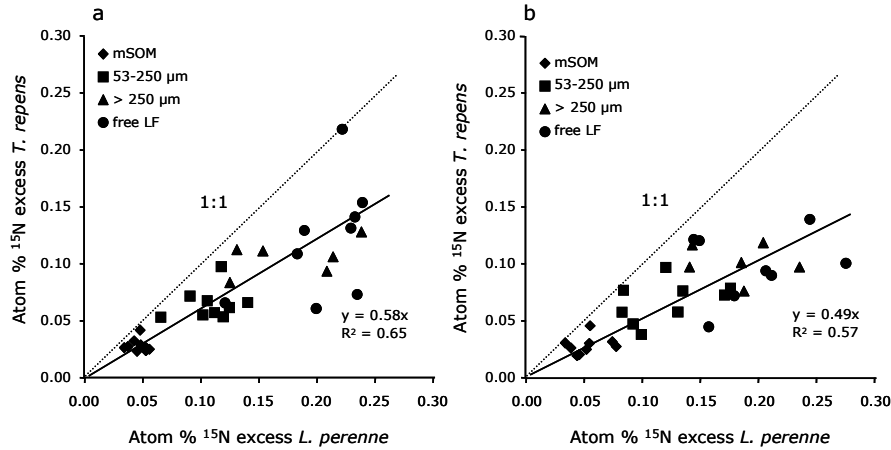


Figure 3.1 a,b: The ^{15}N atom% excess in free LF, iPOM and mSOM of all aggregate size classes under *L. perenne* against *T. repens*, for low N treatments under (a) ambient and (b) elevated CO_2 . Data points represent ^{15}N atom% excess values for the two plant species within individual rings. The 1:1 line indicates the situation in which no ^{15}N dilution under *T. repens* compared to *L. perenne* would occur.

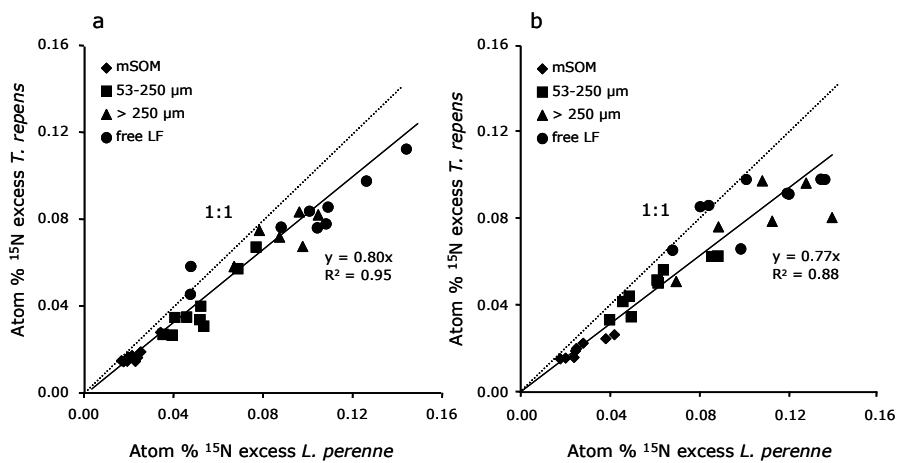


Figure 3.2 a,b: The ^{15}N atom% excess in free LF, iPOM and mSOM of all aggregate size classes under *L. perenne* against *T. repens*, for high N treatments under (a) ambient and (b) elevated CO_2 . Data points represent ^{15}N atom% excess values for the two plant species within individual rings. The 1:1 line indicates the situation in which no ^{15}N dilution under *T. repens* compared to *L. perenne* would occur.

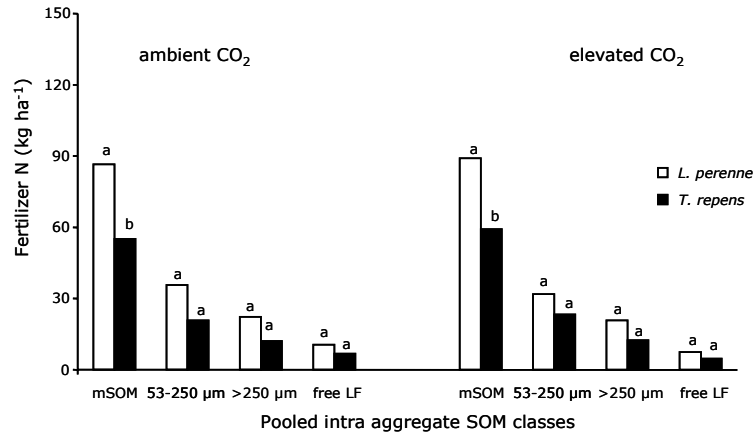


Figure 3.3: Amount of fertilizer N in pooled intra-aggregate SOM classes under *L. perenne* and *T. repens* for low N treatments and both ambient and elevated CO₂. Values followed by a different lowercase letter are significantly different within SOM class and N treatment and between species.

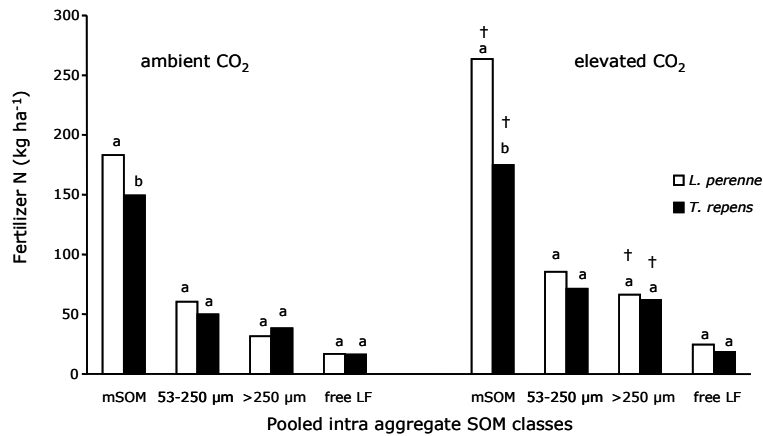


Figure 3.4: Amount of fertilizer N in pooled intra-aggregate SOM classes under *L. perenne* and *T. repens* for high N treatments and both ambient and elevated CO₂. Values followed by a different lowercase letter are significantly different within SOM class and N treatment and between species. Values followed by † indicate a significant difference within species, N treatment and SOM class, and between CO₂ treatment.

Table 3.3: Fraction of new C (f_c) in SOM fractions under *T. repens* and *L. perenne* under elevated CO₂ as affected by N fertilizer application (n=3).

Aggregate size (μm)	SOM class (μm)	<i>T. repens</i>		<i>L. perenne</i>	
		Low N	High N	Low N	High N
< 53		0.11 ± 0.04†	0.10 ± 0.03	0.16 ± 0.03	0.11 ± 0.00
53–250		0.11 ± 0.04	0.12 ± 0.01	0.18 ± 0.04	0.16 ± 0.04
	mSOM	0.11 ± 0.02	0.17 ± 0.03	0.15 ± 0.02	0.09 ± 0.02
	53–250	0.38 ± 0.08	0.39 ± 0.07	0.40 ± 0.02	0.34 ± 0.02
	free LF	0.36 ± 0.09	0.48 ± 0.08	0.48 ± 0.05	0.41 ± 0.02
250–2000		0.22 ± 0.06	0.21 ± 0.04	0.20 ± 0.05	0.24 ± 0.01
	mSOM	0.12 ± 0.02	0.13 ± 0.02	0.14 ± 0.03	0.12 ± 0.00
	53–250	0.44 ± 0.08	0.46 ± 0.08	0.40 ± 0.06	0.37 ± 0.02
	250–2000	0.59 ± 0.03	0.71 ± 0.10	0.51 ± 0.07	0.52 ± 0.07
	free LF	0.58 ± 0.03	0.78 ± 0.12	0.67 ± 0.05	0.60 ± 0.07
>2000		0.31 ± 0.08	0.24 ± 0.04	0.21 ± 0.06	0.31 ± 0.07
	mSOM	0.20 ± 0.03	0.19 ± 0.01	0.13 ± 0.04	0.20 ± 0.01
	53–250	0.53 ± 0.08	0.54 ± 0.05	0.30 ± 0.01	0.44 ± 0.06
	>250	0.73 ± 0.04	0.79 ± 0.04	0.49 ± 0.08	0.64 ± 0.07
	free LF	0.52 ± 0.14	0.67 ± 0.02	0.40 ± 0.09	0.59 ± 0.09
ANOVA					
		<i>T. repens</i>		<i>L. perenne</i>	
N		NS		NS	
Fraction		**		**	
N * fraction		NS		*	
Species				NS	
N				NS	
Fraction				**	
Species*N				NS	
Species * fraction				NS	
N* fraction				NS	
N*species* fraction				*	
Between aggregates	<53/53-250 μm	NS		NS	
	53-250/250-2000 μm	*		NS	
	250-2000/>2000 μm	NS		NS	
Within aggregates, between POM	53-250 μm	**		**	
	250-2000 μm	**		**	
	>2000 μm	**		**	
Within POM, between aggregates	mSOM	NS		NS	
	53-250 μm	**		NS	
	>250 μm	**		NS	
	free LF	**		**	

† Mean ± SE; *, ** Significant at the 0.05 and 0.01 probability level, respectively.

Aggregates and POM - ¹³C

The f_C values in the aggregates and in iPOM fractions were not significantly affected by plant species as a main effect (Table 3.3). The highest f_C values were found in the largest aggregate sizes. The large iPOM fraction and free LF turned over fastest, while f_C values were higher for mSOM. The N treatments did not significantly affect the f_C values in the aggregates or POM fractions under *T. repens*, but under *L. perenne* with high N, the f_C values increased relative to those under low N in the POM classes in aggregates >2000 μm (Table 3.3). No differences in the total new C content were found between species for any pooled intra-aggregate SOM fractions (data not shown).

For both *L. perenne* and *T. repens* under elevated CO₂, a linear relation between f_C and f_N was found among the SOM classes for both N treatments (Figure 3.5a,b) with R² values between 0.84 and 0.88. For both N treatments, the $f_C : f_N$ ratio was higher under *T. repens* than under *L. perenne*: 7.48 vs. 3.04 for the low N treatment, and 2.37 vs. 1.47 for the high N treatment, respectively (Figure 3.5a,b). The largest difference in the f_N values between *L. perenne* and *T. repens* occurred in SOM fractions with the highest f_C values.

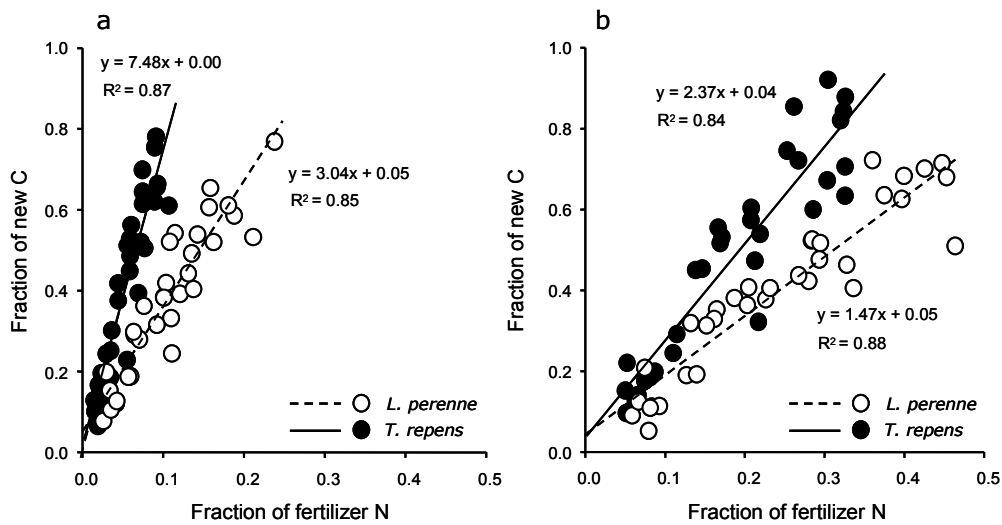


Figure 3.5 a,b: Fraction of fertilizer N against fraction of new C in free LF, iPOM and mSOM of all aggregate size classes under *L. perenne* and *T. repens*, for elevated CO₂ treatments under low (a) and high (b) N treatments.

3.4 Discussion

C dynamics- whole soil

As observed in other studies (Hu et al. 2001), we also found a trend of increased total soil C under elevated CO₂ (Table 3.1). Comparing changes in total C under FACE conditions, Kimball et al. (2002) noted that in all 13 reported studies, total C increased under elevated CO₂ when N did not limit growth. However, none of the increases in total soil C were significant. Detecting changes in total soil C content remains difficult as the annual net input of C in the soil is small relative to the total C pool already present in the soil (Hungate et al. 1996).

The growth of N₂-fixing legumes is normally not limited by a lack of available soil N. As soil C input in an N₂-fixing system is less dependent on available soil N, a more pronounced increase in soil C input and subsequently soil C content would be expected in an N₂-fixing system than in an N-limited non-N₂-fixing system. However, in our study, the *L. perenne* system receiving low rates of ¹⁵N-fertilizer had similar total soil C content as the N₂-fixing systems. The lack of an increase in total soil C in response to the higher C input under *T. repens* compared to *L. perenne* suggests there is a concomitant increase in the turnover rate of old and new soil C under *T. repens*. This suggestion is corroborated by the significantly smaller old C (> 9 years) pool under *T. repens* compared to *L. perenne* (Table 3.1).

N dynamics - whole soil

After 9 years, the soil N pool under *T. repens* was significantly diluted in ¹⁵N compared to *L. perenne* for both fertilizer N rates (Table 3.2). As N₂ fixation decreased the ¹⁵N enrichment of *T. repens* biomass compared to the values measured in *L. perenne* biomass (Zanetti et al. 1996), the input of fixed N in *T. repens* plant components contributed to the ¹⁵N dilution of the overall soil N pool. Nitrogen derived from N₂-fixation entered the soil as rhizodeposition and via root turnover, or as residue from above ground plant material. Hogh-Jensen and Schjoerring (2001) reported in a field experiment a rhizodeposition of 9 g N m⁻² under *L. perenne* against 71 g N m⁻² under *T. repens*. As much as 84% of N in the rhizodeposition under *T. repens* was derived from N₂ fixation, making rhizodeposition an important source of unlabeled N. Zanetti et al. (1996) found at this FACE site, that the stolons of *T. repens* contained up to 3.97 g of fixed N m⁻², making stolons an important input of fixed N in the soil. Furthermore, as *L. perenne* and *T. repens* were frequently defoliated, the above ground biomass below cutting height and harvest losses that were left in the field also contributed to the input of fixed N in the soil.

Recovery of fertilizer N in the top 10 cm of the soil was low and never exceeded 8 % of the total amount of fertilizer N applied. The recovery was unaffected by

elevated CO₂ (Table 3.2). In another study conducted at the same FACE site using the same two species grown in large containers and receiving identical rates of fertilizer N, the recoveries of fertilizer N after 4 years in the soil were similarly low: from 3.0 to 5.3 % under low N treatments and between 2.5 and 8.9 % under high N treatments (Hartwig et al. 2002). As in our current study, soil fertilizer N recovery was higher under *L. perenne* than under *T. repens*. The finding that the N₂-fixing *T. repens* sequestered less fertilizer N in the soil than the non-N₂-fixing *L. perenne* was unexpected. N₂-fixing systems may be inherently more leaky for inorganic N than non-N₂-fixing systems. Differences in root architecture and biomass between the N₂-fixing and non-N₂-fixing plant may also contribute to differences in fertilizer N recovery. Hartwig et al. (2002) reported that after 4 years of exposure to elevated CO₂ and high rates of N applications, the dry matter of roots under *L. perenne* was 891 g m⁻² versus 89 g m⁻² for *T. repens*. Since the interval between application of fertilizer N and plant N-uptake determines the length of exposure of fertilizer N to losses through leaching and denitrification, an extended root system could potentially lead to smaller losses of fertilizer N.

Elevated CO₂ did not increase total fertilizer N recovery in the soil (Table 3.2). A similar result was found by Hartwig et al. (2002). In open-topped chambers in a tall grass prairie, fertilizer N recovery in the 5-15 cm soil depth was significantly higher under elevated CO₂ than under ambient CO₂: 43.8 versus 34.9 % (Williams et al. 2001). However, the increased recovery of fertilizer N under the elevated CO₂ concentration may have been an artifact, since the fertilizer N recovery under non-chamber ambient CO₂ conditions (42.0 %) did not differ significantly from elevated CO₂ conditions.

In a microcosm system planted to *Danthonia richardsonii*, no significant CO₂ effect on ¹⁵N-NH₄NO₃ losses was observed after 4 years (Lutze and Gifford 2000). In a sandstone grassland with moderate soil fertility, the recovery of added ¹⁵N in the soil decreased under elevated CO₂ (Hu et al. 2001). Prolonged elevated CO₂ has been reported to significantly increase soil C (Lutze and Gifford 1998; Williams et al. 2000), whereas others found no significant increases albeit an upward trend in total soil C (Table 3.1; Hu et al. 2001). An increase in fertilizer-¹⁵N recovery in the soil without a concomitant increase in total soil C would be less likely. However, an increase in the recovery of fertilizer N in the soil following an increase in total soil C under elevated CO₂ would be expected due to the strong relationship between f_N and f_C , independent of fertilization rate (Van Groenigen et al. 2002).

N dynamics - aggregates and iPOM

The largest differences in the ¹⁵N enrichment between species occur in coarse POM and free LF (Figure 3.2). Since these SOM fractions are mainly derived from root material (Six et al. 1998), these data suggest an input of fixed N through root turnover

under *T. repens*. Although the coarse POM and free LF showed the highest degree of ^{15}N enrichment, it is important to consider both the turnover rate and the size of the various SOM fractions. Because the majority of N and C in the soil is found in the mSOM fraction (Van Groenigen et al. 2002), mSOM is also the fraction with the lowest ^{15}N enrichment (Figures 3.1 and 3.2).

When the intra-aggregate fractions are pooled across all aggregate size classes, the largest difference in fertilizer N content between plant species is observed for mSOM. This suggests that the significant difference between species in the ^{15}N dilution of the total SOM pool is largely caused by the incorporation of fertilizer N in the mSOM pool under *L. perenne* (Figures 3.3 and 3.4). These data corroborate findings by Balabane (1996) and Balabane and Balesdent (1995), who reported a strong sink of both fertilizer N and plant derived N in mSOM fractions.

As elevated CO_2 did not affect the recovery of fertilizer N in mSOM under *L. perenne* and *T. repens* in the low N treatments, the overall N dynamics were largely unaffected by prolonged elevated CO_2 (Figure 3.3; Chapter 2). Although the ^{15}N enrichment of the individual iPOM fractions under both species receiving high N treatments were largely unaffected by prolonged elevated CO_2 (Figures 3.1 and 3.2), elevated CO_2 increased the amount of fertilizer N recovered in mSOM and to a smaller extent in iPOM $>250\ \mu\text{m}$ for both species (Figure 3.4). An increase in the total recovery of ^{15}N -fertilizer in the mSOM fraction under elevated CO_2 without a change in the f_{N} value corresponded to an increase in total N in the mSOM fraction (data not shown). Of all our SOM fractions, mSOM can be considered the most important for sequestration of N and is most sensitive to changes in atmospheric CO_2 concentration.

Linking C and N dynamics

Regardless of aggregate size class or treatment, both f_{C} and the ^{15}N enrichment of intra-aggregate SOM classes increase according to mSOM < small iPOM < coarse POM and free LF. This strongly suggests that the intra-aggregate SOM fractions differentiate kinetically different fractions of SOM (Six et al. 2001; Van Groenigen et al. 2002). In intra-aggregate SOM fractions under both *L. perenne* and *T. repens*, f_{C} is strongly correlated with the fraction of fertilizer N (Figure 3.5a,b). The higher $f_{\text{C}} : f_{\text{N}}$ ratio under *T. repens* than under *L. perenne* indicates that less fertilizer N was involved in sequestering new C under *T. repens*. This implies that fixed N fulfilled a similar role in sequestering new C as fertilizer N. That is, whether N enters the system as inorganic N (fertilizer N) or as organic N (fixed N that is incorporated into organic compounds), once N has been accumulated in the plant, both fixed N and fertilizer N fulfill a similar role in sequestering newly fixed C in SOM. As anticipated, N_2 fixation in the *T. repens* systems decreased under high rates of fertilizer N applications (Zanetti

et al. 1996), thereby increasing the role of fertilizer N in sequestering new C (Figures 3.5a,b).

Resh et al. (2002) reported an increase in total soil C under N₂-fixing trees compared to non-N₂-fixing trees. Since the difference in total soil C contents between N₂-fixing and non N₂-fixing species was positively correlated to the soil N accretion under the N₂-fixers ($R^2=0.83$), Resh et al. (2002) concluded that fixed N contributed to the sequestration of C. Neff et al. (2002) reported that fertilizer N additions stabilize old C pools and increase the decomposition of labile C pools under alpine tundra. Conversely, in our experiment the size of the old (>9 years) soil C pool was not affected by fertilizer N additions and was significantly smaller under *T. repens* than under *L. perenne* (Table 3.1). Therefore, it seems that in a *T. repens* system under elevated CO₂, neither N₂ fixation nor soil N availability are the main factors controlling soil C sequestration. A decrease in the retention of the old C pool under *T. repens* compared to *L. perenne* might have been caused by changes in microbial activity under elevated CO₂. Montealegre et al. (2002) reported that the number of metabolically active bacteria increased with elevated atmospheric CO₂ in bulk soil under *T. repens*, but that the number of metabolically active bacteria in bulk soil of *L. perenne* remained unaffected.

N₂ fixation in long-term experiments

A widely accepted method to estimate N₂ fixation is by the ¹⁵N-isotope dilution method (Fried and Broeshart 1975; Rennie and Rennie 1983). A ¹⁵N-labeled source, often fertilizer N, is applied to both the N₂-fixing plant and a non-N₂-fixing reference plant. By determining the dilution of ¹⁵N in the N₂-fixing plant in reference to the non-N₂-fixing reference plant, the percentage of total N in the N₂-fixing plant that is derived from N₂ fixation can be estimated. The assumption is that both the N₂-fixing plant and a non-N₂-fixing reference plant accumulate ¹⁵N and ¹⁴N in the same ratio as those two isotopes occur in the soil mineral N pool.

At our study site, N₂ fixation was an important source of N for *T. repens* (Zanetti et al. 1998; Lüscher et al. 2000). Under elevated CO₂ 274 kg ha⁻¹ yr⁻¹ of the N in the above ground biomass of *T. repens* was derived from N₂ fixation at the low N fertilizer rate, and 192 kg ha⁻¹ yr⁻¹ at the high rate of N fertilization (Zanetti et al. 1996). Under ambient CO₂ these values were 254 and 147 kg ha⁻¹ yr⁻¹, respectively. Estimates for N₂ fixation were based on the ¹⁵N-isotope dilution method, using *L. perenne* as the reference crop (Zanetti et al. 1996).

However, as fertilizer-¹⁵N taken up by *T. repens* is diluted by fixation of atmospheric N₂, the ¹⁵N content of newly formed SOM should be lower in the *T. repens* system than in the *L. perenne* system. In successive growing seasons, as more symbiotically fixed N enters the *T. repens* system, an overall decrease in the isotopic ¹⁵N signature of its total SOM pool and in the various SOM fractions relative to the *L.*

perenne system would be manifested. Because of the large amount of N in the SOM pool, its ^{15}N -isotopic signature will change slowly.

Our data suggest that the ^{15}N dilution of the soil N pool under *T. repens* compared to *L. perenne* was caused both by the input of fixed N and the loss of fertilizer N. The overall ^{15}N dilution in SOM fractions under *T. repens* (Figures 3.1 and 3.2) suggest that soil N taken up by *T. repens* has a lower ^{15}N content than soil N taken up by *L. perenne*. Consequently, a decrease in the $\text{atom}\%^{15}\text{N}$ values in *T. repens* plant material over the years can not be contributed solely to N_2 fixation. Therefore, the ^{15}N -isotope dilution method overestimates N_2 fixation in the long term.

Results from long-term experiments in which legumes and reference crops were grown separately seem to provide indirect evidence that N_2 fixation in plant material may be overestimated. In a four-year experiment, Heichel et al. (1984) annually applied ^{15}N -labeled fertilizer to separate plots of alfalfa and two reference crops. The amount of annually fixed N_2 significantly increased from 126 to 198 kg N ha⁻¹ yr⁻¹ over a 4 year period. Hartwig et al. (2002) annually added 560 kg N ha⁻¹ to both *T. repens* and *L. perenne*. After four years, the average annual N_2 -fixation in *T. repens* under elevated CO_2 remained as high as 198 kg N ha⁻¹. As the observed high rates of N_2 -fixation were based on the ^{15}N -isotope dilution method, the results were likely affected by ^{15}N dilution of the soil N pool under legumes. The effect of different soil ^{15}N concentrations under legumes and reference crops on N_2 -estimates could be partly avoided by planting both species in close vicinity so that both species have access to the same available soil N pool (Boddey et al. 1995).

If N_2 fixation were to be the only process affecting f_{N} values of SOM which differed between *T. repens* and *L. perenne*, then the ratios of the f_{N} values in the two systems (Figures 3.1 and 3.2) could be used to estimate the long-term contribution of fixed N to the SOM in the *T. repens* systems. However, the greater loss of ^{15}N fertilizer from the *T. repens* system over time compared to *L. perenne* suggests that even in the absence of N_2 fixation *T. repens* would generate residues with lower f_{N} values than *L. perenne*. This effect confounds the estimation of fixed N input to the soil by ^{15}N isotope dilution of SOM pools.

3.5 Conclusions

The use of ^{15}N -labeled fertilizer in a FACE experiment, exposed to elevated ^{13}C -depleted CO_2 for 9 consecutive years provided a unique opportunity to compare N and C dynamics under *T. repens* and *L. perenne*. Both in the *L. perenne* and *T. repens* systems, the sequestration of new C and fertilizer N were strongly linked and fixed N plays a similar role in sequestering new C as fertilizer N. The dynamics of new C and N into the various SOM fractions was similar for both species, with higher C and N turnover rates in the larger intra-aggregate SOM size classes and lower turnover rates

for the mSOM fractions. No significant increases in total soil C and N were observed under either *L. perenne* or *T. repens* indicating that changes in total soil N and C do occur very slowly, or not at all. Significant increases in total soil C and N following prolonged exposure to elevated CO₂ will also be more difficult to detect because of the variability in total soil N and C content between the FACE rings. Although N₂ fixation was a major source of N for *T. repens*, the presence of N₂ fixation per se did not lead to higher soil N and C content compared to a low N fertilized *L. perenne* system. Apparently, other factors than N₂ fixation exert a higher control on soil C and N stabilization in the *T. repens* system. After 9 years the recovery of fertilizer N in the soil (0-10 cm) was significantly higher in the *L. perenne* than in the *T. repens* system. A lower root density, possibly combined with a relative high N rhizodeposition may have caused an increase in the loss of fertilizer N under *T. repens*. As a consequence, the dilution of ¹⁵N in the SOM pools under *T. repens* over time can not be entirely attributed to N₂ fixation. Therefore, no valid estimates of the contribution of fixed N in the SOM fractions can be made.

4 **Decomposition of ^{14}C -labeled roots in a pasture soil exposed to 10 years of elevated CO_2**

Abstract

The net flux of soil C is determined by the balance between soil C input and microbial decomposition, both of which might be altered under prolonged elevated atmospheric CO_2 . In this study, we determined the effect of elevated CO_2 on decomposition of grass root material (*Lolium perenne* L.). ^{14}C -labeled root material, produced under ambient (35 Pa p CO_2) or elevated CO_2 (70 Pa p CO_2) was incubated in soil for 64 days. The soils were taken from a pasture ecosystem which had been exposed to ambient (35 Pa p CO_2) or elevated CO_2 (60 Pa p CO_2) under FACE-conditions for 10 years and two fertilizer N rates: 140 and 560 kg N ha⁻¹ yr⁻¹. In soil exposed to elevated CO_2 , decomposition rates of root material grown at either ambient or elevated CO_2 were always lower than in the control soil exposed to ambient CO_2 , demonstrating a change in microbial activity. In the soil that received the high rate of N fertilizer, decomposition of root material grown at elevated CO_2 decreased by approximately 17% after incubation for 64 days compared to root material grown at ambient CO_2 . The amount of $^{14}\text{CO}_2$ respired per amount of ^{14}C incorporated in the microbial biomass ($q^{14}\text{CO}_2$) was significantly lower when roots were grown under high CO_2 compared to roots grown under low CO_2 . We hypothesize that this decrease is the result of a shift in the microbial community, causing an increase in metabolic efficiency. Soils exposed to elevated CO_2 tended to respire more native SOC, both with and without the addition of the root material, probably resulting from a higher C supply to the soil during the 10 years of treatment with elevated CO_2 . The results show the importance of using soils adapted to elevated CO_2 in studies of decomposition of roots grown under elevated CO_2 . Our results further suggest that negative priming effects may obscure CO_2 data in incubation experiments with unlabeled substrates. From the results obtained, we conclude that a slower turnover of root material grown in an 'elevated- CO_2 world' may result in a limited net increase in C storage in ryegrass swards.

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4.1 Introduction

The current rise in atmospheric CO₂ is expected to affect the flow of C and N through terrestrial ecosystems. Most prolonged elevated-CO₂ studies reported a non-significant trend towards an increase in the average soil C content when N was not limiting, suggesting that net soil C storage might occur (Kimball et al. 2002). Gifford (1994) suggested that soil C sequestration following increased C assimilation by plants could offset increases in atmospheric CO₂. As changes in soil C dynamics will be mediated through the soil micro-organisms, net soil C storage is ultimately determined by the balance between soil C input and decomposition rates.

Numerous investigators found greater rates of soil microbial respiration under elevated CO₂ than under ambient CO₂ (Rice et al. 1994; Sowerby et al. 2000; Phillips et al. 2002). Since substrate availability is one of the key factors driving microbial metabolism, plant growth response to elevated CO₂ and subsequent increases in litter production probably contributed to the increase in soil microbial respiration (Zak et al. 2000). From a meta-analysis, Norby et al. (2001) suggested that the change of litter quality produced under elevated CO₂ has a limited effect on C dynamics. However, their analysis mainly included studies using above-ground plant material, whereas lower decomposition rates for roots grown under elevated CO₂ have been reported (Cotrufo and Ineson 1995; Van Ginkel et al. 2000). Gorissen and Cotrufo (2000) found that lower decomposition rates for litter produced under elevated CO₂ could not be properly explained by changes in the C:N ratio, a view that is supported by Ross et al. (2002). Therefore, it was suggested that CO₂-induced changes in decomposition of grasses occur via other pathways which remain unclear.

An increase in root biomass, total rhizodeposition, and possible changes in plant tissue chemistry alter the substrates available for microbial metabolism and could therefore affect soil microbial biomass (Cotrufo and Gorissen 1997) and composition (Klironomos et al. 1996). Cotrufo and Gorissen (1997) showed that the increased microbial biomass under three different grass species was proportional to the increased root mass under elevated CO₂. Montealegre et al. (2002) observed a 1.4-fold increase in the proportion of respiring vs. total bacteria under *L. perenne* after four years of FACE conditions. Hu et al. (2001) also reported an increase in microbial C under elevated CO₂, but a decrease in specific microbial activity. A soil microbial response following prolonged elevated CO₂ concentrations that will affect decomposition rates might be expected, since tissue quality will deteriorate following increased plant growth rates (Norby et al. 2001).

Low N availability might limit the microbial response to elevated CO₂ (Hu et al. 2001) and changes in N availability might also shift patterns of microbial decomposition. Neff et al. (2002) reported that 10 years of N additions accelerated decomposition of light fractions of soil organic matter, but stabilized mineral-associated fractions. Van Ginkel et al. (2000) concluded that the altered

decomposition under elevated CO₂ was not due to a change in internal microbial metabolism. However, long-term exposure to elevated CO₂ and/or N additions, as in the FACE experiment, possibly do affect microbial metabolism and subsequently C dynamics. A valid criticism is that most of the studies on decomposition of plant litter are carried out with a soil microbial population that is not adapted to prolonged elevated CO₂ (Norby et al. 2001). The most suitable comparison would consist of decomposition of 'ambient CO₂'-grown plant material in an 'ambient CO₂'-soil with 'elevated CO₂'-grown plant material in an 'elevated CO₂'-soil. As soil microbial metabolism might lag behind changes in organic matter input, the effect of possible changes in the microbial population should be studied in prolonged experiments (Ross et al. 2002).

When addressing the effect of elevated CO₂ on decomposability of plant material, many studies focused on above-ground plant material. However, roots form the main source for building up soil organic matter (Balesdent and Balabane 1996). Root growth is generally more stimulated by elevated atmospheric CO₂ than shoot growth (Van Ginkel et al. 1997; Hebeisen et al. 1997). As grass roots decompose more slowly than leaves (Gorissen and Cotrufo 2000), it seems likely that the effect of elevated CO₂ on soil C input is for the greater part exerted through C allocation to roots.

The metabolic quotient ($q\text{CO}_2$) i.e., microbial respiration to microbial biomass ratio, indicates the energy needed for maintenance of microbial biomass (Anderson and Domsch 1993). This ratio varies according to the composition and physiological state of the microflora, as affected by differences in pH (Anderson and Domsch 1993), temperature, and several other environmental conditions (Anderson and Gray 1991). Miller and Dick (1995) found a negative correlation between $q\text{CO}_2$ and aggregate size, which they suggested was partly caused by a larger C availability in macro-aggregates.

¹⁴C-labeled plant material has been useful in studying C fluxes in plant/soil systems. The ¹⁴C label allows quantifying the contribution of plant-derived C to CO₂ fluxes and SOM pools (Van Ginkel et al. 2000; Gorissen and Cotrufo 2000) and the turnover of C through the microbial biomass (Ladd et al. 1995). Analogous to $q\text{CO}_2$, the metabolic quotient $q^{14}\text{CO}_2$ is an indicator of the metabolic efficiency of microbial biomass decomposing ¹⁴C-labeled substrate (Bottner et al. 1998).

The aims of this study within a FACE-experiment were to determine (i) the effects of elevated CO₂ on decomposability of root material, and (ii) the long-term (> 10 yr) effects of elevated CO₂ and N additions on the soil microbial activity. The use of ¹⁴C-labeled root material produced under ambient or elevated CO₂ concentrations enabled us to determine the metabolic quotient of the soil microbial community as affected by 10 years of elevated atmospheric CO₂. We hypothesize that microbial communities quickly respond to a change in quality of substrate input, resulting in a slower decomposition of roots produced under elevated CO₂. We further hypothesize

that prolonged elevated CO₂ affects the response of soil microbes to additional root-C input. The use of ¹⁴C-labeled material enabled us to distinguish between the contributions of added root material and native SOC to the total CO₂ flux.

4.2 Materials and methods

Soil

Soil was sampled in 2002 from a 10-years-old FACE experiment at the Swiss Federal Institute of Technology (ETH) in Eschikon, Switzerland (Zanetti et al. 1996). The FACE experiment consists of six rings (diameter 18m): three control rings with ambient air (35 Pa pCO₂), and three rings that receive additional CO₂ (totaling 60 Pa pCO₂) during daytime. The soil was a fertile, clay loam, Eutric Cambisol. Experimental plots of *L. perenne* were established using a split-plot design with CO₂ as the main plot treatment and N fertilizer as the sub-plot treatment. Fertilization rates are 140 kg ha⁻¹ (low N) and 560 kg ha⁻¹ N (high N) per year, applied as NH₄NO₃ in 5 splits during the growing season following cutting of swards. Although these fertilization rates are high compared to many other agro-ecosystems, they represent realistic rates for the region, and are not uncommon. Further details about the experimental site are reported by Zanetti et al. (1996).

Samples were collected in the fall of 2002 at a depth of 0-10 cm. From each plot, four 5-cm diameter cores were collected and bulked. Soil samples were air-dried (approximately 40°C; 48 h) and sieved through a 2-mm sieve, after which large pebbles were removed by hand. Large roots were removed with tweezers. The soil was stored at 5°C to minimize microbial activity pending the decomposition experiment. Subsamples of the soil were ball milled and soil carbonates were removed using HCl fumigation as described by Harris et al. (2001). Total C and N concentrations of the subsamples were determined at the UC Davis Stable Isotope Facility using a continuous flow, isotope ratio mass spectrometer (CF-IRMS, PDZ-Europa Scientific, Sandbach, UK) interfaced with a CN sample converter.

¹⁴C Root labeling

L. perenne seedlings were grown in a sandy loam soil in 0.65-L pots at two CO₂ concentrations (35 and 70 Pa pCO₂) in growth chambers, in which a continuously ¹⁴C-labeled atmosphere was maintained (Gorissen et al. 1996). The specific activity of the ¹⁴CO₂ was 0.84 +/- 0.07 kBq mg C⁻¹. No mineral N was added during the growth period. Further details about the growing conditions are reported by Van Ginkel et al. (1997). After 45 days, the roots were harvested. As up to 90% of the young fine roots can be lost upon conventional root washing procedures (Kuzyakov et al. 1999), roots

were only gently washed, causing some of the adhering soil to stick to the roots. After drying at 70°C, the roots were ground in a micro hammer mill (Culatti AG, Zurich, Switzerland) with a 1 mm sieve and stored until used for the decomposition study. The ¹⁴C-carbon content of roots was determined using a modified wet combustion method (Van Ginkel et al. 2000) followed by liquid scintillation counting. Dried and ground roots (30 mg) were digested in 5 ml of a 10% (w/v) solution of K₂Cr₂O₇, dissolved in a mixture of concentrated H₂SO₄ and H₃PO₄. The CO₂ evolved was trapped in 10 ml of a 0.5 M NaOH solution. A mix of 0.5 ml of the NaOH solution and 3 ml of Ultima Gold (Packard) was counted on a liquid scintillation counter (Tri-Carb 2100TR; Packard). The roots were analyzed in fourfold for total C and N at the UC-Davis Stable Isotope Facility.

Decomposition experiment

Soils from the FACE-experiment were incubated as (1) root-free soil and, (2) root-free soil mixed with the ground ¹⁴C-labeled roots. Fifty grams of soil were moistened to a water content of 16% (w/w) and amended with the *L. perenne* roots (0.500 ± 0.001 g per beaker). All treatment combinations for the soil and ¹⁴C-labeled roots were included, resulting in 12 treatment combinations: 4 of unamended soil and 8 treatments of soil with ¹⁴C-labeled roots (Table 4.1).

Table 4.1: Overview of all incubations of soil subjected to 10 years of ambient or elevated CO₂ and two N treatments, with *L. perenne* root material grown at two concentration levels of CO₂.

	Soil		Roots
	CO ₂	N-rate	CO ₂
1	Ambient	Low	None
2		High	None
3	Elevated	Low	None
4		High	None
5	Ambient	Low	Ambient
6		High	Ambient
7	Elevated	Low	Ambient
8		High	Ambient
9	Ambient	Low	Elevated
10		High	Elevated
11	Elevated	Low	Elevated
12		High	Elevated

Each beaker was placed in a 1-L glass jar together with a centrifuge tube containing 10 ml 1.0 M NaOH to trap the respired CO₂. Four jars with only the NaOH solution were included in the experiment as blanks. Two ml of de-ionized water were added at the bottom of the jars to prevent the soil samples from drying. The jars were closed airtight and incubated in the dark for 64 days at a constant temperature of 25°C.

After 1, 2, 4, 8, 18, 32, 46, and 64 days, the jars were opened to replace the NaOH solutions and prevent depletion of oxygen. The solutions were analyzed for total CO₂ and ¹⁴C concentrations. The total amount of respired CO₂ was analyzed by titration with 0.1 M HCl according to Van Ginkel et al. (2000). Liquid scintillation counting was used to determine the ¹⁴C concentrations of the solutions.

¹⁴C-labeled soil microbial biomass

After 64 days, the amount of ¹⁴C-labeled soil microbial biomass was determined using the fumigation extraction method (Vance et al. 1987). Briefly, two subsamples of 10 grams soil per incubation jar were fumigated for 24 h with chloroform. At the same time as the fumigation began, two 10-gram subsamples were extracted with 0.5 M K₂SO₄. After fumigation the same procedure was followed for the fumigated subsamples. The extracts were analyzed for ¹⁴C using liquid scintillation counting.

Root-derived respiration

The fraction of C of the ¹⁴C-labeled roots that was decomposed is defined as

$$\% \text{ root } ^{14}\text{C respired} = \left(\frac{^{14}\text{C respired (Bq)}}{^{14}\text{C added (Bq)}} \right) * 100 \quad (4.1)$$

The total amount of respired C derived from the ¹⁴C-labeled roots is defined as

$$R(\text{mol}) = \frac{^{14}\text{C respired (Bq)}}{\text{specific activity } ^{14}\text{C roots (Bq/mol)}} \quad (4.2)$$

The total amount of respired native SOC is defined as

$$\text{SOC}_{\text{resp}}(\text{mol}) = \text{total CO}_2(\text{mol}) - R(\text{mol}) \quad (4.3)$$

The metabolic quotient $q^{14}\text{CO}_2$ is defined as

$$q^{14}\text{CO}_2 = \frac{^{14}\text{C respired (Bq)}}{^{14}\text{C in microbial biomass (Bq)}} \quad (4.4)$$

Statistical analysis

Our experiment included two CO₂ treatments and two N treatments for the soil, and two CO₂ treatments for the roots. Each treatment combination of roots and soil had 3 replicates. The experiment had a split-plot design, with soil CO₂ as the main plot treatment and N fertilization and root CO₂ as the sub-plot treatments. Data were analyzed with the PROC MIXED procedure of SAS (Littell et al. 1996) with replicates as a random effect, and elevated CO₂ level for roots, and elevated CO₂, and N level for the soil as fixed effects. For ambient CO₂-soils that received low N treatments, results derived from ¹⁴C data are based on two replicates, due to a consistently outlying replicate. Statistical analyses were run on rank-transformed data. We conducted an exploratory analysis and found that instances where assumptions of equal variance and normality were not strictly met did not affect the interpretation of the ANOVA results with raw data. Treatment effects are considered significant at $P < 0.05$ if not indicated differently.

4.3 Results

Soil and roots

The average soil C concentration of the root-free soil was 29.4 g C kg⁻¹ (Table 4.2). Ten years of elevated atmospheric CO₂ and N treatments did not significantly affect soil C concentrations ($P=0.57$). After 64 days, the average amount of CO₂ respired from the control soils was 0.77 g CO₂-C kg⁻¹. Soils that had been exposed to elevated atmospheric CO₂ tended to respire more CO₂: low N +23%, high N +15% ($P=0.06$), whereas N treatments had no effect on control soil respiration (Table 4.2). The ambient-CO₂ root material had a significantly higher N concentration than elevated-CO₂ roots, resulting in a lower C:N ratio (Table 4.3).

Table 4.2: Initial C and N concentrations, C:N ratio and C mineralized after 64 days of soil subjected to 10 years of ambient or elevated CO₂ and two N treatments (n=3).

Soil CO ₂	N-rate	C	N	C:N	SOC _{resp}
		(g kg ⁻¹)			
		(g kg ⁻¹ ; 0-64 days)			
Ambient	Low	28.7 ± 4.0†	3.16 ± 0.44	9.1 ± 0.0	0.68 ± 0.03
	High	27.3 ± 3.5	2.95 ± 0.38	9.2 ± 0.0	0.73 ± 0.06
Mean		28.0 ± 2.4	3.05 ± 0.27	9.2 ± 0.0	0.70 ± 0.03
Elevated	Low	31.7 ± 4.2	3.35 ± 0.41	9.4 ± 0.1	0.84 ± 0.10
	High	29.7 ± 5.3	3.22 ± 0.54	9.2 ± 0.2	0.83 ± 0.08
Mean		30.7 ± 3.1	3.28 ± 0.30	9.3 ± 0.1	0.84 ± 0.06
Grand mean		29.3 ± 2.0	3.17 ± 0.20	9.2 ± 0.1	0.77 ± 0.03
ANOVA					
Soil CO ₂		NS	NS	NS	*
Soil N		NS	NS	NS	NS
Soil CO ₂ * soil N		NS	NS	NS	NS

† Mean ± SE * Significant at the 0.1 probability level.

Table 4.3: C and N concentration, C:N ratio of *L. perenne* root material grown at two levels of CO₂ concentration.

Roots CO ₂	C	N	C:N
	g kg ⁻¹		
Ambient	229 ± 1.1 a†	8.7 ± 0.0 a	26.4 ± 0.2 a
Elevated	216 ± 1.0 b	6.2 ± 0.0 b	34.6 ± 0.1 b

† Mean ± SE. Different letters within a column represent significant difference (*t*-test, *P*<0.01).

Root decomposition

During the incubation, an average of 68.6% of added root C was respired (Table 4.4). After 1 day, high-CO₂ roots decomposed significantly more slowly than low-CO₂ roots. However, during the rest of the incubation period, the effect of elevated CO₂ on root decomposition depended on the amount of N fertilizer applied to the soil.

Table 4.4: Percentage of C mineralized from *L. perenne* root material grown at two concentration levels of CO₂ after 1, 2, 4, 8, 18, 32, 46, and 64 days of incubation in *L. perenne* soil subjected to 10 years of ambient or elevated CO₂ and two N treatments (n=3, except for ambient CO₂-soils receiving low N treatments, for which n=2).

Soil CO ₂	N-rate	Roots CO ₂	Root-C mineralized (%) after day							
			1	2	4	8	18	32	46	64
Ambient	Low	Ambient	11.1	18.4	33.2	49.8	60.9	65.9	68.3	70.2
		Elevated	7.9	13.7	28.1	46.3	58.7	64.2	67.0	69.5
	High	Ambient	11.5	21.1	39.2	55.8	67.3	73.6	76.7	79.3
		Elevated	7.9	14.8	31.1	47.8	58.3	63.8	66.5	68.9
Mean			9.6	17.0	32.9	49.9	61.3	66.9	69.6	72.0
Elevated	Low	Ambient	9.6	15.7	27.4	42.8	53.4	58.6	61.7	64.1
		Elevated	6.5	11.7	22.7	39.9	53.2	59.7	63.6	66.4
	High	Ambient	10.7	18.2	30.5	48.8	61.2	67.0	70.2	72.8
		Elevated	6.5	11.4	21.3	37.5	48.0	52.5	55.5	58.0
Mean			8.3	14.2	25.5	42.2	54.0	59.5	62.7	65.3
Grand mean			9.0	15.6	29.2	46.1	57.7	63.2	66.2	68.6
ANOVA										
Soil CO ₂			NS	NS	*	***	***	**	*	NS
Soil N			NS	NS	NS	NS	NS	NS	NS	NS
Soil CO ₂ * soil N			NS	NS	NS	NS	NS	NS	NS	NS
Roots CO ₂			***	***	***	***	***	***	***	***
Soil CO ₂ * roots CO ₂			NS	NS	NS	NS	NS	NS	NS	NS
Soil N*roots CO ₂			NS	**	**	**	***	***	***	***
Soil CO ₂ * soil N*roots CO ₂			NS	NS	NS	NS	NS	NS	NS	NS

*, **, *** Significant at the 0.1, 0.05 and 0.01 probability level, respectively.

At the high rate of N fertilizer, roots produced under high CO₂ decomposed 17% more slowly after incubation for 64 days than roots produced under low CO₂. This decrease in cumulative ¹⁴C-CO₂ production was caused by lower decomposition rates of high-CO₂ roots compared to low-CO₂ roots during the first 8 days of the experiment (results not shown). Between day 8 and day 32, the cumulative ¹⁴C-CO₂ production was significantly lower for elevated-CO₂ soils compared to ambient-CO₂ soils. Although at 46 days, the decrease in cumulative ¹⁴C-CO₂ production in soils exposed to elevated CO₂ was only marginally significant ($P=0.09$), the tendency for decomposition to be slower in elevated-CO₂ soils compared with ambient-CO₂ soils remained until the end of the experiment (Table 4.4). The highest average respiration rates were found for roots grown under ambient CO₂ and incubated in ambient-CO₂ soils that received high rates of N fertilizer. The lowest respiration rates were found for high-CO₂ roots that were incubated in elevated-CO₂ soils that received high rates of N-fertilizer. When we look specifically at one of the most interesting comparisons of this study, i.e., decomposition of ‘ambient-CO₂’-grown plant material in an ‘ambient-CO₂’-soil with ‘elevated-CO₂’-grown plant material in an ‘elevated-CO₂’-soil, we observe that decomposition in an ‘elevated-CO₂ world’ is slower than in an ‘ambient-CO₂ world’ (Figure 4.1). An N-fertilizer interaction, however, exists.

After 64 days, 24% of all CO₂ respired from soils with added roots was soil-derived (data not shown). The addition of substrate significantly decreased the amount of soil-derived CO₂-C respired during the incubation from 0.77 to an average from all treatments of 0.59 g C kg⁻¹ (Table 4.5). The addition of root material caused a decrease in SOC respiration during the first 18 days, after which the decomposition rates of SOC in root-free and root-amended soil became similar (Figure 4.2). Within soils that received root materials, both soil CO₂ and root CO₂ treatments affected the respiration of SOC. High-CO₂ roots tended to decrease soil-derived respiration compared to ambient-CO₂ roots, averaging 0.60 and 0.57 g C kg⁻¹ respectively ($P=0.06$). Soils that were exposed to elevated CO₂ tended to release more soil-derived C than ambient-CO₂ soils, averaging 0.69 vs. 0.49 g C kg⁻¹ ($P=0.07$), respectively.

Soil microbial biomass

After 64 days, an average of 7.6% of the root C was incorporated in the microbial biomass (Table 4.5). The effect of root CO₂ treatment depended on the amount of N fertilizer applied. At a low rate of N fertilizer applications, more root C produced under elevated CO₂ was incorporated in the microbial biomass than that produced under ambient CO₂, i.e., 8.3% of the high-CO₂ versus 6.5 % of the low-CO₂ root C. Soil CO₂ treatments did not affect the amount of root C that was incorporated in the microbial biomass (Table 4.5). After 64 days, the $q^{14}\text{CO}_2$ ranged from 7.9 to 10.4 across treatments. The metabolic quotient was significantly lower for high-CO₂ roots; 8.2 and 10.1, respectively. Soil CO₂ and N treatments did not affect $q^{14}\text{CO}_2$.

Table 4.5: SOC respired after 64 days, % C root-C incorporated in soil microbial biomass, and $q^{14}\text{CO}_2$ for *L. perenne* root material grown at two CO₂ levels after 64 days of incubation in *L. perenne* soil subjected to 10 years of ambient or elevated CO₂ and two N treatments (n=3, except for ambient CO₂-soils receiving low N treatments, for which n=2).

Soil CO ₂	N-rate	Roots CO ₂	SOC _{resp} (g kg ⁻¹ ; 0-64 days)	Root-C (%) in SMB	<i>q</i> ¹⁴ CO ₂
Ambient	Low	Ambient	0.56 ± 0.05†	6.8 ± 0.6	10.4 ± 0.9
		Elevated	0.46 ± 0.05	8.9 ± 0.6	7.9 ± 0.9
	High	Ambient	0.47 ± 0.02	7.9 ± 0.4	10.1 ± 0.7
		Elevated	0.46 ± 0.02	8.3 ± 0.7	8.4 ± 0.7
Mean			0.49 ± 0.02	8.0 ± 0.3	9.2 ± 0.5
Elevated	Low	Ambient	0.72 ± 0.08	6.2 ± 0.2	10.4 ± 0.7
		Elevated	0.66 ± 0.06	7.7 ± 0.6	8.6 ± 0.5
	High	Ambient	0.67 ± 0.11	7.8 ± 1.0	9.6 ± 1.1
		Elevated	0.69 ± 0.12	7.3 ± 0.7	8.1 ± 0.6
Mean			0.69 ± 0.04	7.3 ± 0.4	9.2 ± 0.4
Grand mean			0.59 ± 0.03	7.6 ± 0.2	9.2 ± 0.3
ANOVA					
Soil CO ₂			*	NS	NS
Soil N			NS	NS	NS
Soil CO ₂ * soil N			NS	NS	NS
Roots CO ₂			*	***	***
Soil CO ₂ * roots CO ₂			NS	NS	NS
Soil N*roots CO ₂			NS	***	NS
Soil CO ₂ * soil N*roots CO ₂			NS	NS	NS

† Mean ± SE; *,*** Significant at the 0.1 and 0.01 probability level, respectively.

4.4 Discussion

Elevated-CO₂-adapted soils and decomposition of SOC and roots

Our results show the importance of including soils adapted to elevated CO₂ in studies on root decomposition under elevated CO₂. Average decomposition rates of both ambient- and elevated-CO₂ root material were always lower in the soil exposed to elevated CO₂ (Table 4.4). The lower decomposition of roots in soils exposed to elevated CO₂ is in accordance with the results of Ross et al. (2002). They found that soil exposed to elevated CO₂ respired more native SOC, but had lower initial rates of decomposition of added grass litter. Gahrooei (1998) also reported that *Quercus cerris* L. litter decomposed more slowly in a CO₂-enriched environment. Our results are inconsistent with Sowerby et al. (2000), who concluded from a litterbag experiment that litter produced under elevated CO₂ and incubated in soil exposed to elevated CO₂

decomposed more rapidly than litter produced under ambient CO₂ and incubated in soil exposed to ambient CO₂. However, as their litter produced under elevated CO₂ contained a significantly larger amount of soluble C, the increased C loss from this litter might have been partly caused by leaching. Van der Krift et al. (2002) provided isotopic evidence that decomposition rates based on mass C loss measurements in litter bag experiments may not be accurate, due to leaching of C.

Ross et al. (2002) suggested that a decrease in litter decomposition in soil exposed to high CO₂ (Table 4.4) might have been caused by preferential metabolism by the decomposer populations of easily decomposable soil C. Indeed, Hu et al. (2001) reported an increase in available C for microbes under annual grasslands after 5 years of elevated CO₂. This explanation is supported by our findings that SOC-derived CO₂ respiration was higher in soil exposed to high CO₂ concentrations compared to soils exposed to ambient CO₂, independent of the addition of roots (Table 4.5). It is likely that more C is available in the soil exposed for 10 years to elevated CO₂ than in soil exposed to ambient CO₂.

Elevated-CO₂-grown roots and decomposition

Within high-N soil treatments, the highest decomposition rates were measured for roots grown under ambient CO₂ and incubated in ambient-CO₂ soils. Roots produced under elevated CO₂ incubated in ambient-CO₂ soils decomposed more slowly, but the lowest decomposition rates were found for roots grown under elevated CO₂ incubated in elevated-CO₂ soil (Table 4.4). This finding indicates that decomposition in an 'elevated-CO₂ world' is possibly slower than in an 'ambient-CO₂ world' (Figure 4.1).

Furthermore, our results indicate that at different stages of the incubation, different factors controlled cumulative decomposition rates. Whereas root decomposition during the first day was significantly affected only by whether roots were produced under ambient or elevated CO₂, the cumulative decomposition towards the end of the incubation period was controlled by the rate of N-fertilizer application and by whether the soil had been exposed to elevated CO₂ (Table 4.4). These results show the complexity of belowground processes that occur under elevated CO₂ and different nutrient regimes, and that the results of incubation experiments remain highly dependent on the duration of the incubation period (Norby et al. 2001). Yet, when roots were produced under elevated CO₂, the cumulative CO₂ respiration was lower throughout the incubation, independent of the rate of N applied. This result indicates that some yet-to-be-determined ecological process, which occurred consistently, is involved during the decomposition of litter produced under elevated CO₂.

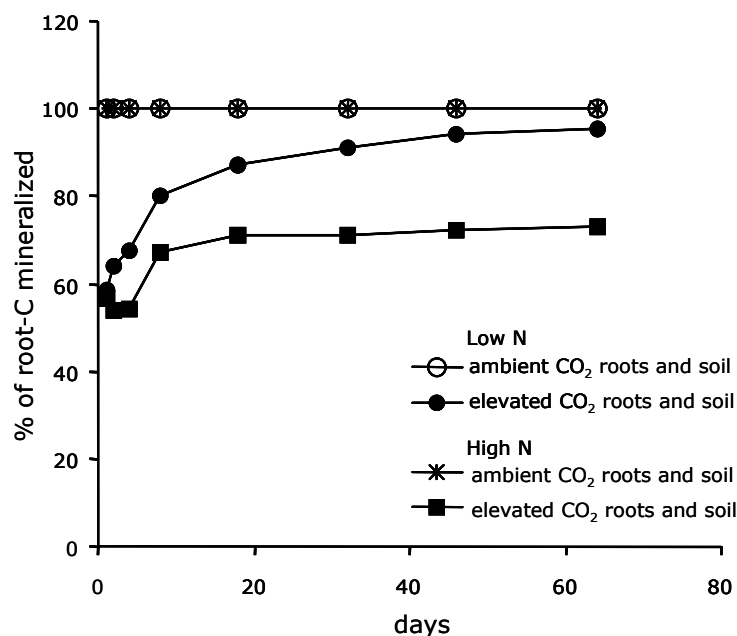


Figure 4.1: Decomposition of root material in an ‘elevated-CO₂ world’ (i.e., elevated-CO₂ roots in elevated-CO₂ soil) as a percentage of decomposition in an ‘ambient-CO₂ world’ (i.e., ambient-CO₂ roots in ambient-CO₂ soil) as affected by two rates of N application during an incubation at 25°C for 64 days. Please note that the rate of decomposition in the ‘ambient-CO₂ world’ is not identical for both rates of N application.

Our results point to a slower rate of litter turnover under elevated atmospheric CO₂, possibly leading to increased soil C-sequestration. Yet, the soil C data show no significant increase in C concentration after 10 years of elevated CO₂ (Table 4.2). From a meta-analysis, Kimball et al. (2002) concluded that small increases in soil C concentration are likely to occur under elevated atmospheric CO₂. However, increases in soil C are difficult to detect in individual FACE-experiments because of the large size of the native SOM pool compared to the pool of new C, spatial variability (Hungate et al. 1996), and possible changes in soil bulk density. Indeed, numerous researchers reported a non-significant increase in soil C content under prolonged elevated CO₂ (Leavitt et al. 1996; Hungate et al. 1997a; Van Kessel et al. 2000a,b). However, significant increases have been reported (Williams et al. 2000). This implies that even when sensitive methods, such as used in our incubation experiment, detect changes in the rate of decomposition, the resulting effect on C-sequestration might remain unnoticed under field conditions for relatively long periods of time. Similar results were found using a physical fractionation procedure of FACE soils (Six et al. 2001). Although Six et al. (2001) found no effect of elevated CO₂ on total soil C

contents, the C contents of particulate organic matter (POM) increased significantly when the rate of N application was high.

De Graaff et al. (2004) conducted a ^{13}C -labeled incubation similar to our experiment, with both soils and above-ground material obtained from the ETH-FACE-site. Although elevated CO_2 significantly increased the C:N ratios of the residues, it did not affect the rate of decomposition. However, different effects on the rates of decomposition of roots and above-ground residue produced under elevated CO_2 , which seemed to be independent of the C:N ratio, have been reported (Gorissen and Cotrufo 2000). In our experiment, elevated CO_2 had a relatively strong positive effect on the C:N ratio of the roots (Table 4.3). Root material grown at the same FACE site also has a higher C:N ratio than above-ground material (Van Kessel et al. 2000a), and its C:N ratio increased under elevated CO_2 (Jongen et al. 1995). Although differences in C:N ratios are disputed as an appropriate general indicator of the rate of decomposition (Gorissen and Cotrufo 2000; Ross et al. 2002), they may reflect differences in the 'quality' of root and above-ground residue as it affects the rate of decomposition. Unfortunately, the decomposition of roots and above-ground material grown under FACE conditions in soil exposed to prolonged elevated CO_2 has not yet been compared in one single study.

Soil microbial activity

A decrease in the $q^{14}\text{CO}_2$ can be caused by either an increased efficiency in the use of added substrate for microbial growth (Harden et al. 1993) or a slower turnover of microbial biomass (Ladd et al. 1995). Compared to bacteria, fungi have higher C assimilation efficiencies and slower turnover rates (Adu and Oades 1978; Holland and Coleman 1987). Correspondingly, a strong negative correlation between the fungal:bacterial ratio and $q\text{CO}_2$ has been reported (Sakamoto and Oba 1994; Blagodatskaya and Anderson 1998). Fungi are generally regarded as the main decomposers of more recalcitrant plant material (Paul and Clark 1996). Therefore, an enhanced fungal community could have contributed to the decrease in $q^{14}\text{CO}_2$ following the addition of more recalcitrant roots produced under high CO_2 concentrations. Coûteaux et al. (1991) reported an invasion of white-rot fungi upon the incubation of high- CO_2 litter, whereas low- CO_2 litter treatments remained unaffected. In a similar incubation experiment, Van Ginkel et al. (2000) reported lower rates of decomposition of roots produced under elevated CO_2 , but they found no change in the $q^{14}\text{CO}_2$. Different amounts of C added to the soil (approximately 0.15 g kg^{-1} soil vs. 2.2 g kg^{-1} soil) and subsequent effects on N availability, and/or differences in incubation temperature (14°C vs. 25°C) between the experiment by Van Ginkel et al. (2000) and our current experiment may have affected the $q^{14}\text{CO}_2$ differently.

Several field experiments suggest that altered plant growth under elevated CO₂ stimulates fungi more strongly than bacteria (Hungate et al. 2000; Rillig et al. 2000; Klammer et al. 2002; Phillips et al. 2002). However, fatty acid profile analyses showed inconsistent results in other field experiments (Zak et al. 1996; Kandeler et al. 1998). Our experimental design did not allow us to determine whether decreased litter quality contributes to a microbial shift under elevated CO₂ under field conditions. Yet, our results indicate a change in soil microbial metabolism following the addition of litter produced under elevated CO₂, causing a slower rate of litter turnover. In the long term, such a microbial response would result in an increase in C sequestration.

Priming/conserving effect

Our data strongly suggest a conserving (negative priming) effect for SOM-C upon the addition of *L. perenne* roots (Tables 4.2 and 4.5). These results are in accordance with Kuzyakov et al. (1997), who found that the addition of both fresh and dried root material resulted in conserving of SOM-C. In our experiment, the conserving of SOM-C was marginally affected by the CO₂ treatments of the root material (Table 4.5). Similar conserving effects are likely to occur regardless of the isotopic signature of the substrate and soil. Therefore, the precise contribution of residue C in non-labeled experiments will become obscured. As such, our results confirm the value of using C isotopes in incubation studies.

Both priming and conserving effects on SOM-C decomposition have been reported upon the addition of plant residue (Kuzyakov et al. 2000). Conserving effects have been attributed to a microbial shift from the native SOM to more easily decomposable material (Sparling et al. 1982; Reid and Goss 1983), whereas positive priming effects have generally been attributed to an increase in microbial activity. Apparent conserving effects might occur as the result of an artifact, such as the use of non-homogeneously ¹⁴C-labeled substrate (Kuzyakov 2002). However, the roots used in our experiment were homogeneously labeled (Van Ginkel et al. 1997). As the rate of SOC decomposition in soils with and without roots gradually converge (Figure 4.2), a temporary microbial shift to easily decomposable components when roots are added seems likely. As the microbial communities associated with the decomposition of readily available C and recalcitrant SOC are different (Fontaine et al. 2003), such a shift would only apply to a part of the soil microbial community.

In most agro-ecosystems, the input of C in the soil will increase under elevated atmospheric CO₂ (Kimball et al. 2002). This, together with a conserving effect from increased plant residues under elevated CO₂ might lead to a preservation of older SOM. Whether such a stabilization will occur under field conditions is, however, unclear. The amount of root material added in our experiment was high compared to the amount of SOM present, thereby possibly causing artifacts. In the field, rhizodeposition is an important part of C input, and rhizosphere priming will affect C

dynamics (Kuzyakov 2002). Although our experiment did not take into account rhizosphere priming, an overall conserving effect of SOM following the addition of roots is in accordance with Cardon et al. (2001) and Lekkerkerk et al. (1990). They reported that under elevated CO_2 , the soil microbial community appeared to shift from consuming older soil C to utilizing easily decomposable rhizodeposits derived from increased root biomass.

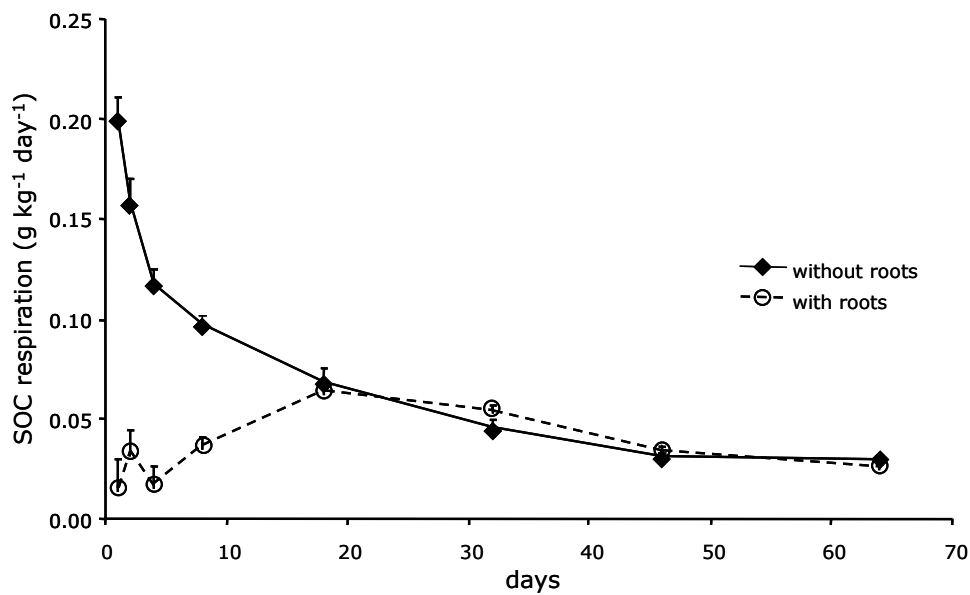


Figure 4.2: SOC-derived respiration of soil subjected to 10 years of ambient or elevated CO_2 and two N treatments (averaged over soil CO_2 and N treatments) with and without the addition of roots grown at two CO_2 levels (averaged over root CO_2 treatments) during an incubation at 25°C for 64 days.

4.5 Conclusions

The combination of ^{14}C -labeled plant material grown under elevated atmospheric CO_2 and soil from a long-term FACE-project provided a unique opportunity to study litter decomposition and soil organic matter turnover as affected by elevated CO_2 . Overall, decomposition of roots in an ‘elevated- CO_2 world’ was slower than in an ‘ambient- CO_2 world’. In soils that received high N treatments, roots grown under high CO_2 decomposed more slowly than low- CO_2 roots. In soils that received low N treatments, decomposition of high CO_2 -roots was only temporarily retarded. Root material grown under elevated CO_2 increased the substrate use efficiency of the microbial biomass under both rates of N application, possibly because of an increased participation of fungal biomass in the decomposition. Additional research will be required to

determine whether such an increase in substrate use efficiency following prolonged exposure to elevated CO₂ occurs under field conditions. More specifically, future studies should address changes in the composition and size of the soil microbial community under prolonged elevated CO₂ and different N treatments, and the consequences for C stabilization. The current experiment was performed on soils which received a relatively high dose of N fertilizer. As the effect of root CO₂ treatment on decomposition rates and microbial C incorporation interfered with N treatments, different results might be expected for soils with a low N availability. Our data suggest a shift in factors controlling C decomposition during the incubation. Consequently, a great deal of caution should be applied when extrapolating results from short-term litter incubations to field situations in predicting ecosystem responses. As the addition of roots induced the conserving of SOM-C, with its extent depending on whether the roots were produced under ambient or elevated CO₂, one should be even more cautious interpreting data from incubation studies when no C isotopes are used.

5 **Elevated CO₂ does not favor a fungal decomposition pathway**

Abstract

We examined the effect of prolonged elevated CO₂ on the concentration of fungal- and bacterial-derived compounds by quantifying the soil contents of the amino sugars glucosamine, galactosamine and muramic acid. Soil samples were collected from three different terrestrial ecosystems (a grassland, an aspen forest and a soybean/corn agroecosystem) that were exposed to elevated CO₂ under FACE conditions. Amino sugars were extracted from bulk soil and analyzed by gas chromatography. Elevated CO₂ did not affect the size or composition of the amino sugar pool in any of the systems. However, high rates of fertilizer N applications decreased the amount of fungal derived residues in the grassland system. Our findings imply that the relative abundance of fungi and bacteria is largely unaffected by elevated CO₂ in terrestrial ecosystems.

5.1 Introduction

The current rise in atmospheric CO₂ might indirectly affect the soil microbial community through its effect on the vegetation in terrestrial ecosystems. Elevated atmospheric CO₂ tends to stimulate plant N uptake and/or increase litter C:N ratios, thereby gradually decreasing soil mineral N availability (Luo et al. 2003). Moreover, lower leaf conductance under elevated CO₂ can reduce evapotranspiration, thereby increasing soil water content (e.g. Volk et al. 2000) and further changing the environmental conditions for soil microorganisms.

The qualitative effect of elevated CO₂ on the soil microbial community remains widely debated (Niklaus et al. 2003). Therefore, the difference in fungal versus bacterial responses to elevated CO₂ is pertinent. Fungal cell walls are more recalcitrant and decompose more slowly than bacterial cell walls (Nakas and Klein 1979; Six et al. 2006). Fungi produce organic binding agents and entangle soil particles, thereby stimulating aggregate formation and providing physical protection of SOM (Tisdall 1994). Moreover, fungi have been shown to deposit litter C within these aggregates (Frey et al. 2003). For these reasons, soil C storage is expected to be more persistent when the soil food web becomes increasingly dominated by fungi (Beare et al. 1992; Six et al. 2006).

Fungi are regarded as the main decomposers of recalcitrant litter with high C:N ratios (Paul and Clark 1996). Low soil N availability may also stimulate fungi over bacteria because fungal biomass has a higher C:N ratio and fungi can translocate soil inorganic N (Frey et al. 2003). On these grounds, it has been hypothesized that rising CO₂ levels would induce a shift towards the fungal pathway (Klironomos et al. 1996).

This hypothesis has proven difficult to test under field conditions. Soil microbes adapt quickly to changes in temperature, moisture and C availability, causing seasonal shifts in the soil microbial community composition. Thus, a single sampling of the microbial population may not fully reveal its response to prolonged elevated atmospheric CO₂. As microbial cell walls turn over much slower than living microbial biomass (Guggenberger et al. 1999), their abundance might be a more useful indicator of time-averaged soil microbial responses.

Microbial cell walls partly consist of amino sugars. The sugar derivative N-acetyl muramic acid is exclusively found in the peptidoglycan of prokaryotic cell walls, where it alternates with N-acetyl glucosamine at a 1:1 ratio. Fungal cell walls contain chitin, a polymer of N-acetyl glucosamine. Galactosamine on the other hand is predominantly derived from bacterial cell walls (Amelung 2001). As such, the relative abundance of these three amino sugars can be used to determine fungal and bacterial contributions to SOM dynamics (Zhang and Amelung 1996; Amelung 2001).

We aimed to determine the impact of elevated CO₂ on the soil concentration of bacterial and fungal residues. To test whether elevated CO₂ affects the microbial community similarly across a range of ecosystems, we included soil samples from

three different systems under FACE conditions; a trembling aspen (*Populus tremuloides*) forest, a soybean (*Glycine max*)/corn (*Zea mays*) rotation and a fertilized *Lolium perenne* grassland. The experiment on the fertilized grassland included two N addition levels, allowing us to test the effect of soil N availability on the soil microbial community as well.

5.2 Materials and methods

Site characteristics of the three FACE systems are summarized in Table 5.1. The Swiss FACE experiment included two soil N availability treatments; plots in each ring were fertilized with either 140 (low N) or 560 kg N ha⁻¹yr⁻¹ (high N). At each site, soil samples were taken from all ambient rings and all rings receiving elevated CO₂. Samples were collected in the spring of 2002 at the Swiss FACE, the two other systems were sampled in the fall of 2002. Samples were taken to a depth of 0-20 cm at the FACTS-II, and at 0-10 cm at the other sites.

Table 5.1: Overview of experimental conditions at the three experimental sites

	FACTS-II	SoyFACE	SwissFACE
Location	Rhineland (WI) USA	Urbana-Champaign (IL) USA	Eschikon Switzerland
Species	trembling aspen	soybean/corn rotation	rye grass
Soil type	loamy Alfisol	clay loam Mollisol	clay loam Mollisol
MAT (°C)	4.7	10.8	8.6
MAP (mm)	793	954	1108
Duration experiment at time of sampling	6 years	3 years	10 years
CO ₂ enrichment	560 ppm (day only)	550 ppm (24 h day ⁻¹)	600 ppm (day only)
# rings	3 elevated CO ₂ 3 ambient	4 elevated CO ₂ 4 ambient	3 elevated CO ₂ 3 ambient
Ring diameter	30 m	20 m	18 m
Sampling depth	0-20 cm	0-10 cm	0-10 cm

Soil samples were sieved (8mm) and oven-dried, after which large roots and stones were removed by hand. The C contents of bulk soil were measured according to Van Groenigen et al. (2003). Amino sugars were extracted from bulk soil according to the method of Zhang and Amelung (1996). The amino sugars were then transformed into aldononitrile derivatives according to Guerrant and Moss (1984). Within 10 days, the aldononitrile derivatives were analyzed on an Agilent 6890 gas chromatograph, equipped with an Agilent DB5 column.

For our calculations, we assumed a 40% C content for glucosamine and galactosamine, and a 43% C content for muramic acid (Simpson et al. 2004). To calculate fungal and microbial derived C, we assumed that all galactosamine and muramic acid was produced by bacteria, and that glucosamine and muramic acid occur in equal amounts in bacterial residues. We calculated total amino sugar contents as the sum of glucosamine, galactosamine and muramic acid.

We analyzed our data using a mixed model ANOVA in the SAS system for Windows V8. For each site, blocks (i.e. field plots) were included as a random effect, and CO₂ as a fixed effect. In the case of Swiss FACE, N treatment was included as a second fixed effect. Differences between means were identified using paired t-tests. Treatment effects were considered significant at $P < 0.05$.

5.3 Results and discussion

Total soil amino sugar contents ranged between 337 and 1224 mg C kg⁻¹ soil across systems (Figure 5.1), or 3.0 and 5.2 % of the total soil C pool. These values correspond well with previously reported results for grassland, forests and agricultural systems (e.g. Simpson et al. 2004; Turrion et al. 2002). Differences between sites are most likely due to differences in soil mineralogy, land use and climate, all of which can affect soil amino sugar concentrations (Guggenberger et al. 1999; Amelung et al. 1999). The composition of the soil amino sugar pool was similar in all systems: glucosamine comprised between 58 and 66% of the total amino sugar-C pool, galactosamine contributed 31-36%, and the contribution of muramic acid ranged between 3 and 7% (Figure 5.1). These results are similar to findings of many other studies (e.g. Turrion et al. 2002; Simpson et al. 2004; Glaser et al. 2006).

Elevated CO₂ did not affect the concentration of any of the amino sugars, or the size of the total amino sugar pool in either the grassland, the aspen forest, or the corn/soybean rotation (Figure 5.1). Accordingly, the contribution of fungal and bacterial amino sugars to the soil C pool in these drastically different ecosystems was unaffected by elevated CO₂ (Figure 5.2). However, in the grassland ecosystem, high N soils contained significantly less fungal-derived amino sugars than low N soils ($P=0.05$). When the CO₂*N interaction was included in the statistical model, the overall N effect was marginally significant ($P=0.06$). Nitrogen additions especially reduced fungal residues under ambient CO₂.

Our results corroborate Glaser et al. (2006), who found that at the Swiss FACE site, elevated CO₂ does not affect the soil amino sugar pool composition in low N treatments. Moreover, our findings agree with measurements on living microbial biomass. Elevated CO₂ as a main effect did not affect saprophytic fungi biomass at the Swiss FACE site (Řezáčová et al. 2005). Similarly, fatty acid profile analyses

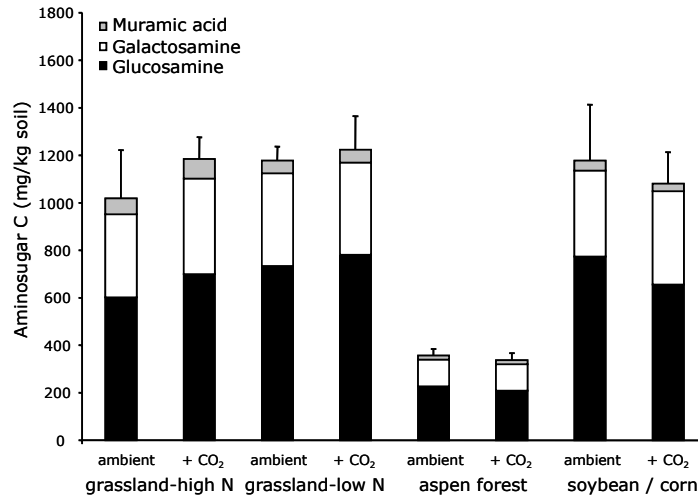


Figure 5.1: Soil concentrations of muramic acid, galactosamine and glucosamine in three different terrestrial ecosystems (grassland, forest and agricultural), as affected by prolonged elevated CO₂. Note that the Swiss FACE experiment included low and high N treatments. Error bars indicate standard errors of the mean for total amounts of amino sugar C.

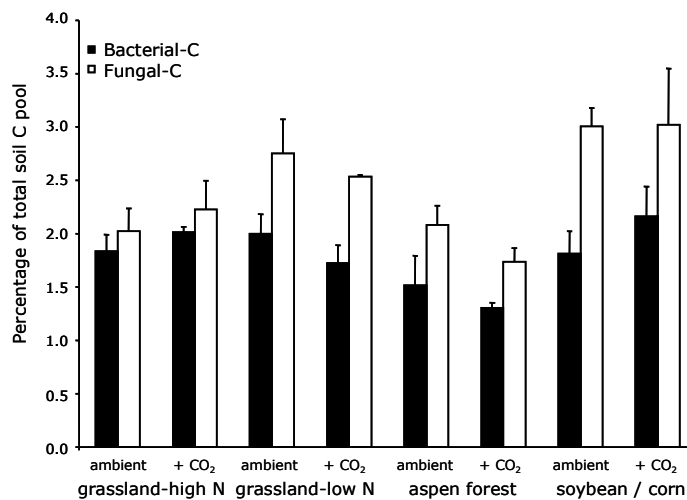


Figure 5.2: Fungal residue C and microbial residue C in three different terrestrial ecosystems (grassland, forest and agricultural), as affected by prolonged elevated CO₂. Results are expressed as a percentage of soil C contents. Note that the Swiss FACE experiment included low and high N treatments. Error bars indicate standard errors of the mean. Fungal C = fungal derived glucosamine-C; Bacterial C = bacterial derived glucosamine-C + galactosamine-C + muramic acid-C. At the Swiss FACE site, high N soils contained significantly less fungal residue C than low N soils. No other significant effects were observed.

suggest that elevated CO₂ did not significantly affect the relative abundance of saprophytic fungi at the FACTS-II site (Chung et al. 2006), although there was a trend towards increased fungal activity.

A recent meta-analysis suggests that elevated CO₂ stimulates mycorrhizal abundance in many ecosystems, with an average increase of +47% (Treseder 2004). This conclusion seems inconsistent with our finding that elevated CO₂ does not increase the amount of fungal-derived residues. Yet, by taking out fine roots from the soil, we probably removed mycorrhizae derived residue. Thus in our study, the amino sugar pool composition predominantly reflects treatment responses of saprophytic fungi and bacteria.

Nitrogen additions might also affect the presence of saprophytic fungi. Řezáčová et al. (2005) found that at the Swiss FACE site, high N fertilization decreased the presence of saprophytic fungi under ambient CO₂ concentrations. Moreover, N additions reduced the abundance of saprophytic fungi in an incubation experiment (Bossuyt et al. 2001) and in a field study on grassland soils (Bradley et al. 2006). The mechanism behind these microbial responses is not well understood. One explanation would be that only under low soil N availability, saprophytic fungi have an advantage over bacteria through competition for nutrients. Fungal hyphae enable them to utilize spatially separated C and N resources, by transporting soil inorganic N into C-rich residues (Beare et al. 1992; Frey et al. 2003). Obviously, this competitive advantage disappears when N is abundant. High N additions might also have a direct toxic effect on some saprophytic fungi by inhibiting their enzymes (Fog 1988). Regardless of the exact cause, our findings strongly imply that N additions affect the stabilization of SOM through its effect on the soil microbial community.

Amino sugar analyses only provide a broad indication of microbial responses, as they do not consider fungal and bacterial diversity. Yet, due to the relative slow turnover of microbial cell walls, amino sugars are a valuable indicator of time-averaged microbial responses. This is especially the case in elevated CO₂-studies where large temporal variation in soil microbial responses is often observed (e.g. Hungate et al. 2000). Our data indicate that in three drastically different ecosystems, elevated CO₂ did not affect the relative abundance of fungi and bacteria.

6 The impact of elevated atmospheric CO₂ on soil C and N dynamics: a meta-analysis

Abstract

Field experiments are a valuable tool for predicting the effect of future CO₂ concentrations on SOM dynamics. Using meta-analytic techniques, we reviewed the effect of CO₂ enrichment on SOM dynamics under field conditions. Our analysis summarized the effects of 65 studies and covered results from both OTC and FACE experiments. Averaged over all studies, soil C contents increased by 4.1% under elevated CO₂. A relatively strong increase in potential mineralizable C (+11.1%) and soluble C (+9.4%) suggests that the rise in soil C is largely due to expanding labile C pools. The effect of CO₂ enrichment on soil C contents depended on N availability; elevated CO₂ only increased soil C contents in experiments that received 30 kg N ha⁻¹ yr⁻¹ or more. Short-term experiments on planted vegetation showed a larger range of soil microbial responses to elevated CO₂ than long-term experiments on natural vegetation. Microbial C contents and microbial respiration increased by 8.5% and 18.0% under elevated CO₂, respectively. The effect of CO₂ enrichment on microbial biomass and respiration tended to be higher for fertilized studies. However, since soil C storage under elevated CO₂ depends on N additions, we conclude that at high N fertilization rates the CO₂ response for soil C input outweighs that of microbial respiration. Elevated CO₂ stimulated gross N immobilization by 29.8%, whereas gross N mineralization rates remained unaffected. These results, together with a 7.2% increase in microbial N contents under elevated CO₂ in long-term studies, suggest that higher CO₂ levels enhanced microbial N demand. An increase in microbial N demand under elevated CO₂, together with the dependency on N additions for soil C storage under elevated CO₂ are in line with the progressive nitrogen limitation theory.

Based on: Van Groenigen KJ, de Graaff MA, Six JW, Harris D, Kuikman P, Van Kessel C (2006a). In: *Managed ecosystems and CO₂ case studies, processes and perspectives* (Eds. Nösberger J, Long SP, Norby RJ, Stitt M, Hendrey GR, Blum H), pp 373-392. Springer-Verlag, Berlin Heidelberg

6.1 Introduction

The current rise in atmospheric CO₂, a consequence of human activities such as fossil fuel burning and deforestation, is thought to stimulate plant growth in many ecosystems (Bazzaz and Fajer 1990). Gifford (1994) suggested that the resulting increase in C assimilation by plants and its subsequent sequestration in the soil could counterbalance CO₂ emissions. However, higher plant growth rates in a CO₂-rich world can only be sustained if the soil supplies plants with additional nutrients (Zak et al. 2000; Luo et al. 2004). Therefore, the effect of elevated CO₂ on soil N availability is of key importance when predicting the potential for C storage in terrestrial ecosystems.

In short-term experiments, soil N availability can decrease (Diaz et al. 1993) or increase (Zak et al. 1993) under elevated CO₂, depending on the response of the soil microbial community. Moreover, plants under elevated CO₂ can increase N uptake at the expense of microbial N consumption (Hu et al. 2001). Clearly, the impact of higher CO₂ levels on C and N dynamics in terrestrial ecosystems depends on a set of complex interactions between soil and plants. Also, the establishment of equilibrium between SOM input and decomposition can take up to decades or longer. Therefore, we need long-term experiments under realistic field situations to predict changes in ecosystems under future CO₂ concentrations.

The use of open-top chambers (OTC) and free-air carbon dioxide enrichment (FACE) techniques allowed for CO₂ fumigation studies under far more realistic conditions than before (Rogers et al. 1983; Hendrey 1993). Over the past two decades, many OTC and FACE experiments have been conducted, covering a wide range of terrestrial ecosystems. Soil characteristics related to C and N cycling have been studied in most of these experiments, but no clear pattern has emerged that allows us to generalize about CO₂ enrichment effects on SOM dynamics (Zak et al. 2000).

By affecting soil C and N dynamics, ecosystem management practices could influence an ecosystem's response to increased atmospheric CO₂ concentrations. For example, the addition of fertilizer N might affect both the input and loss of soil C by affecting the CO₂ response of plant growth (Oren et al. 2001) and decomposition rates (Rice et al. 1994; Niklaus and Körner 1996). Intensive soil disturbances can also affect soil C and N dynamics, as they disrupt aggregates containing physically protected SOM (Six et al. 2002). Thus, we should take into account differences in ecosystem management when we compare results from CO₂ enrichment studies.

The sensitivity of individual experiments to detect changes in soil C is low because of high spatial variability and the large size of the soil C pool compared to the input of C (Hungate et al. 1996). The statistical power to identify changes in SOM pools across individual experiments might be increased by a quantitative integration of research results. 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 enable to test whether there are significant differences in the mean CO₂ response between categories of studies (Hedges and Olkin 1985).

Meta-analyses have recently been used to summarize the effect of elevated CO₂ on plant physiology, litter quality and decomposition rates (Curtis and Wang 1998; Norby et al. 2001; Ainsworth et al. 2002). In comparison, few researchers have used meta-analysis to summarize CO₂ effects on SOM dynamics (Jastrow et al. 2005). For this review we compiled the available data from FACE and OTC experiments on a number of soil characteristics related to soil C and N cycling. Using meta-analytic techniques, we compared the effect of CO₂ enrichment on these characteristics between several levels of ecosystem management.

6.2 Materials and Methods

Database compilation

Data were extracted from 65 published studies on SOM dynamics in FACE and OTC experiments. The response variables included in the meta-analysis are listed in Table 6.1. Whenever values were reported in tables, they were taken directly from the publication. Results presented in graphs were digitized and measured to estimate values for the particular pool or flux. Data reported on an area base were converted to a weight base, using soil density data whenever available. In all other cases, equal bulk soil density in current ambient and elevated CO₂ treatments was assumed.

Table 6.1: List of response variables included in the meta-analysis and their abbreviations, as used in figures.

Parameter	Definition
C	Soil C content
N	Soil N content
C:N	Soil C:N ratio
MicC	Microbial C content
MicN	Microbial N content
rCO ₂	Microbial respiration, measured in short-term (<15 days) incubations
MinN	N mineralization rates, measured in short-term (<30 days) incubations
GNI	Gross N immobilization, measured by ¹⁵ N pool dilution methods
GNM	Gross N mineralization, measured by ¹⁵ N pool dilution methods
C _{max}	Potential mineralizable C, measured in long-term (>55 days) incubations
C _{sol}	Soluble C

To make meaningful comparisons between experiments, a number of restrictions were applied to the data. Data were included for soil layers ranging in depth from 0–5 cm to 0–40 cm. When data were reported for multiple depths, we included results

that best represented the 0-10 cm soil layer. As our review focuses on mineral soils, measurements on forest litter layers, marsh and rice paddies were excluded from the database. The elevated CO₂ levels of the experiments included in the data base ranged from 450 ppm to 750 ppm. Data were not corrected for the degree of CO₂ enrichment. When more than one elevated CO₂ level was included in the experiment, only the results at the level that is approximately twice the ambient CO₂ level were included. The duration of the CO₂ treatment had to be at least 100 days (the approximate length of a growing season in the temperate zone). 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. Reich et al. (2001) reported the effect of elevated CO₂ on N mineralization under 16 grassland species, which were grown both in monocultures and in mixtures. If results would be included for all separate species, their experiment would dominate the MinN data set. Therefore, only the results for the 16 species mixture plots were used. For OTC experiments, data from the control chambers rather than the non-chamber control plots were included as the results at ambient CO₂ concentrations. In case these were available, data for blower controls in FACE experiments were included as the results at ambient CO₂ concentrations.

Results on C and N fluxes were all based on incubation data (laboratory and in situ). Data for microbial biomass were based on the fumigation-extraction method (Vance et al. 1987) and the substrate induced respiration technique (Anderson and Domsch 1978). Data for soluble C were based on extractions using either cold water, or 0.5 M solutions of KCl or K₂SO₄. For total soil C and N contents, only the most recent data for each study were incorporated. For data on microbial biomass and activities, time series from the most recent year of measurement were included whenever available. In these cases, the average values at ambient and elevated CO₂ levels were calculated over time.

Table 6.2: Categorical variables used to summarize experimental conditions; and the values they could assume in the analysis of between-group heterogeneity.

	Level 1	Level 2	Level 3
Method	OTC	FACE	
N fertilization (kg ha ⁻¹ yr ⁻¹)	<30	30-150	>150
Vegetation (a)	Planted	Natural	
Vegetation (b)	Herbaceous	Woody	
Vegetation (c)	Crops	Non-crops	

Experimental conditions were summarized by a number of categorical variables: type of exposure facility, N addition and vegetation type (Table 6.2). Vegetation was characterized as either herbaceous or woody, crop or non-crop and planted or natural. Vegetation was considered planted if it was sown or placed into the soil less than

10 years before the start of the CO₂ fumigation. Thus, all experiments on planted vegetation were conducted on physically disturbed soil.

The duration of each experiment (i.e. years of CO₂ fumigation) was also included in the database. The number of observations on microbial responses to elevated CO₂ was relatively low compared to observations on soil C and N contents. To ensure that each N fertilization class was well represented, we decided to pool the two highest classes for these response variables.

Statistical analyses

The data set was analyzed with meta-analytic techniques described by Curtis and Wang (1998) and Ainsworth et al. (2002), using the statistical software MetaWin ver. 2.1 (Rosenberg et al. 2000). The natural log of the response ratio (r = response at elevated CO₂/response at ambient CO₂) was used as a metric for all variables and is reported as the percent change at elevated CO₂ ($[r-1] \times 100$). A mixed model was used for our analysis, based on the assumption that a random variation in CO₂ responses occurred between studies.

The effect of elevated CO₂ on total soil C content, soil N content and soil C:N ratios was analyzed using a weighted parametric analysis. In this analysis, each individual observation was weighted by the reciprocal of the mixed-model variance, which was the sum of the variance of the natural log of the response ratio and the pooled within-class variance (Curtis and Wang 1998). Results from studies that did not report standard deviation were included conservatively by assigning them the minimum weight calculated from other studies in the data set, according to Norby et al. (2001).

To test whether differences in CO₂ responses could be explained by experimental conditions, results were compared between categories of studies. In the weighted analysis, the total heterogeneity for a group of comparisons (Q_t) was partitioned into within-class heterogeneity (Q_w) and between class heterogeneity (Q_b), according to Curtis and Wang (1998). The impact of experiment duration was tested as a continuous variable. For this analysis, Q_t was partitioned in heterogeneity, explained by the regression model (Q_m) and the amount of residual error heterogeneity.

For all other response variables, the standard deviations were often not available. Because standard deviations are required for a weighted parametric analysis, an unweighted analysis using resampling techniques was conducted on these variables instead. In the unweighted analysis, 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). To assess the effect of experiment duration on variability in microbial CO₂ responses, we compared short-term (1-2 growing seasons) and long-term (>2 growing seasons) experiments.

In both the unweighted and weighted analyses, the CO₂ effect on a response variable was considered significant if the 95% confidence interval did not overlap 0, and marginally significant if the 90% confidence interval did not overlap 0. Means of categories were considered significantly different if their 95% confidence intervals did not overlap.

6.3 Results

Soil C and N contents

Elevated CO₂ increased total soil C contents significantly by 4.1% (Figure 6.1), but the CO₂ response depended on fertilizer N additions (Table 6.3). In experiments that received less than 30 kg N ha⁻¹ yr⁻¹, soil C contents were unaffected. However, soil C accumulation became apparent with increasing inputs of N. Experiments receiving between 30 and 150 kg N ha⁻¹ yr⁻¹ showed a significant increase in soil C at elevated CO₂ (+4.3%), whereas experiments receiving >150 kg N ha⁻¹ yr⁻¹ showed a significant CO₂ response of +8.1%. Within the N fertilization classes, none of the variables affected the CO₂ response for soil C.

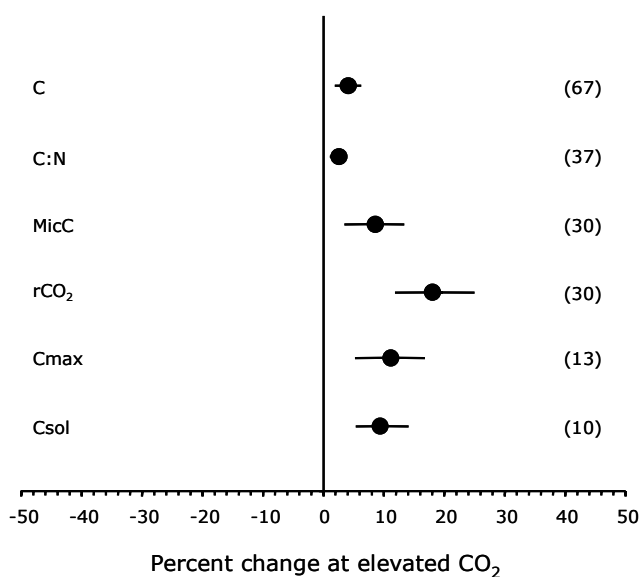


Figure 6.1: The effect of elevated CO₂ on several indicators of soil C cycling. See Table 6.1 for definition of abbreviations. Mean \pm 95% confidence interval. The number of observations representing each response variable appears in parenthesis.

As the data set was divided, not every categorical variable was represented in each sub-group. The data set of experiments receiving $>150 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ contained no experiments on natural vegetation. Moreover, this subgroup was heavily biased towards herbaceous plants; only one of the studies was carried out on woody plants. However, across the whole soil C data set, the CO₂ response did not differ between herbaceous and woody species or between planted and natural vegetation (Table 6.3). None of the other categorical variables, or the continuous variable time affected the CO₂ response for soil C content either.

Table 6.3: Between group heterogeneity (Q_b) for the CO₂ response of total soil C and N contents and C:N ratio. For the continuous variable time, the heterogeneity explained by the regression model (Q_m) is reported. Response variables are represented by k observations * $P < 0.05$, ** $P < 0.01$.

Variable	k	Method	N	Vegetation (a) planted/natural	Vegetation (b) herbaceous/woody	Vegetation (c) crops/non-crops	Time
C	67	0.03	7.56*	0.47	2.19	1.99	2.24
N	48	0.00	1.37	0.62	1.03	0.16	0.68
C:N	37	0.40	5.78	11.50**	0.89	5.34*	0.39

Elevated CO₂ increased soluble C by 9.4%. Soil N concentrations significantly increased by 2.8% under elevated CO₂, with none of the categorical variables affecting the CO₂ response (Figure 6.2). Averaged over all experiments, elevated CO₂ increased soil C:N by 2.4% (Figure 6.1). The CO₂ response with respect to C:N differed between experiments on planted and natural vegetation (Table 6.3); only under planted vegetation did elevated CO₂ significantly increase soil C:N (+4.0%). A significant Q_b was found for the distinction between crops and non-crops. Nevertheless, the CO₂ response for soil C:N did not differ between these categories (i.e. their 95% confidence intervals overlapped). Experiments on crops were represented by only two studies with highly different CO₂ responses. Thus, of all categorical variables, the distinction between planted and natural vegetation best explained the variation in CO₂ effects on soil C:N.

Microbial biomass and activity

Microbial respiration and potential mineralizable C increased by 18.0% and 11.1% under elevated CO₂, respectively. Microbial C also increased, but showed a smaller response (+8.5%). The CO₂ response for microbial respiration and microbial C tended to be stronger for high N than for low N treatments (Figure 6.3), but the differences between fertilization classes were not significant.

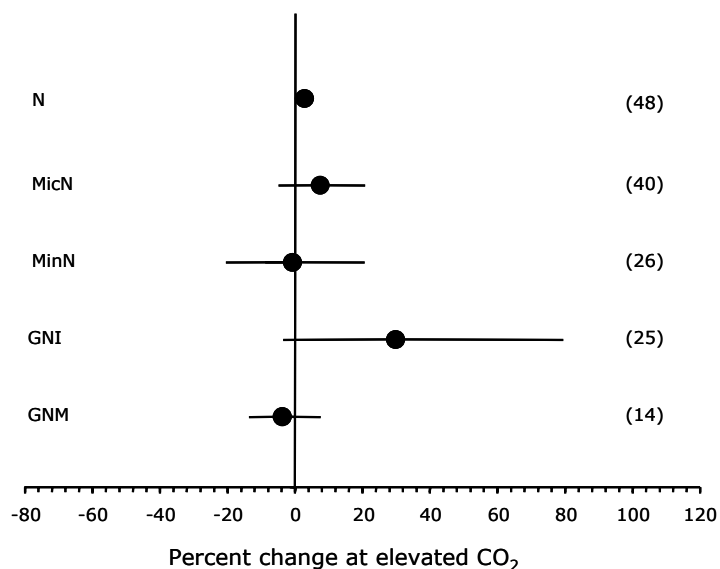


Figure 6.2: The effect of elevated CO_2 on several indicators of soil N cycling. See Table 6.1 for definition of abbreviations. Mean $\pm 95\%$ confidence interval. The number of observations representing each response variable appears in parenthesis.

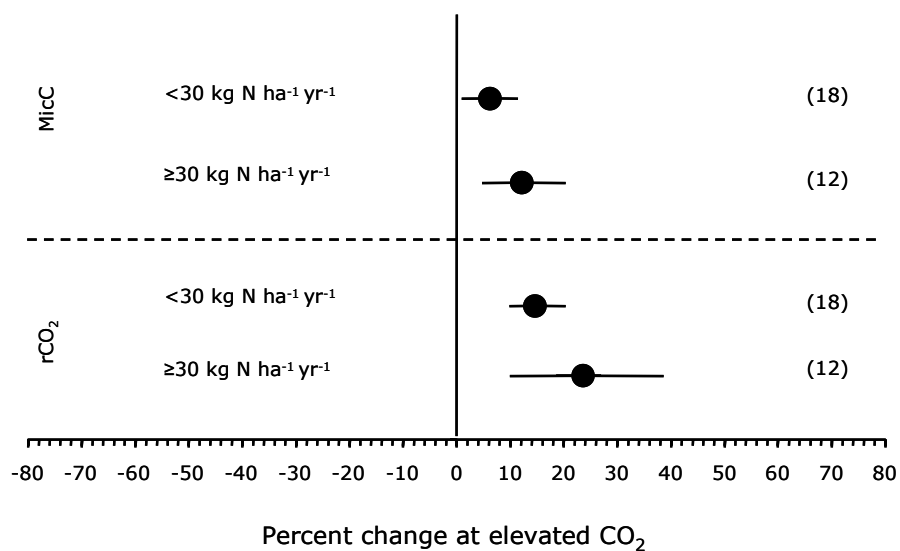


Figure 6.3: The effect of elevated CO_2 on microbial C and microbial respiration, as affected by N addition. See Table 6.1 for definition of abbreviations. Mean $\pm 95\%$ confidence interval. The number of studies representing each response variable appears in parenthesis.

The effect of elevated CO₂ on microbial N pools and fluxes was characterized by large confidence intervals, indicating large differences in CO₂ responses between studies (Figure 6.2). Of all variables relating to soil N dynamics, only gross N immobilization was marginally significantly affected by elevated CO₂ (+29.8%). Although CO₂ enrichment had no overall effect on microbial N contents, it significantly increased microbial N in long-term studies (Figure 6.4b). As with microbial C studies, most of the short-term studies on microbial N involved planted vegetation. The CO₂ response for microbial N, N mineralization, and to a lesser extent, microbial C and microbial respiration showed relatively large confidence intervals for short-term experiments (Figure 6.4a,b).

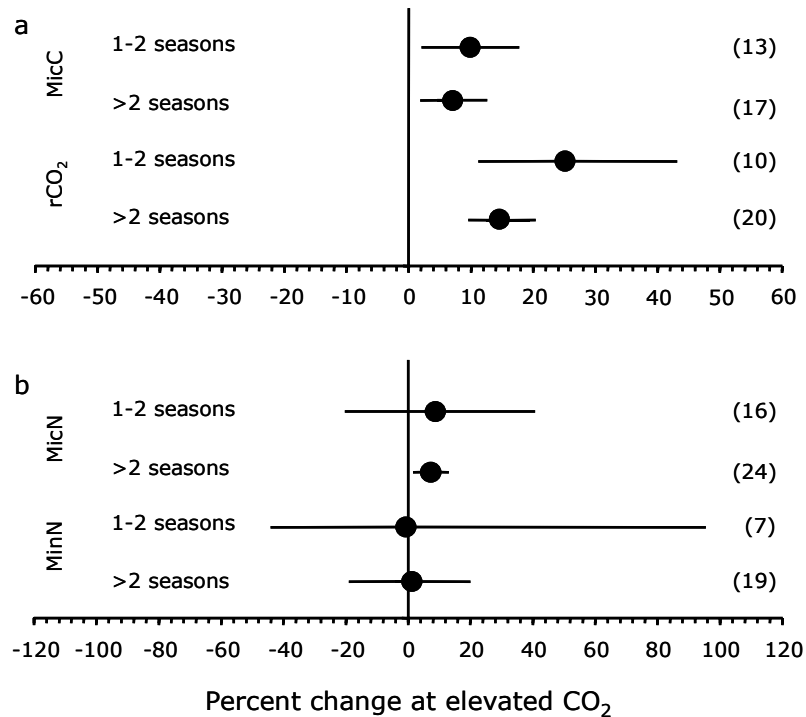


Figure 6.4 a,b: The effect of elevated CO₂ on microbial C and microbial respiration (a) and microbial N and N mineralization (b) in short- and long-term studies. See Table 6.1 for definition of abbreviations. Mean \pm 95% confidence interval. The number of observations representing each response variable appears in parenthesis.

6.4 Discussion

Soil C contents

The overall increase in soil C pools under elevated CO₂ suggests a potential for soil C sequestration. However, the average 4.1% increase in total soil C is small, taking into consideration the spatial variability in individual field experiments. Not surprisingly, only a small number of experiments reported significant increases in soil C under elevated CO₂ (Rice et al. 1994; Wood et al. 1994; Prior et al. 1997, 2004, 2005; Williams et al. 2000; Hagedorn et al. 2001).

Our estimate of the effect of CO₂ enrichment on total soil C has several constraints. First, initial soil C contents in ambient and elevated CO₂ plots might differ, thereby affecting the measured CO₂ effect on soil C contents (Schlesinger and Lichter 2001). In individual experiments, however, the chance of larger initial C contents in ambient plots is equivalent to the chance of larger initial C contents in plots receiving elevated CO₂. Pre-existing differences are therefore expected to cancel each other out in large data sets such as the one used in the current meta-analysis.

Secondly, our estimate solely concerns soil C in the top soil layer. As most of the new C enters the soil in the top layer, this is where the CO₂ effect on soil C is expected to be strongest. Also, treatment effects on soil C stocks might differ between soil depths. For instance, Mack et al. (2004) found that N fertilization increased SOM in the top layer of an arctic tundra soil, whereas it caused SOM at lower depths to decline. Thus, great caution is required when extrapolating the CO₂ effect on soil C in the top layer to lower depths.

The increase in soluble C under elevated CO₂ suggests that leaching of C might increase. Leached C either accumulates at lower depths or ends up in the groundwater, thereby contributing to C sequestration. However, a total C analysis of the top 75 cm of a grassland soil after 10 years of elevated atmospheric CO₂ showed no indication of increased precipitation of dissolved organic C (DOC) at lower depths (Van Kessel et al. 2005). In a model forest under elevated atmospheric CO₂, losses of C through DOC leaching were small compared to soil C inputs (Hagedorn et al. 2002). These results suggest that losses of DOC do not form a main part of the ‘missing carbon sink’.

Elevated CO₂ only increased soil C contents in experiments that received 30 kg N ha⁻¹ yr⁻¹ or more. These results corroborate models predicting that additional ecosystem C storage under future CO₂ concentrations will be limited by N availability (Hungate et al. 2003). Moreover, they suggest that previous estimates of soil C sequestration under elevated CO₂ might be overly optimistic (Jastrow et al. 2005). It should be noted that the initial soil N availability of individual experiments has not been taken into account in our meta-analysis. Differences in initial soil N availability can affect the impact of elevated CO₂ on plant growth, and thus soil C input.

Unfortunately, there is no indicator on the nutritional status of the soil that was available for all experiments.

As plant growth under elevated CO₂ is often limited by nutrient availability (Curtis and Wang 1998; Oren et al. 2001), we argue that the dependence of soil C storage on N fertilization is mainly caused by its effect on soil C input. Nitrogen additions can increase soil C stabilization (Neff et al. 2002), thereby exacerbating increases in soil C following a rise in soil C inputs. However, to our knowledge, not one study has found a positive interaction between elevated CO₂ and N fertilization for stabilization of new C. Thus, we found no evidence that suggests the positive effect of N fertilization on soil C sequestration under elevated CO₂ is related to its effect on C stabilization *per se*.

The positive effect of elevated CO₂ concentrations on potential mineralizable C is relatively strong compared to its effect on soil C contents, suggesting that the rise in soil C is mainly due to expanding labile C pools. As these pools are typically small and have high turnover rates, their contribution to soil C sequestration is limited. A substantial increase in soil C requires that additional C entering the soil is stabilized in long-lived pools. Thus, the protective capacity of soils largely determines the potential to sequester C under elevated CO₂ in the long term (Six et al. 2002). Ongoing soil disturbance decreases the physical protection of new SOM, thereby reducing the stabilization of C (Paustian et al. 2000). Yet, crops and non-crops did not differ in the CO₂ response for soil C (Table 6.3), suggesting that ongoing physical soil disturbance did not interact with the effect of CO₂ enrichment. One explanation for the lack of such an interaction is that CO₂ fumigation studies are too short for differences in the physical stabilization of C to affect total C contents. Also, most experiments on crops are heavily fertilized. The positive effect of N fertilization on soil C input could potentially compensate for the negative effect of soil disturbance on soil C accumulation.

As elevated CO₂ generally increases soil C input rates, its effect on soil C contents is expected to grow over time in most individual experiments. Therefore, we hypothesized that the effect of elevated CO₂ on soil C contents was stronger in long-term studies. In fact, all studies that found a significant increase in soil C under elevated CO₂ were at least 2 years old. Yet, experiment duration did not affect the CO₂ response for soil C in the total data set, nor in any of the N fertilization classes (Table 6.3). Apparently, differences between experiments are too large to detect a time \times CO₂ interaction.

Several meta-analyses suggest that the effect of elevated CO₂ on plant growth is stronger for trees than for herbaceous plants (Ainsworth et al. 2005; De Graaff et al. 2006). Our data show that this difference does not translate into a stronger CO₂ response for soil C under woody plants. A possible explanation for the lack of difference between plant life forms is that the residence time of assimilated C is relatively short for herbaceous plants (Schlesinger 1997). Thus in this category an

increase in plant growth could rapidly result in increases in soil C input, and thereby promote soil C sequestration. Given that CO₂ fumigation studies typically last only a few years, a relative large increase in soil C inputs for herbaceous plants might compensate for a relative small increase in plant growth.

Microbial biomass and activity

In our meta-analysis, CO₂ enrichment significantly increased soil microbial C contents and microbial respiration as measured by incubation. An increase in atmospheric CO₂ also stimulated microbial respiration in an intact plant-soil system (Hungate et al. 1997a) and enzymatic activities under natural grassland (Ebersberger et al. 2003), all pointing to enhanced microbial activity. Numerous studies found increases in soil C input under elevated CO₂ (Hungate et al. 1997a; Hoosbeek et al. 2004; Pendall et al. 2004). As soil microorganisms are generally C-limited (Anderson and Domsch 1978), an increase in C availability most likely contributed to the rise in microbial activity. In water-limited ecosystems, a rise in atmospheric CO₂ can also enhance microbial activity through soil moisture feedbacks (Ebersberger et al. 2003; Pendall et al. 2003), due to an increase in water use efficiency of plants.

In nutrient-poor grasslands and prairies, the effect of elevated CO₂ on microbial respiration is generally small, but strongly increases when fertilizer N is added (Rice et al. 1994; Niklaus and Körner 1996; Hungate et al. 1997a). Nonetheless, our meta-analysis did not reveal significant differences in the CO₂ response of microbial respiration between N fertilizer classes. We argue that the lack of significance is due to differences in initial soil N availability between experiments, which are obscuring the effect of N additions. However, the significant increase in soil C contents in fertilized ecosystems under elevated CO₂ suggests that a possible positive effect of N additions on the CO₂ response for microbial respiration does not outweigh the increase in soil C input.

The confidence intervals associated with the CO₂ responses for microbial biomass and activity are relatively large in short-term studies (Figure 6.4a,b). In these studies, soil C input largely consists of rhizodeposition. Whereas rhizodeposition generally increases under elevated CO₂ (Hu et al. 1999), the magnitude of the CO₂ response differs strongly between species (e.g. Paterson et al. 1996), possibly contributing to the variety of microbial responses. Also, short-term studies mostly involved planted vegetation and were thus conducted on physically disturbed soil. Soil disturbance stimulates the release of physically protected native SOM, a process that might affect microbial responses to elevated CO₂. Thus, we do not know whether it is the short duration of experiments per se or soil disturbance that broadens the array of microbial responses. Nonetheless, our results suggest that one should be cautious when using results from short-term experiments on planted vegetation for long-term predictions on the ecosystem level.

Soil N dynamics

The significant increase in soil C:N ratios under elevated CO₂ suggests a potential decrease in soil N availability. However, the large variability in CO₂ responses of microbial N contents and N transformation rates makes it hard to predict how future increases in atmospheric CO₂ concentrations will affect soil N cycling. On the one hand, the marginally significant increase in gross N immobilization under elevated CO₂ suggests a rise in microbial N demand. On the other hand, 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 by enhanced turnover of microbial N. However, in our meta-analysis, elevated CO₂ increased microbial N contents in long-term experiments. This implies that, over time, a rise in atmospheric CO₂ will increase the amount of N immobilized in the microbial biomass.

These findings are in line with Luo et al. (2004), who suggested that when elevated CO₂ stimulates biomass production in unfertilized ecosystems, the resulting increase in soil C inputs will gradually reduce N availability. This process is called progressive nitrogen limitation (PNL). The negative effect of PNL might be temporarily alleviated by increased efficiency of plant N uptake, due to increased fine root production (Mikan et al. 2000) or increased mycorrhizal colonization of roots (Rillig et al. 2000). However, the rise in plant growth and soil C input resulting from these adaptations increases the soil C:N ratio, thereby further enhancing microbial competition for N. Therefore, CO₂-induced mechanisms that increase plant N uptake without a net ecosystem gain of N are self-limiting (Hungate et al. 2003).

In theory, PNL can be postponed or alleviated in ecosystems where elevated CO₂ increases biological N₂ fixation and/or increases N retention (Luo et al. 2004). The significant increase in soil N contents under elevated CO₂ implies that one or both of these processes is indeed stimulated. To our knowledge, a sustained increase in symbiotic N₂ fixation under elevated CO₂ has only been measured in fertilized systems (Zanetti et al. 1996; Ross et al. 2004). In an unfertilized scrub oak community, the initial doubling of N₂ fixation was followed by a decrease in following years, which was attributed to Mo limitation (Hungate et al. 2004). In unfertilized natural grasslands, elevated CO₂ did not stimulate N₂ fixation due to P limitations (Niklaus et al. 1998). These results suggest that the ability of symbiotic N₂ fixation to prevent PNL will be constrained by supporting nutrients.

Elevated CO₂ decreased soil N leaching in a number of forest ecosystems (Hungate et al. 1999; Hagedorn et al. 2000; Johnson et al. 2004), suggesting a positive effect on N retention. The effect of elevated CO₂ on trace N gas losses is less clear. The emission of N₂O in a natural desert ecosystem was not affected by elevated CO₂ (Billings et al. 2002), but NH₃ volatilization sporadically increased. Hungate et al.

(1997b) found that NO emissions from natural grassland decreased under elevated CO₂. Similarly, Mosier et al. (2003) found that NO emissions from a fertilized shortgrass steppe were lower in plots that had previously been subjected to increased CO₂ concentrations. In both cases, the reduction in gaseous N losses was explained by an increase in microbial immobilization of N.

Williams et al. (2001) found that elevated CO₂ increased ¹⁵N retention in SOM under a tallgrass prairie, which was explained by enhanced microbial N demand. In the same experiment, soil C contents increased significantly under elevated CO₂ (Williams et al. 2000). Conversely, Niklaus and Körner (2004) found that ¹⁵N retention in a native grassland was not affected by elevated CO₂. In the same experiment, a relative small stimulation of soil C input and limited stabilization of new C prevented increases in soil C contents under elevated CO₂. From these results, we conclude that microbial N immobilization is a main mechanism involved in N retention in nutrient poor soils under elevated CO₂. However, an increase in N retention under elevated CO₂ requires an increase in soil C. Effectively, the additional C sequestered under elevated CO₂ is used to retain more N, so that the N cycle tracks the C cycle (Thornley and Cannell 2000).

It is important to note that all experimental studies finding PNL applied a step-increase in atmospheric CO₂. In the real world, a gradual increase in atmospheric CO₂ would allow more time to adjust the N status of the soil, possibly decreasing the severity of PNL (Luo et al. 2004). In unfertilized systems, the gain of N through increased retention under elevated CO₂ is limited by the amount of atmospheric N deposition. In these systems, the net gain of N will thus be small (Thornley and Cannell 2000). It may take decades to centuries for ecosystems to reach a new equilibrium, where gain of N through retention has compensated for the CO₂-induced decrease in soil N availability and factors other than N availability are limiting the ecosystem's response to elevated CO₂.

6.5 Future research needs

Recently, several papers discussed future research needs regarding SOM dynamics under elevated atmospheric CO₂ (Luo et al. 2004; Pendall et al. 2004; Jones and Donnelly 2004). One of the general conclusions was that the extrapolation of results from field experiments to long-term predictions for actual ecosystems continues to be a main challenge. Longer durations of experiments and combined experimental and modeling studies will contribute to more accurate predictions on the fate of SOM in a CO₂-rich world.

More experiments on natural ecosystems are needed, as they are currently underrepresented. This is especially the case for forest ecosystems: to date, no data are available on the effect of increased atmospheric CO₂ on SOM dynamics in mature,

natural forests. The recently developed WebFACE technique (Pepin and Körner 2002) enables one to study the effect of elevated CO₂ on tall canopy trees. This technique is currently used to study the effect of CO₂ effects on plant physiology (Körner et al. 2005), but may be used for studying SOM dynamics in the future.

Our meta-analysis strongly suggests that additional N is needed to sequester C under elevated CO₂. Thus, in unfertilized ecosystems, a mechanistic understanding of N supply processes is essential to predict the effect of elevated CO₂ on SOM dynamics. Whereas available data suggest that elevated CO₂ will have a limited effect on N₂ fixation in natural ecosystems (Hungate et al. 2004; Niklaus et al. 1998), more experiments on a wider number of ecosystems are needed before definitive conclusions can be drawn. The effect of elevated CO₂ on N retention has been studied by following the fate of isotopically labeled inorganic N in grassland and prairie soils (Williams et al. 2001; Niklaus and Körner 2004; Van Kessel et al. 2006). The same technique might be used to study N retention in other ecosystems.

We also need more insight into the effect of elevated CO₂ on soil C stabilization mechanisms. Soil aggregation has been found to increase under elevated CO₂ (Rillig et al. 1999; Six et al. 2001; Prior et al. 2004), thereby potentially increasing physical SOM stabilization. An increase in [CO₂] might also affect chemical SOM stabilization by its effect on litter quality (e.g. Parsons et al. 2004). The long-term impact of these feedback mechanisms on C sequestration is unclear and requires more experimental data for verification. Finally, our meta-analysis suggests that physical soil disturbances can influence the effect of elevated CO₂ on SOM dynamics. To our knowledge, the combined effect of physical soil disturbance and increased atmospheric CO₂ has only been studied in one experiment (Prior et al. 2005). Such experiments might enable us to reveal the relative importance of atmospheric CO₂ concentrations and ecosystem management practices for soil C sequestration.

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7 Element interactions limit soil C storage

Abstract

Rising levels of atmospheric CO₂ are thought to increase C sinks in terrestrial ecosystems. The potential of these sinks to mitigate CO₂ emissions, however, may be constrained by nutrients. Using meta-analysis we found that elevated CO₂ only causes accumulation of soil C when N is added at rates well above typical atmospheric N inputs. Similarly, elevated CO₂ only enhances N₂ fixation, the major natural process providing soil N input, when other nutrients (e.g. phosphorus, molybdenum, and potassium) are added. Hence, soil C sequestration under elevated CO₂ is constrained both directly by N availability, and indirectly by nutrients needed to support N₂ fixation.

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7.1 Introduction

Numerous studies have reported a surge in plant growth following an abrupt rise in atmospheric CO₂ (Schimel 1995; Mooney et al. 1991). If increased C assimilation by plants is translated into increased soil organic C, terrestrial ecosystems might help mitigate rising anthropogenic CO₂ emissions (Gifford 1994). Simulation models project a wide range of responses of soil C sinks to elevated CO₂. Some models suggest that low nutrient availability will preclude soil C storage (Hungate et al. 2003; Rastetter et al. 1997), while others maintain that soil C can accumulate even when nutrient supplies are low (Cannell and Thornley 1998). Nitrogen fixation, the main source of natural N input in terrestrial ecosystems (Galloway et al. 2004), has been invoked as a process that can potentially diminish N limitation in nutrient poor systems. Elevated CO₂ has been found to increase N₂ fixation (Zanetti et al. 1996), which could provide additional N to support C accumulation in soil. However, N₂ fixation by plants can be limited by the availability of other nutrients such as molybdenum, phosphorus and potassium (Vitousek et al. 2002).

Until now, empirical evidence to evaluate the effect of nutrient availability on soil C storage under elevated CO₂ has been lacking. The effects of nutrient availability and elevated CO₂ are difficult to discern in individual experiments because of high spatial variability in soil C and nutrients, and because of the large amount of C in the soil relative to input rates (Hungate et al. 1996; Schlesinger and Lichter 2001). A quantitative integration of results across multiple studies can overcome some of these problems.

In the current study, we used meta-analysis to summarize the effect of atmospheric CO₂ enrichment on soil C, using 80 observations from 41 published and unpublished studies. We also summarized the effect of elevated CO₂ on standing root biomass for these studies, using corresponding data for 56 observations on soil C. We divided the studies into three categories of N availability based on N fertilization rates: I) up to 30 kg N ha⁻¹ yr⁻¹, comparable to maximum atmospheric N depositions in the US and most of the EU (Holland et al. 2005), II) between 30 and 150 kg N ha⁻¹ yr⁻¹, typical of extensive agriculture in the US (FAO 2004), and III) more than 150 kg N ha⁻¹ yr⁻¹, typical for intensive agriculture in the EU (FAO 2004). Similarly, we also evaluated the potential of N₂ fixation to supply extra soil N input under increased CO₂ concentrations, using 92 observations in 25 published and unpublished studies. We compared studies that received additional non-N nutrients, and studies that did not. The databases for soil C, root biomass, N₂ fixation, and the results of the meta-analyses can be found in Data Sets 1-7, which are published as supporting information on the PNAS web site.

7.2 Results and discussion

Elevated CO_2 had no effect on soil C in ecosystems receiving up to 30 kg N $\text{ha}^{-1} \text{yr}^{-1}$ (Figure 7.1a). Soil C accumulation became apparent with increasing inputs of N. Elevated CO_2 increased soil C by 2.1 % per year at N additions between 30 and 150 kg $\text{ha}^{-1} \text{yr}^{-1}$ and by 2.9 % per year at N additions above 150 kg N $\text{ha}^{-1} \text{yr}^{-1}$. Whether under natural or planted vegetation, in intact or disturbed soils, using woody or herbaceous species: elevated CO_2 only increased soil C at N additions exceeding 30 kg $\text{ha}^{-1} \text{yr}^{-1}$ (Data Set 4). These results, spanning an array of experimental conditions and terrestrial ecosystems, provide powerful evidence that additional N is needed if C is to be stored in soil under elevated CO_2 .

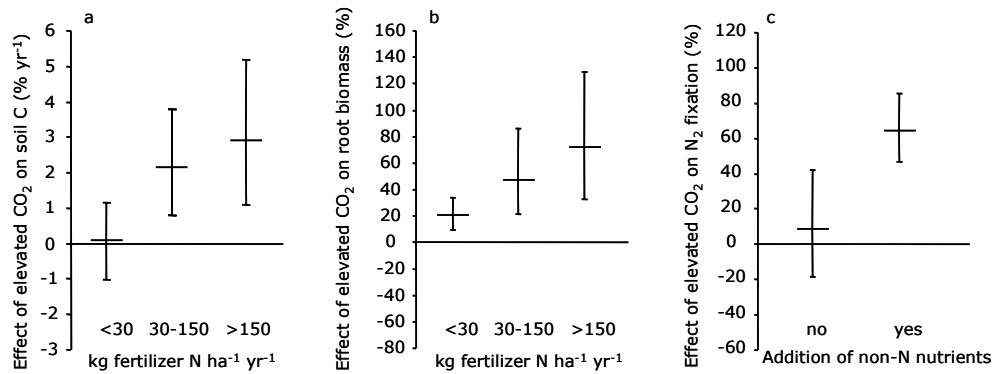


Figure 7.1 a,b,c: The effect of elevated CO_2 on soil C contents, root biomass and N_2 fixation. **a**, Change in soil C contents as affected by N fertilization. There is a significant difference between N fertilization classes ($P=0.02$). The values for <30, 30-150 and >150 kg N $\text{ha}^{-1} \text{yr}^{-1}$ are based on 43, 25, and 12 observations, respectively. **b**, Change in root biomass as affected by N fertilization. There is a significant difference between N fertilization classes ($P=0.03$). The values for <30, 30-150 and >150 kg N $\text{ha}^{-1} \text{yr}^{-1}$ are based on 29, 17, and 10 observations, respectively. **c**, Change in N_2 fixation as affected by nutrient additions. There is a significant difference between studies that received additional non-N nutrients (43 observations), and studies that did not (49 observations) ($P=0.02$). All observations are weighted by experiment duration and number of replicates. All error bars represent 95% confidence intervals.

Why is the soil C response to elevated CO_2 restricted by N availability? As an increase in plant growth under elevated CO_2 causes N to accumulate in litter and plant biomass, soil N availability is expected to limit plant growth under elevated CO_2 in the long term without the addition of exogenous N (Finzi et al. 2002). By contrast, N fertilization can sustain increases in plant growth and thus soil C input under elevated

CO₂ (Curtis and Wang 1998; Oren et al. 2001). Indeed, soil N availability limited plant growth under elevated CO₂ for the studies contributing to our soil C data base; the response of root biomass to elevated CO₂ increased with N additions (Figure 7.1b). As new C enters mineral soil mainly through the root system, these results suggest that the effect of N availability on soil C responses to elevated CO₂ were caused by differences in soil C input.

When ecosystems under elevated CO₂ are subject to N stress, soil microbes might mobilize N through increased decomposition of native soil organic matter (Lekkerkerk et al. 1990; Zak et al. 1993; Pendall et al. 2003). This so-called priming effect could explain why even in unfertilized experiments, elevated CO₂ significantly increases plant biomass (Figure 7.1b). Yet, as priming simultaneously increases soil N availability but reduces the soil C reservoir, its potential to accommodate soil C storage is probably small. In fact, the increase in root biomass under elevated CO₂ did not lead to C storage in studies that received less than 30 kg ha⁻¹ yr⁻¹ of N (Figure 7.1a). Thus, while N limitation alone does not always preclude positive plant growth responses to elevated CO₂, the interaction between the C and N cycles in terrestrial ecosystems rapidly constrains the CO₂ effect on soil C contents.

Elevated CO₂ can also promote plant N uptake by growing fine roots and mycorrhizae (Zak et al. 2000; Pregitzer et al. 1995; Klironomos et al. 2005). However, the potential for such redistributions of N to accommodate C sequestration is small because the resulting rise in plant growth will further increase readily available C and therefore microbial N demand (Diaz et al. 1993). Hence, mechanisms that increase plant N uptake without a net ecosystem gain of N are likely to be constrained by ecological stoichiometry (Sterner and Elser 2002) and therefore self-limiting (Luo et al. 2004). Thus, the potential for C storage under elevated CO₂ is highest for ecosystems where plant growth is not limited by N availability. In all other cases, other sources of N are needed to support plant growth and sequestration of soil C.

Nitrogen fixation has been suggested to be one of the other N sources (Zanetti et al. 1996). However, our meta-analysis shows that elevated CO₂ had no effect on N₂ fixation under conditions representative of most of Earth's terrestrial ecosystems: with no fertilizer additions, with intact soils, and with naturally occurring plant communities, the response of N₂ fixation to elevated CO₂ was indistinguishable from zero (Data Set 6).

Across the entire dataset, elevated CO₂ increased N₂ fixation when other nutrients were also added (Figure 7.1c). These results suggest that stimulation of N₂ fixation by elevated CO₂ is constrained by the availability of nutrients other than N. In all but one case, non-N nutrient additions included both phosphorus and potassium, which are essential for N₂ fixation (Vitousek et al. 2002). Because non-N nutrients were always added in combination (Data Set 3), we cannot test which elements were especially important in releasing N₂ fixation from nutrient limitation. Previous studies have suggested that CO₂ stimulation of N₂ fixation is restricted by the availability of

phosphorus (Niklaus et al. 1998; Edwards et al. 2006) and molybdenum (Hungate et al. 2004). Our findings imply that such nutrient constraints are a general feature of N₂ fixation responses to elevated CO₂.

We found no evidence that N fertilization suppressed the response of N₂ fixation to elevated CO₂ (Data Set 6), even though addition of N fertilizer frequently depresses N₂ fixation (Woodward 1992). Because N addition often occurred in combination with additions of other elements (32 out of 45 observations), this result may in part reflect the positive effects of additions of other nutrients overwhelming the negative effects of added N. However, in the subset of observations where we could assess the influence of added N in isolation (in the absence of other nutrient supplements), N fertilization had no effect on the response of N₂ fixation to elevated CO₂ (Data Set 6). Thus, the absence of a response of N₂ fixation to elevated CO₂ without additions of other nutrients was not an artefact caused by N additions.

Elevated CO₂ also increased N₂ fixation in experiments with disturbed soils, an effect comparable in magnitude to that observed by adding non-N nutrients (Data Set 6). Soil disturbance likely operates through its direct effect on nutrient availability, as it generally decreases soil organic matter contents and liberates nutrients (Haas et al. 1957). Planted communities showed a significantly stronger CO₂ response for N₂ fixation than natural ecosystems. Yet, all studies on natural communities were performed on intact soils, and none of them received non-N nutrient supplements. The CO₂ response between natural and planted communities did not differ on intact and unfertilized soil (Data Set 6). Thus, planting did not affect the CO₂ response of N₂ fixation *per se*. Together, these results suggest that strong responses of N₂ fixation to elevated CO₂ depend on the availability of non-N nutrients.

The effect of CO₂ on N₂ fixation decreased with experiment duration (Data Set 6, $P=0.001$). One possible mechanism for this decline is identical to that reducing soil N availability in response to elevated CO₂: following an increase in plant growth, micronutrients required for N₂ fixation accumulate in litter and plant biomass (Johnson et al. 2003). The resulting decrease in non-N-nutrient availability limits the response of N₂ fixation to elevated CO₂ or in some cases even turns a stimulation into a suppression (Hungate et al. 2004). In addition, light limitation, often absent in short-term experiments, is likely to become more pronounced over time under elevated CO₂ (Grashoff et al. 1995). Together, the dependency of N₂ fixation on supporting nutrients, the lack of a response in natural ecosystems and the decreasing CO₂ response over time strongly imply that the role of N₂ fixation in providing additional N needed for C storage under elevated CO₂ will be small.

7.3 Conclusions

Results presented here show that nutrient limitations of plant growth under elevated CO₂ extend to soil C accumulation and N₂ fixation. Our analysis thus provides empirical corroboration for the largely untested hypothesis that large C accumulations only occur with increased inputs or reduced losses of N (Hungate et al. 2003; Rastetter et al. 1997; Sterner and Elser 2002) and broadens it to non-N nutrients. Together, these conceptual, theoretical and empirical approaches suggest a limited potential for rapid C storage in the terrestrial biosphere following an increase in atmospheric CO₂.

7.4 Materials and methods

Data collection

We extracted results for soil C contents, root biomass and N₂ fixation from atmospheric CO₂ enrichment studies, conducted in the field, in growth chambers or in glass houses (Data Sets 1-3). We included observations of the effect of elevated CO₂ that met several criteria. First, the duration of the experimental CO₂ treatment had to be at least 100 days (the approximate length of a growing season in the temperate zone). Second, means and sample sizes had to be available for ambient CO₂ treatments (between 300-400 ppm) and elevated CO₂ treatments (450-800 ppm). Estimates of variance were tabulated when available, but were not required for inclusion in the analysis. Third, details of experimental conditions needed to be specified. We only included studies that reported experiment duration, soil sampling depth, plant species and the type of experimental facility. Studies also needed to indicate N fertilization rates. Most studies applied N additions directly to the soil, rather than the canopy. This method of N fertilization is bypassing the possibility of foliar N uptake. Consequently, the lower threshold for N effects on soil C storage and root biomass in our meta-analysis is merely an approximation of atmospheric deposition rates.

Finally, studies needed to indicate whether they involved experiments in pots (i.e. any container with dimensions <1 m) or in ecosystems. We made a distinction between studies on intact and disturbed soils. The latter category included all pot studies, studies on reconstructed soils, and studies that applied tillage during CO₂ enrichment.

Because we examined how effects of elevated CO₂ varied with experimental conditions, we included separate observations of elevated CO₂ effects from a single ecosystem under different experimental treatments (e.g., in multifactorial studies). When studies involved more than one level of CO₂ enrichment, we only included results at the level that is approximately twice the ambient CO₂ concentration.

To increase sensitivity for detecting small effects on soil C, we included only surface soil samples, ranging in depth from 0-5 to 0-30 cm. When studies reported soil C contents for multiple depths, we included results that best represented the 0-10 cm soil layer. Our analysis focuses on mineral soils; we thus excluded measurements on forest litter layers, marshes and bogs. Because soil C accumulates slowly over time and effects of CO₂ enrichment on soil C are often difficult to discern, we only included measurements after the longest exposure period for each study. Soil C contents were all included in the database as a weight percentage. We converted results reported on an area basis to a weight basis, using soil density data whenever these were available. When soil density data were not reported, we converted results assuming soil bulk density of 1 g cm⁻³ in ambient CO₂ and elevated CO₂ treatments. We included root biomass data for the whole sampled soil depth. When root biomass data were reported for multiple years, we only included data from the year closest to the year of the corresponding soil C observation.

All forms of biological N₂ fixation (i.e. free-living and symbiotic bacteria, symbiotic actinomycetes and cyanobacteria) were included. N₂ fixation was determined by acetylene reduction, ¹⁵N dilution, or N contents of plant tissue when atmospheric N₂ was the only available N source. We explicitly examined the temporal dependence of the N₂ fixation response for multi-year studies, using one estimate per treatment combination per year. While such measurements are not independent, this approach makes it possible to test whether the responses of N₂ fixation change through time. Eliminating non-independence by restricting the dataset to one estimate per ecosystem-treatment combination did not substantially alter the results (Data Set 7). While this analysis did reveal a stronger response of N₂ fixation in woody compared to herbaceous vegetation, experiments using woody vegetation were dominated by the use of disturbed soils and the addition of non-nitrogenous fertilizers (19 out of 24 observations). In the absence of soil disturbance and non-N nutrient supplements, N₂ fixation did not respond to elevated CO₂ in either woody or herbaceous plants (Data Set 7).

Meta-analysis

Data Sets 1-3 were evaluated by using meta-analysis (Hedges and Olkin 1985). We used the natural log of the response ratio ($r = \text{response at elevated CO}_2 / \text{response at ambient CO}_2$) as a metric for the response of N₂ fixation and root biomass to elevated CO₂. These results are reported as the percentage change under elevated CO₂ ($[r-1]*100$). The accumulation of soil C in response to an increase in C input follows a logarithmic pattern over time, yet, in the first 5-10 years, accumulation rates will be approximately linear (Schlesinger 1990). As the average duration of CO₂ exposure in the meta-analysis was 3.6 years, we assumed linear changes in soil C over time. Accordingly, we used the natural log of the time-adjusted response ratio $r_t = ([r-$

$1]/y)+1$ as a response metric, with y as the length of the study in years. We assumed that most of the soil C input occurs during the growing season. Thus, in order to prevent overestimation of annual changes in the soil C pool in short-term studies (<1 year), we used a minimum of $y=1$. Results are reported as the percentage change per year under elevated CO_2 ($[r_t-1]*100$).

Well-replicated and long-term studies provide more reliable estimates of ecosystem CO_2 responses (Hungate et al. 1996). Thus, we weighted the response metrics by replication and experimental duration, using the function $F_C = (n_a * n_e) / (n_a + n_e) + (y * y) / (y + y)$, with n_a and n_e as the number of replicates under ambient and elevated CO_2 . Using other weighting functions, such as weighting solely by the number of replicates (Adams et al. 1997) or weighting all studies equally, did not affect the outcome of our analysis (Data Sets 4-7). The weighting function conventionally used in meta-analyses, i.e. weighting by the inverse of the pooled variance (using largest observed variance for missing estimates) yielded similar results. However, this function calculated weights that differed up to three orders of magnitude in size (supporting material, Data Sets 1-3). By assigning extreme importance to individual observations, average effect sizes were largely determined by a small number of studies. Thus, we favour the alternative weighting functions as they assigned less extreme weights.

We used a mixed model for analysis of all three data sets, based on the assumption that random variation in CO_2 responses occurred between studies (14) and bootstrapping (4999 iterations) to calculate 95% confidence intervals around mean effect sizes for categories of studies. P-values for differences between categories of studies and for correlation with experiment duration were calculated using resampling techniques incorporated in MetaWin 2.1 (Rosenberg et al. 2000).

8 General discussion and conclusions

8.1 Introduction

Due to human activities such as deforestation and the combustion of fossil fuels, the concentration of CO₂ in the atmosphere is rising at ever increasing rates. Higher atmospheric concentrations of CO₂ and other greenhouse gases lead to climate change. But, elevated CO₂ often stimulates plant growth, creating the possibility to increase C storage in terrestrial ecosystems and to mitigate emissions of greenhouse gases. Soils in particular hold great promise as a C sink, since the soil C pool has a slow turnover time relative to C in vegetation and atmosphere. An increase in soil C storage can be achieved through higher rates of C input, a decrease in decomposition rates, or a combination of both. However, the intricate link between soil C and N dynamics could affect the potential of these two pathways of C sequestration. A rise in soil C input without a concomitant increase in N input will increase the C:N ratio of SOM, and might thereby stimulate N immobilization by microbes. On the other hand, a decrease in decomposition of organic matter will slow down the release of inorganic N that is needed to sustain plant growth and soil C input.

Soil N availability limits plant growth throughout many regions of the Earth. Thus, a key question is: can soils under increased atmospheric CO₂ concentrations provide additional N to accommodate the input and storage of C in soil? Elevated CO₂ affects the physiological activities of plants and microbes in various ways, and the net effect of these responses on soil N availability is still unclear. To predict ecosystem behavior under future CO₂ concentrations, we therefore need to quantify the interactions between soil C- and N pools, plants and the soil microbial population.

In this dissertation, I investigated the effect of rising CO₂ levels on SOM dynamics at different levels of soil N availability. Most of my research involved field studies, in which atmospheric CO₂ concentrations were increased to levels expected in the second half of the 21st century. Field studies now provide our most realistic estimates of how real ecosystems will respond to rising CO₂ concentrations. However, they are often frustrated by spatial variability and the large size of organic matter pools relative to the fluxes of C and nutrients. I followed two approaches to overcome this problem. Firstly, I used isotopic labeling to trace C and N entering the soil. By distinguishing new C and N, I could reduce confounding effects related to high background concentrations of native SOM. As a second approach, I summarized data from a large number of CO₂ enrichment experiments, using a statistical technique called meta-analysis (see Chapter 1.4.2). This technique enabled me to identify CO₂ effects that might go unnoticed in individual studies, due to a lack of statistical power.

To synthesize the main findings of my research, I will return to the original hypotheses stated in the outline of this thesis. First, I will assess whether and by what mechanism soil C accumulates under elevated CO₂. Second, I will discuss to what extent a CO₂-induced increase in soil C input affects N availability, and how this affects soil C storage on longer time scales. Third, I will assess whether elevated CO₂ can stimulate soil C storage indirectly through effects on soil biota and soil architecture. Finally, I will indicate how the findings of my research can be used to update projections on future soil C storage.

8.2 Testing hypotheses

H1: Increased soil C input and decreased rates of litter decomposition will cause soil C contents to increase under elevated atmospheric CO₂ concentrations

Soil C contents are ultimately determined by the balance between soil C input and microbial respiration (Chapter 1.2). Thus, to assess the net effect of elevated CO₂ on soil C storage, I will first evaluate its impact on these two fluxes. Measuring soil C input remains problematic, as it requires new C to be traced into the soil. Therefore, the response of plant growth to elevated CO₂ has been suggested as a proxy for soil C input (e.g. Zak et al. 1994). Responses of root growth in particular might be a representative proxy in short-term experiments, as root-derived materials are an immediate substrate for microbial activity (Zak et al. 2000).

Table 8.1 summarizes the effect of elevated CO₂ on root growth and microbial respiration at Swiss FACE, the field experiment central to Chapters 2 through 4. As data on CO₂ responses are derived from different years of the experiment, cautiousness is warranted when comparing CO₂ response of the fluxes. That said, the response of root growth to elevated CO₂ clearly exceeds the response of microbial respiration, suggesting a potential for soil C storage.

Are these findings reflected in CO₂ effects on soil C contents? Soils from the Swiss FACE site have been sampled and analyzed for soil C contents for the last 9 years of the CO₂ enrichment experiment. The absolute effect of elevated CO₂ on soil C contents differed from year to year, but it was mostly positive (Van Kessel et al. 2000b; Van Kessel et al. 2006). Averaged over N addition and species treatments, soil C contents after 9 years were 8% higher in plots that received additional CO₂ (Chapter 3). Yet, as in many other field studies, differences between ambient and elevated CO₂ plots were not significant. I submit that elevated CO₂ stimulated soil C storage in the Swiss FACE to some extent, but that the experiment lacks statistical power to detect small increases in soil C.

Large spatial variation and high background concentrations of soil C hamper the statistical power of many CO₂ field experiments. For instance, Hungate et al. (1996) calculated that in a certain annual grassland, a CO₂-induced increase in soil C input of 35% would not result in detectable changes in soil C for at least 8 years. At the same site, a 7% raise in soil C input would cause an increase in soil C contents that is too small to detect, regardless of the length of the experiment. The Swiss FACE experiment has fewer replications (n=3 vs. n=10) and higher CV values for soil C contents (25% vs. 13%) than the experiment described by Hungate et al. (1996). Thus, even if elevated CO₂ would markedly stimulate soil C storage at the Swiss FACE site, the resulting increase in soil C might remain undetected.

Table 8.1 also shows results of recent meta-analyses, including those of Chapters 6 and 7, that summarized the effect of elevated CO₂ on microbial respiration, root growth and soil C contents. The average CO₂ responses differ between meta-analyses, due to variation in weighting procedures and criteria to include observations. Nonetheless, a clear pattern emerges when we compare the analyses: root growth responds stronger to elevated CO₂ than microbial respiration. Averaged over all reported field studies, elevated CO₂ significantly increases soil C contents. Thus, it appears that CO₂ responses of root growth and microbial respiration found in Swiss FACE are typical for many ecosystems, and that an increase in soil C input is a driving force behind soil C storage under elevated CO₂.

Table 8.1: An overview of CO₂ effects (%) on root biomass, microbial respiration and soil C contents; results of meta-analyses and the Swiss FACE experiment. Bold characters indicate significant CO₂ responses.

Reference	Root biomass	Microbial respiration	Soil C contents
Swiss FACE			
De Graaff et al. 2004	ND	16.8	ND
Hebeisen et al. 1997	40.4	ND	ND
Van Groenigen et al. 2003	ND	ND	8.2
Meta-analyses			
De Graaff et al. 2006	28.3	17.7	2.9*
Jastrow et al. 2006	ND	ND	5.6
Luo et al. 2006	31.6	ND	5.6
Van Groenigen et al. 2006a	ND	18.0	4.1
Van Groenigen et al. 2006b	34.6	ND	3.2*

* CO₂ responses were originally corrected for experiment duration, and reported as yearly changes in the soil C pool.

Numbers reported here are calculated from the original data base.

ND = Not determined

Elevated CO₂ may also stimulate soil C storage by decreasing litter quality, thereby slowing decomposition rates. In fact, Chapter 4 shows that plant material grown under elevated CO₂, incubated in soil adapted to elevated CO₂ decomposed slower

than ambient plant material in ambient soil (Figure 4.1). These results corroborate Jongen et al. (1995), who found that elevated CO₂ increased C:N ratios and retarded *in situ* decomposition of *L. perenne* roots in Swiss FACE plots.

However, I submit that these findings are of little importance for soil C storage under elevated CO₂. Firstly, the effect of elevated CO₂ on litter quality under field conditions is typically small. In Chapter 4, elevated CO₂ increased the C:N ratio of roots from lab-grown plants by 30%. But, a recent meta-analysis by Luo et al. (2006) shows that elevated CO₂ increased root C:N ratio's by 5% and 11% in OTC and FACE experiments respectively. Moreover, a meta-analysis by Norby et al. (2001) shows that elevated CO₂ does not affect litter quality (expressed as lignin content) in field studies.

Secondly, long-term studies show that differences in residue quality have a limited effect on the amount of litter that eventually becomes stabilized (Rasmussen and Collins 1991). Similarly, Latter et al. (1998) found that various types of litter contributed equally to soil C accumulation in the long term, despite initial differences in decomposition rates. These findings suggest that on longer time scales, elevated CO₂ will affect soil C concentrations mostly through its effect on the quantity, rather than the quality of SOM input.

H2: Increased soil C input under elevated CO₂ decreases the availability of N in soils

As discussed under H1, elevated CO₂ can stimulate soil C storage through an increase in soil C input. But, how does this affect soil N availability? Let us first consider the results of the meta-analysis in Chapter 6. This meta-analysis shows that a rise in atmospheric CO₂ increases microbial respiration, but does not affect net N mineralization (Figures 6.1, 6.2). In other words, elevated CO₂ stimulates microbial activity, but the N that is subsequently released from substrates is largely retained in microbial biomass. These results are in line with the significant increase in N immobilization rates under elevated CO₂ (Figures 6.2, 6.4b). If these microbial responses are sustained, soils will provide little or no additional N under elevated CO₂.

At the same time, increased plant growth under elevated CO₂ causes N to accumulate in litter and biomass (e.g. Finzi et al. 2002). In concert, these processes can gradually decrease soil N availability (Luo et al. 2004), thereby reducing the response of plant growth to elevated CO₂. This so-called Progressive Nitrogen Limitation (PNL) has been observed in several field experiments. For instance, Oren et al. (2001) found that over the course of five years, the response of plant growth to elevated CO₂ in an unfertilized pine forest became increasingly limited by N availability. Reich et al. (2006) reported similar results for a series of perennial grassland species.

In theory, PNL can be alleviated by a number of ecosystem responses. Some studies suggest that CO₂-induced priming of native SOM can increase soil N

availability (Zak et al. 1993), thereby supporting plant growth and soil C input under nutrient poor conditions. Yet, CO₂-induced priming is probably a transient process, as the amount of physically unprotected native SOM that can be accessed is small. In fact, isotopic data suggest that the CO₂ induced priming in a poplar plantation (Hoosbeek et al. 2004) decreases over time (Hoosbeek, personal communication). If these findings are representative for other ecosystems, they suggest that priming merely delays, not averts CO₂-induced N limitation of plant growth.

Plants might temporarily alleviate PNL by stimulating fine root production (Mikan et al. 2000) and mycorrhizal colonization of roots (Rillig et al. 2000), thereby increasing the efficiency of N uptake. However, the rise in plant growth and soil C input resulting from these adaptations increases the soil C:N ratio, thereby further enhancing microbial demand for N. Therefore, CO₂-induced mechanisms that increase plant N uptake without a net ecosystem gain of N are self-limiting (Hungate et al. 2003).

In the Swiss FACE experiments, elevated CO₂ significantly stimulated N₂ fixation by *T. repens* (Zanetti et al. 1996; Soussana and Hartwig 1996). Fixed N entering the system fulfilled a similar role in soil C sequestration as fertilizer N (Chapter 3). As such, a CO₂-induced increase in N₂ fixation can alleviate PNL. However, the meta-analysis of Chapter 7 shows that elevated CO₂ does not increase N₂ fixation in natural ecosystems. Across the entire dataset, elevated CO₂ only increased N₂ fixation when other nutrients were also added (Figure 7.1c). These results suggest that stimulation of N₂ fixation by elevated CO₂ is constrained by the availability of nutrients other than N. Moreover, the effect of CO₂ on N₂ fixation decreased with experiment duration. One possible mechanism for this decline is similar to PNL: following an increase in plant growth, micronutrients required for N₂ fixation accumulate in litter and plant biomass (Johnson et al. 2003).

In summary, results of the meta-analyses described in Chapter 6 are in line with the PNL theory, and suggest that soil N availability will gradually decrease under elevated CO₂. Whereas adjustments in plant physiology might help to alleviate N limitation, such changes will only be temporal.

H3: Additional N input stimulates plant growth and C sequestration under elevated atmospheric CO₂

As discussed under H2, elevated CO₂ negatively affects soil N availability in many field experiments. Given the wide spread N limitation of plant growth, a key question is: Can soils under future CO₂ concentrations sequester C without additions of N? Elevated CO₂ affects ecosystem processes via a multitude of mechanisms at different timescales. Thus, in answering this question, it is important to distinguish between long and short-term ecosystem responses.

Short term (years to decades)

The effects of nutrient availability and elevated CO₂ are difficult to discern in individual experiments because of high spatial variability in soil C and nutrients, and because of the large amount of C in the soil relative to input rates (Hungate et al. 1996; Schlesinger and Lichter 2001). Therefore, I decided to assess the effect of soil N availability on soil C storage through meta-analysis. Meta-analytical techniques allow comparing CO₂ responses between experimental classes. By categorizing studies based on the amounts of N that were added, one can evaluate the role of soil N availability in CO₂ responses.

Table 8.2 summarizes results of Chapters 6 and 7 and two other recent meta-analyses. Absolute CO₂ effects differ per meta-analysis, for the same reasons as in Table 8.1. The meta-analyses used different definitions for low and high soil N availability, further contributing to differences in CO₂ responses. Nonetheless, a clear pattern emerges when we compare the CO₂ responses of low and high N studies: elevated CO₂ does not stimulate soil C storage in studies receiving little or no additional N.

Table 8.2: Results of meta-analyses summarizing CO₂ effects (%) on root biomass, microbial respiration and soil C contents, as affected by soil N availability.

Reference	Soil N availability	Root biomass	Microbial respiration	Soil C contents
De Graaff et al. 2006	Low	14.6	14.4	0.7*
	High	33.7	22.3	6.9*
Luo et al. 2006	Low	39.0	ND	-4.3
	High	52.0	ND	3.4
Van Groenigen et al. 2006a**	Low	ND	14.7	2.2
	High	ND	23.1	6.4
Van Groenigen et al. 2006b**	Low	20.9	ND	-0.3*
	High	55.8	ND	7.4*

* CO₂ responses were originally corrected for experiment duration, and were reported as yearly changes in the soil C pool. Numbers reported here were calculated from the original data base.

** The original meta-analysis distinguished three classes of soil N availability. In this overview, CO₂ responses for the two highest classes of soil N availability were combined.

ND = Not determined

The meta-analysis by De Graaf et al. (2006) includes CO₂ responses of both root growth and microbial respiration, allowing an assessment of the pathways by which nutrient availability controls soil C storage. Under low N availability, elevated CO₂ increased belowground biomass by 14.6%. This response was not sufficient to counterbalance a 14.4% increase in microbial respiration, so that soil C contents remain unaffected. Under high N availability, belowground biomass response to elevated CO₂ exceeded the increase in microbial respiration, and soil C contents

increased. Thus, high N availability stimulates soil C storage under elevated CO₂ because it increases the CO₂ response of plant growth, but has little effect on the CO₂ response of microbial respiration. In the meta-analyses described by Chapter 7 and Luo et al. (2006), an increase in soil N availability caused a considerable increase in CO₂ responses for root growth. Thus, these results are in line with the conclusions drawn from De Graaff et al. (2006).

The meta-analyses in Table 8.2 grouped experiments according to N additions and did not take into account the initial soil N availability of individual experiments. Just as fertilizer N additions, high initial soil N availability will increase the effect of elevated CO₂ on plant growth and soil C input. Some models suggest that in ecosystems with fertile soils, the redistribution of N from soil to plant biomass allows for considerable C accumulation under elevated CO₂ (e.g. Tateno and Chapin 1997). Therefore, one cannot conclude from Table 8.2 that soil C contents will not increase under elevated CO₂ in all unfertilized soils. Yet, our analyses clearly show that, at least over the duration of a CO₂ enrichment experiment, the potential of soils to buffer rising levels of atmospheric CO₂ is constrained by soil N availability.

Long term (decades to centuries)

Several studies show that N accumulations during primary and secondary successions occurred alongside C accumulations (e.g. Vitousek 2004). Moreover, Figure 2.9 indicates that in the Swiss FACE experiment, the dynamics of new soil C and fertilizer N are highly correlated under elevated CO₂. These results imply that large C accumulations under elevated CO₂ can only occur with increased inputs or reduced losses of N. At the same time, they suggest that ecosystems exposed to elevated CO₂ have the intrinsic capability to stimulate N accumulation by C input (Luo et al. 2006).

Ironically, processes that reduce soil N availability under elevated CO₂ might cause soil N to accrue on longer time scales. As discussed in Chapter 6, increased plant N uptake and microbial N immobilization are two driving factors behind PNL. Yet, by decreasing standing pools of inorganic N, these processes might reduce N losses through leaching and gas emissions. For instance, Hungate et al. (1997b) found that NO emissions from natural grassland decreased under elevated CO₂. The decline in gaseous N losses was explained by an increase in microbial immobilization of N. Due to an increase in microbial N demand and higher plant N uptake, elevated CO₂ decreased soil N leaching in a number of forest ecosystems (e.g. Hagedorn et al. 2000; Johnson et al. 2004).

Williams et al. (2001) found that elevated CO₂ increased soil N retention under a tallgrass prairie, which was explained by enhanced microbial N demand. In the same experiment, soil C contents increased significantly under elevated CO₂ (Williams et al. 2000). Conversely, Niklaus and Körner (2004) found that N retention in native grassland was not affected by elevated CO₂. In that same experiment, a relatively small

stimulation of soil C input and limited stabilization of new C prevented increases in soil C contents under elevated CO₂. These results together with Figure 2.9 suggest that an increase in N retention under elevated CO₂ requires an increase in soil C. Effectively, the additional C sequestered under elevated CO₂ is used to retain more N, so that the N cycle tracks the C cycle (Thornley and Cannell 2000).

It is currently unclear at what time scale CO₂-induced N retention can accumulate substantial amounts of soil N. Even though Swiss FACE is one of the longest running CO₂ enrichment experiments of its kind, results on N retention are ambiguous. Because elevated CO₂ increased harvestable plant biomass (and presumably soil C input) in the high N treatment in most years (Schneider et al. 2004), I expected a simultaneous increase in fertilizer N retention. Instead, I found that the CO₂ response for N retention differed strongly between years. In year 8, elevated CO₂ significantly increased fertilizer N contents of soil under *L. perenne* by 54% (Table 2.3). One year later, the effect of elevated CO₂ was 3 times smaller, and not significant (Table 3.2). These results might be partly explained by inter-annual variations in soil C input; whereas elevated CO₂ increased harvestable biomass by 24% in year 8, CO₂ responses were approximately twice as small in year 9 (Schneider et al. 2004). Differences in sample preparation might also have affected the results; samples were passed through a 2 mm sieve prior to total SOM measurements in year 9, but not in year 8. The sieving probably removed additional root material, which typically has high f_N values.

The meta-analysis in Chapter 6 indicates that elevated CO₂ significantly increases soil N contents (Figure 6.2), albeit to a smaller extent than soil C contents. These data suggest that some N retention can occur over the course of a CO₂ enrichment experiment. Yet, it is important to note that the meta-analysis in Chapter 6 includes studies on fertilized ecosystems. The effect of elevated CO₂ on soil C input is relatively strong in these systems, and due to the strong relationship between f_N and f_C , an increase in N retention would be expected.

When plants receive fertilizer, CO₂-induced N retention is no longer required to maintain increased plant growth. In unfertilized systems on the other hand, N retention will be the main pathway by which N accumulates. In these systems, the gain of N through increased retention is limited by the amount of atmospheric N deposition. Therefore, at low levels of N deposition the net gain of N will probably be small (Thornley and Cannell 2000). In fact, a meta-analysis by De Graaff et al. (2006) suggests that elevated CO₂ does not significantly affect soil N concentrations under low N availability. It may take decades to centuries for ecosystems to reach a new equilibrium, where gain of N through retention has compensated for the CO₂-induced decrease in soil N availability, and factors other than N availability are limiting the ecosystem's response to CO₂. As such, nutrient constraints will limit soil C storage in the near future.

H4: Under elevated atmospheric CO₂ more soil C ends up in pools with slow turnover rates

For soils to act as a C sink, additional C needs to be stored in pools with slow turnover rates (Hungate et al. 1997a). Yet, the meta-analysis in Chapter 6 shows that average CO₂ responses for potential mineralizable C and soluble C are several times larger than responses for total soil C contents (Figure 6.2). These results suggest that the extra soil C input under elevated CO₂ is mainly allocated to SOM pools with high turnover rates and a low potential for C storage.

Data from the Swiss FACE experiment paint a similar picture; at 20%, the CO₂ response for potential mineralizable C was considerably higher than the response for total soil C contents (Table 4.2). This finding is corroborated by ¹³C data, which can be used to calculate the contribution of new C to the total soil C pool (Chapter 1.4.2). During the first three years of the Swiss FACE experiment, the fraction of new soil C (f_c) in the elevated CO₂ plots increased to 0.21 (Van Kessel et al. 2000b). After that, f_c values remained relatively stable (Chapters 2 and 3; Van Kessel et al. 2006). Apparently, 80% of the total soil C stock consists of pools with slower turnover rates.

Results of soil C measurements on the aggregate level show a similar pattern of soil C accumulation. In the Swiss FACE experiment, elevated CO₂ only increased soil C concentrations of macroaggregates in high N treatments (Figure 2.2). Yet, macroaggregates are considered to have a relatively high turnover rate (e.g. Six et al. 2001).

To summarize, results from meta-analyses and the Swiss FACE experiment both suggest that under elevated CO₂, soil C mostly accumulated in short-lived pools. The remaining soil C stock consists of pools with slow turnover rates, in which new soil C accumulates at lower rates. Thus, results from this dissertation do not support H4. At the same time, they imply that the ultimate potential for soil C storage under elevated CO₂ might not be achieved in field experiments, as these typically last for only a few years. Finally, they suggest that when the response of soil C input to elevated CO₂ is not limited by nutrient availability, soil C storage will be limited by the capacity of the soil to store new C in stable pools.

H5: Under elevated atmospheric CO₂ soil aggregation and the ratio fungal/bacterial biomass will increase, making soils more conducive to soil C storage

As outlined in Chapter 1.2, all effects of elevated CO₂ on the soil are mediated through plants. As the SOM pool is large relative to annual inputs of plant litter and root exudates, it may take years before soils have adapted to an increase in atmospheric CO₂ concentrations. In fact, this dissertation provided several examples showing that responses of SOM dynamics to elevated CO₂ change over time. For

instance, the meta-analysis described in Chapter 6 indicates that elevated CO₂ only increased microbial N demand in long-term experiments (Figure 6.4).

Chapter 1.2 introduced several CO₂-induced mechanisms that might affect soil C storage on longer time scales. Two of these mechanisms are different from CO₂ effects on soil N availability that were discussed previously, in that they do not directly feed back to plant growth and soil C input. Instead, they change soil properties in favor of physical and chemical protection of the soil C pool.

First, elevated CO₂ may stimulate soil aggregation. By forming a physical barrier between microbes and their substrate, an increase in aggregation could stimulate the physical protection of SOM (Six et al. 2000). As such, an increase in soil aggregation is generally regarded as beneficial to soil C storage. Second, elevated CO₂ may affect the composition of the soil microbial population. With respect to soil C storage, differences between bacteria and fungi are fundamental (Six et al. 2006). Fungal cell walls are more recalcitrant and decompose more slowly than bacterial cell walls (Nakas and Klein 1979). Also, fungi produce organic binding agents and entangle soil particles, thereby stimulating aggregate formation (Tisdall 1994). Thus, in theory, elevated CO₂ could stimulate soil C storage by inducing a shift towards the fungal pathway.

To what extent do soils adapted to elevated CO₂ differ from today's soil? Due to its long duration, the Swiss FACE experiment may help to find answers to this question. What follows is a summary of findings at the Swiss FACE site regarding the two aforementioned mechanisms:

Aggregation

Elevated CO₂ increased the amount of macro aggregates in *L. perenne* plots at the Swiss FACE site (Figure 2.1). These findings corroborate results from an earlier study at the same site (Six et al. 2001). Some other long-term field experiments found similar outcomes (Rillig et al. 1999), but opposite results have been reported as well (Del Galdo et al. 2006). Soil aggregate stability depends on many factors that are directly related to plant traits, such as root biomass, root exudation, mycorrhizal infection and rhizosphere soil biota. Moreover, plants can alter soil physical conditions that affect aggregate formation, such as soil moisture (Gordon and Rice 1993; Niklaus et al. 2003). Elevated CO₂ can influence each of these traits, but CO₂ responses are often species-specific (Cotrufo and Gorrisen 1997; Paterson et al. 1996). For this reason, it is not yet possible to generalize about the effect of elevated CO₂ on aggregate stability.

That said, does a CO₂-induced increase in aggregation have a positive impact on soil C storage in the particular case of the Swiss FACE experiment? A rise in atmospheric CO₂ simultaneously stimulated aggregation and soil C input at the experimental site. As such, it is difficult to determine the effect of CO₂-induced aggregation on soil C storage *per se*.

One approach to separate effects of soil C input and aggregation is to compare conventional tillage and no-tillage practices. No-tillage treatments typically increase soil aggregation and decrease soil aggregate turnover (Six et al. 2000). Thus, difference in soil C storage between tillage treatments can be attributed to differences in aggregate behavior, provided that soil C input rates are identical. In fact, several studies have shown that no-tillage practices raise soil C contents, and have attributed it to the stabilization of SOM in microaggregates-within-macroaggregates (Denef et al. 2004, Six et al. 2000). This particular fraction has been shown to be relatively resistant against decomposition (Six et al. 2000; Denef et al. 2004; Simpson et al. 2004).

Thus, if elevated CO₂ causes a larger part of the total soil C to be stabilized in microaggregates-within-macroaggregates, this would entail a positive effect on soil C storage that is independent of its effect on soil C input. Unfortunately, this particular soil fraction was not considered in Chapter 2. To assess the potential of CO₂-induced aggregation in promoting soil C storage, future research efforts should include physical soil fractionation techniques. However, effects of elevated CO₂ on soil aggregation are typically small compared to effects of soil tillage. Thus, the impact of this potential feedback mechanism on total soil C contents can only be studied in long-term experiments.

Microbial community shifts

Some results of Chapter 4 suggest that elevated CO₂ might indeed stimulate fungi over bacteria at the Swiss FACE site. Specifically, the incubation of ¹⁴C labeled roots grown under elevated CO₂ resulted in a decrease of $q^{14}\text{CO}_2$ (i.e. the amount of root C that was respired, divided by the amount that was incorporated in the microbial biomass). Such a decrease indicates either an improved efficiency in the use of added substrate for microbial growth (Harden et al. 1993) or a slower turnover of microbial biomass (Ladd et al. 1995). Sakamoto and Oba (1994) propose that fungi have higher substrate use efficiency than bacteria, but that assertion has recently been questioned by Thiet et al. (2006). On the other hand, fungal biomass is generally accepted to have a slower turnover than bacteria (e.g. Holland and Coleman 1987). Several studies found that incubation of litter produced under elevated CO₂ favors a fungal decomposition pathway (e.g. Coûteaux et al. 1991; Tuchman et al. 2002). As such, fungal dominance forms a likely explanation for the observed decrease in $q^{14}\text{CO}_2$.

Yet, as pointed out under H1, the effect of elevated CO₂ on litter quality in Chapter 4 was large compared to CO₂ responses found under field conditions. Moreover, the amount of roots added to the soil in this experiment exceeded soil C input in the field. As microbial responses depend on the amount of substrate that is added (Bremer and Kuikman 1994), it might be premature to extrapolate the microbial responses found in Chapter 4 to field situations.

In fact, amino sugar analyses in Chapter 5 provided no evidence that elevated CO₂ changed the relative abundance of fungi and bacteria at the Swiss FACE site. Similar results were found at two other field sites (an aspen forest and a soybean/corn rotation), suggesting that elevated CO₂ does not favor saprophytic fungi across a wide range of ecosystems. Thus, the potential of elevated CO₂ to affect soil C storage through a shift in the microbial community appears small.

In summary, soils exposed to long-term FACE conditions do not seem to be more conducive to soil C storage. However, a CO₂-induced increase in aggregation could promote soil C storage in some systems. Unfortunately, the impact of this feedback mechanism is hard to quantify, as elevated CO₂ simultaneously affects soil C input and aggregation.

8.3 Translating experimental results into predictions

Can the findings of this dissertation help us to update estimates for soil C storage under future global change scenarios? The meta-analysis described in Chapter 6 might provide a good starting point, as it summarizes all available data on soil C responses to elevated CO₂.

Field studies typically increase atmospheric CO₂ concentration to levels expected around the year 2100. For a first estimate of soil C storage in the near future, I will assume that FACE and OTC studies mimic the effects of elevated atmospheric CO₂ on soil C contents between the present and the end of the 21st century. Averaged over all experimental observations, elevated CO₂ increased soil C contents by 4.1% (Chapter 6). The Earth's soils contain approximately 1.5×10^{18} gram C (Batjes 1996). Thus, a 4.1% increase of the soil C pool would sequester an additional $(0.041 \times 1.5 \times 10^{18})$ 62×10^{15} gram C. This is roughly the same amount of CO₂-C that has accumulated in the atmosphere over the last decade. In other words, this simple calculation suggests that in the near future, soil C storage as a direct consequence of CO₂ fertilization will be of little importance in alleviating rising CO₂ levels.

Field experiments are too short to measure C accumulation in pools with slow turnover rates, and may therefore underestimate the ultimate potential for soil C storage. A second approach to estimate potential soil C storage does not use soil C data from CO₂ enrichment experiments. Instead, results of long-term studies on the fate of soil C input are combined with meta-analyses on plant growth responses to elevated CO₂. From a series of prolonged field studies (8-35 years old), Rasmussen and Collins (1991) calculated that the proportion of soil C input that is ultimately retained as SOM ranges between 14 and 21%. Moreover, a meta-analysis by De Graaff et al. (2006) suggests that elevated CO₂ increase root growth by 28%. Based on these results, and on the assumptions that:

- the effect of elevated CO₂ on soil C input is proportional to its effect on root growth, and
- the effects of CO₂-induced feedback mechanisms mentioned under H5 are negligible,

one can estimate that under elevated CO₂, soil C contents at steady state will increase by $(0.14 \times 28\%) - (0.21 \times 28\%)$, i.e. 3.9-5.9%. Interestingly, this approach yields results that are similar to the estimate based on CO₂ responses of soil C. This indicates that most of the potential soil C accumulation is achieved over the course of a CO₂ enrichment experiment, and that the potential for soil C storage in slow C pools might be small.

How do these estimates compare to model projections of soil C storage? Cramer et al. (2001) assessed the possible responses of ecosystem processes to rising atmospheric CO₂ concentration and climate change, using six dynamic global vegetation models. The same models were also featured in the Third Assessment Report of the Intergovernmental Panel on Climate Change (IPCC). Each model explicitly represented the interaction of ecosystem C with vegetation dynamics. Cramer et al. (2001) ran these models with three different scenarios: rising CO₂ concentrations, a change in climate, or a combination of both. Figure 8.1 shows the expected size of the soil C pool under all three scenarios up to the year 2100, averaged across the 6 models. A rise in atmospheric CO₂ increases the total soil C pool by approximately 250×10^{15} gram, i.e. 3 times more than the estimates based on field experiments. In the scenario that accounts for both global climate change and rising CO₂ levels, the projection for soil C storage was roughly twice as high as the estimates based on meta-analyses.

There are several possible explanations for the differences between model projections and estimates based on field studies. First, the model projections included the effect of elevated CO₂ on the global distribution of vegetation types. In theory, a shift towards vegetation types associated with high rates of soil C input could cause higher projections of soil C storage. However, the effects of elevated CO₂ on vegetation distribution were minor in most models (Cramer et al. 2001), and are therefore unlikely to cause substantial differences in soil C storage.

A potential source of error in estimates based on meta-analysis stems from the fact that biomes are not proportionally represented in elevated CO₂ research. Overall averages of meta-analyses are biased towards CO₂ responses of systems that are studied most often. One strategy to overcome this problem would be to calculate CO₂ responses for separate biomes. Figure 8.3 shows results of Chapter 6, split up between three classes of ecosystems. The total amounts of organic C in soils of forest, grassland and cropland roughly occur in a ratio of 8:5:2 (IPCC 2001). Weighting the soil C responses of these three biomes accordingly yields an overall increase in soil C of 2.8%. Thus, correcting for the contribution of biomes would only further reduce estimates of soil C storage, and does not explain the difference with model projections.

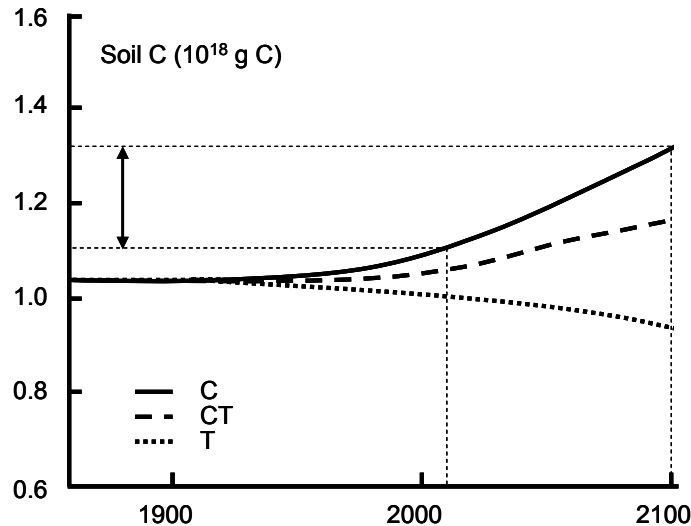


Figure 8.1: Time series of the global soil C pool size, averaged across 6 dynamic global vegetation models. The results of three scenarios are being shown: rising CO₂ concentrations (C), a change in climate (T), or a combination of both (CT). The arrow indicates potential for soil C storage until the year 2100 under scenario C. This figure was redrawn from Cramer et al. (2001).

Table 8.3: An overview of CO₂ effects (%) on soil C contents; results of the meta-analysis in Chapter 6. Results are reported for three classes of ecosystems.

Ecosystem	Mean	95% confidence interval	
		Low	High
Grassland	3.5	1.3	5.8
Forest	0.0	-4.3	4.7
Cropland	12.0	6.0	18.5

Most CO₂ enrichment experiments are conducted on managed ecosystems, while the Earth's land surface is largely covered with natural ecosystems. Thus, another possible explanation for the divergence between estimates based on meta-analyses and model projections is that the former are biased towards managed systems. However, when we restrict the soil C meta-analysis to natural ecosystems, elevated CO₂ stimulates soil C contents to a similar extent (Chapter 7). As such, the dominance of managed ecosystems in elevated CO₂ research cannot explain the differences in estimates either.

Virtually none of the CO₂ enrichment experiments included in the meta-analysis consider possible effects of climate change (i.e. a rise in temperature and changing rainfall patterns) on soils and vegetation. Thus, if elevated CO₂ interacts with climate change in ways that stimulate soil C storage, this might explain the relatively low

estimates of the meta-analysis. However, Shaw et al. (2002) showed in a field experiment that expected climate change scenarios dampen CO₂ responses of plant growth rather than increase them. Moreover, Figure 8.1 suggests that the inclusion of climate change scenarios in model projections reduces the potential for soil C storage.

The most likely explanation for the relatively optimistic model projections would be that models featured in Cramer et al. (2001) do not fully account for N limitation of plant growth and soil C storage. In fact, Hungate et al. (2003) showed that all 6 model projections require more N than can be supplied through soil N input.

Then again, estimates based on meta-analyses might overvalue the impact of soil N limitations on plant growth and soil C storage. In the real world, a gradual increase in atmospheric CO₂ would allow more time to adjust the N status of the soil through N retention, which might decrease the severity of PNL (Luo et al. 2004). However, Hungate et al. (2003) calculated that even a substantial increase of CO₂-induced N retention will allow for little additional C storage in the near future.

It should be mentioned that some other model projections seem to agree with predictions based on the meta-analysis. Using a grassland C model, Van Ginkel et al. (2001) estimated that at the current rate of increases of CO₂ in the atmosphere, temperate grasslands could store 0.036×10^6 g of C per hectare per year (Steinfeld et al. 2006). Thus, according to their model, rising CO₂ concentrations would cause total storage of 3.4×10^6 g of C per hectare between 2007 and 2100. On average, soils in temperate grasslands contain about 100×10^6 g of C per hectare (IPCC 2001). An increase of 3.4×10^6 g of C would equal a 3.4% rise in soil C contents, a result that is similar to my findings in table 8.3.

In summary, field studies strongly suggest that many model projections overestimate the potential for soil C storage. To accurately predict future soil C storage under elevated CO₂, models need to incorporate limitations imposed by nutrient availability.

8.4 Conclusions

By testing hypotheses 1 through 5, this thesis yielded new insights in the processes that affect SOM dynamics under elevated CO₂. It became clear that soil C storage under elevated CO₂ is primarily driven by an increase in plant growth, and thus soil C input. Other pathways, such as a CO₂-induced decrease in litter quality, appear to have a limited impact.

When no fertilizers are added, an increase in soil C input under elevated CO₂ gradually decreases soil N availability. A decline in soil N availability limits the CO₂-response of plant growth, and will therefore constrain soil C storage as well. Plant adjustments such as increased fine root production can temporarily alleviate nutrient constraints, as they increase uptake efficiency. Nitrogen fixation might also increase

under elevated CO₂, thereby providing additional N to support plant growth. But, this process will eventually be constrained by the availability of other nutrients. Ecosystems under elevated CO₂ might reduce N losses, and could thereby accumulate N needed to store additional C. However, the rate of CO₂-induced N retention is probably low.

Addition of fertilizer lifts nutrient constraints, and allows for additional soil C storage under elevated CO₂. Yet, the ultimate potential for soil C storage in fertilized systems is still uncertain. Under elevated CO₂, new C largely accumulates in labile fractions with a low potential for soil C storage. Longer experiments are needed to determine if C will accumulate in stable fractions as well. Calculations suggest that at least in some soils, the potential of CO₂-induced soil C storage is already achieved over the course of a CO₂ experiment. Thus, in fertilized systems, soil C storage under elevated CO₂ will likely be limited by the ability of the soil to protect C from decomposition.

In theory, elevated CO₂ could affect soil properties so that soils become conducive to the protection of C. I discussed two possible mechanisms: a shift towards fungal decomposition, and increased soil aggregation. Whereas I found no indication that elevated CO₂ stimulated fungal activity, it did increase soil aggregation in a Swiss grassland. To adequately study the potential of this mechanism, we need experiments of longer duration, which separate effects of elevated CO₂ on soil C input from effects on aggregation.

I used meta-analyses to predict potential soil C storage under elevated CO₂. My estimates are markedly lower than those based on models used by IPCC, probably because the latter do not consider the effect of N limitations. The highest potential for CO₂-induced soil C storage was found in cropland. These results can be partly explained by the high amounts of fertilizer that most cropping systems receive. Soils under natural vegetation converted to intensive tillage agricultural use typically lose a substantial part of their initial C stocks. By minimizing soil tillage practices, additional C can be stored (Smith 2004). In such a way, cropland could form a main future C sink.

However, one should not infer from these results that fertilization is an effective means to combat the increase in atmospheric CO₂. First, the production of fertilizer is an important source of CO₂ in itself: manufacturing 1 ton of anhydrous ammonia fertilizer requires 33,500 cubic feet of natural gas. Second, highly fertilized ecosystems are likely to lose N through emissions of N₂O (Baggs et al. 2003), a gas with a global warming potential far higher than CO₂.

Research on the effects of elevated CO₂ on soils is far from being finished. For instance, few studies have been done on deserts and tundra, even though these biomes together cover 36% of the Earth's land surface (IPCC 2001). However, with respect to soil C storage, it is questionable if additional experiments in little-studied biomes will lead to new insights. The meta-analysis described in Chapter 7 indicates

that soil N availability limits soil C storage under elevated CO₂ across all experimental classes, strongly suggesting that nutrient constraints of soil C storage are universal.

This dissertation pointed out two limiting factors of future soil C storage under elevated CO₂. In fertilized systems, soil C storage will be limited by the capacity of soil to store C in stable pools. In unfertilized systems, the ultimate potential for CO₂-induced soil C storage will be co-limited by the rate at which ecosystems can accumulate N through N retention. The effect of elevated CO₂ on both these factors can only be studied in prolonged experiments. Thus, to gain further insight in SOM dynamics under elevated CO₂, it would be advisable to extend existing CO₂ enrichment experiments, rather than initiate new ones. To yield realistic estimates on future soil C storage, these experiments should also include treatments that represent the effects of climate change.

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Summary

By burning fossil fuels, cutting down forests and converting soils to agricultural use, humans are rapidly increasing the atmospheric concentration of CO₂. The accumulation of CO₂ and other greenhouse gases in the atmosphere leads to climate change. However, rising levels of atmospheric CO₂ may increase C storage in terrestrial ecosystems, thereby mitigating emissions of greenhouse gases. Whether and by how much C will accumulate in soil remains controversial. On the one hand, numerous studies found a surge in plant growth and soil C input following an increase in atmospheric CO₂. On the other hand, higher plant growth rates in a CO₂-rich world can only be sustained if the soil supplies plants with additional nutrients. Hence, uncertainties about the effect of nutrient limitation cause large differences in projections of future C storage in plant biomass and soil.

In this research, I tried to determine to what extent nutrient availability affects soil C dynamics under elevated CO₂. In order to do so, I focused on long-term field studies, where atmospheric CO₂ concentrations are increased by using fumigation techniques. Most of these studies were conducted on managed ecosystems, which allow for experimental manipulations of the growing conditions, such as the supply of mineral fertilizers. As such, managed ecosystems offer the possibility to study interactions between atmospheric CO₂ and other growth factors.

This dissertation can be roughly divided into two parts. The first part (Chapters 2 through 5) focuses on a single FACE (Free Air Carbon dioxide Enrichment) experiment on fertilized Swiss grassland. Making use of an isotopic tracer in the added fertilizer N and the CO₂ used for fumigation, I could track new C and N into the soil. Whereas N additions and atmospheric CO₂ concentrations did not significantly affect total soil C contents, the sequestration of new C and N under elevated CO₂ were highly correlated. These results suggest that new N was used to sequester new C (Chapters 2 and 3).

In a study on soils from the same Swiss field site, I found that plant material grown under elevated CO₂, incubated in soil exposed to elevated CO₂ decomposed slower than ambient plant material in ambient soil (Chapter 4). Lower decomposition rates combined with an increase in soil C input could lead to an accumulation of soil C. In theory, a CO₂-induced shift in the soil microbial community could also affect soil C storage. Specifically, elevated CO₂ may stimulate the presence and activity of soil fungi, which could benefit soil C storage in multiple ways. However, an amino sugar analysis of soils from Swiss FACE and two other FACE sites suggests that 3 to 10 years of elevated CO₂ had only minor effects on the composition of the soil microbial population (Chapter 5). As such, this potential feedback seems to be of little importance for soil C storage.

Apparently, the effect of elevated CO₂ on soil C stocks is mainly governed by its impact on soil C input. Yet, higher soil C input rates under elevated CO₂ require that soil N availability supports an increase in plant growth. Thus, whether or not ecosystems become C sinks largely depend on how soil N availability is affected by elevated CO₂. In the Swiss FACE experiment, the effect of elevated CO₂ on soil N availability appeared small. Elevated CO₂ increased the total amount of newly added N that was retained in some soil fractions, but only under high fertilization rates. After 9 years, elevated CO₂ did not significantly affect the total retention of added N in the soil (Chapter 3). However, due to spatial variability, the uncertainty related to these results was high.

In fact, spatial variability often prohibits definitive conclusions on the effect of elevated CO₂ on soil organic matter dynamics in field experiments. A quantitative integration of experimental results across multiple studies might tackle this problem. Thus, the second part of this dissertation (Chapters 6 and 7) is devoted to synthesizing available data from CO₂ enrichment experiments. I applied a statistical technique called meta-analysis to combine experimental observations from independent studies, thereby calculating average treatment effects of elevated CO₂.

In Chapter 6, I show that elevated CO₂ does not significantly increase soil N availability, whereas it does increase microbial N demand in long-term experiments. In a second meta-analysis I found that N₂ fixation, the major natural source of ecosystem N, is unresponsive to elevated CO₂ unless other essential nutrients (e.g. P, Mo, K) are added (Chapter 7). Together, these findings suggest that nutrient limitations could gradually restrain plant growth and soil C storage under elevated CO₂.

To directly test the effect of N availability on soil C storage, I summarized all available experimental data on soil C contents under elevated CO₂. In Chapter 7, I show that averaged over all studies, soil C only increases under elevated CO₂ when N is added at rates exceeding typical atmospheric deposition. These results demonstrate that rapid C accumulations under elevated CO₂ are indeed limited by nutrient availability. In the Chapter 8 (General discussion and conclusions), I synthesize findings of all previous chapters. I also discuss possible reasons as to why the results of my meta-analyses are not consistent with model projections of future soil C storage.

Samenvatting

Door fossiele brandstof te gebruiken, bos te kappen en grond te bewerken voor landbouw doet de mensheid de concentratie van CO₂ in de atmosfeer stijgen. De ophoping van CO₂ en andere broeikasgassen in de atmosfeer leidt tot opwarming van de aarde. Oplopende CO₂ concentraties kunnen echter ook koolstofopslag in terrestische ecosystemen bevorderen, en daarmee de uitstoot van broeikasgassen deels compenseren. Het is onduidelijk of en hoeveel C hierdoor in de bodem kan worden vastgelegd. Aan de ene kant hebben talloze studies aangetoond dat een hogere CO₂ concentratie de groei van planten en daarmee de toevoer van C naar de bodem stimuleert. Aan de andere kant kunnen planten in een wereld met meer CO₂ in de atmosfeer alleen harder groeien als de bodem hen van voldoende nutriënten voorziet. De onzekerheid over het effect van de beschikbaarheid aan nutriënten zorgt voor grote verschillen in schattingen naar toekomstige koolstofopslag in bodem en vegetatie.

Met dit onderzoek heb ik geprobeerd te bepalen hoe onder verhoogde CO₂ concentraties de beschikbaarheid van nutriënten de kringloop van C in bodems beïnvloedt. Ik deed dit door me te richten op langdurige veldstudies, waarin de atmosferische concentratie van CO₂ wordt verhoogd door middel van beroking. Het grootste deel van deze veldstudies werd verricht aan beheerde ecosystemen. In deze systemen is het mogelijk groeicondities te manipuleren, bijvoorbeeld door het toedienen van minerale kunstmest. Hierdoor wordt het mogelijk de interactie tussen atmosferisch CO₂ en andere groeifactoren te onderzoeken.

Deze dissertatie valt grofweg in twee delen uiteen. Het eerste deel (Hoofdstuk 2 t/m 5) richt zich grotendeels op een enkel FACE (Free Air Carbon dioxide Enrichment) experiment, gelegen in een met kunstmest behandeld Zwitsers grasland. In dit experiment zijn zowel de toegevoegde kunstmest als de voor beroking gebruikte CO₂ isotopisch gelabeld, zodat ik kon nagaan hoeveel nieuwe C en N er in de bodem terechtkomt. Alhoewel de hoeveelheid toegevoegde N en de concentratie van CO₂ geen significant effect hadden op de totale hoeveelheid C in de bodem, waren de vastlegging van nieuwe C en N sterk met elkaar gecorreleerd. Deze resultaten suggereren dat nieuwe N werd gebruikt om nieuwe C vast te leggen (Hoofdstuk 2 en 3).

In een studie aan bodems uit hetzelfde Zwitserse experiment, bleek dat plantmateriaal dat is gekweekt onder verhoogde CO₂ concentraties en dat werd geïncubeerd in bodems die zijn blootgesteld aan verhoogde CO₂, langzamer wordt afgebroken dan wanneer planten en bodem niet aan hogere CO₂ concentraties zijn blootgesteld (Hoofdstuk 4). Een lagere afbraaksnelheid in combinatie met een hogere toevoer van C zou kunnen leiden tot vastlegging van C in de bodem. In theorie zou een hogere CO₂ concentratie ook de biomassa en activiteit van bodemschimmels

kunnen vergroten, wat op meerdere wijzen de opslag van C in de bodem zou kunnen bevorderen. Een analyse van aminosuikers uit bodems van het Zwitsere FACE experiment en twee andere FACE experiment suggereert echter dat 3 tot 10 jaar van verhoogde CO₂ concentraties maar kleine effecten heeft op de samenstelling van de bodem microbiële populatie (Hoofdstuk 5). Het lijkt er dus op dat dit potentiële terugkoppelingsmechanisme weinig invloed heeft op de vastlegging van C.

Blijkbaar beïnvloedt verhoogde CO₂ het C gehalte van bodems vooral via een effect op de toevoer van C, dus via de groei van planten. Een stijging van de C toevoer vereist dat er genoeg N beschikbaar is om de toegenomen plantengroei te ondersteunen. Of ecosystemen extra C zullen opslaan hangt dus grotendeels af van het effect van verhoogde CO₂ op de beschikbaarheid van N. In dit opzicht lijkt het effect van een hogere CO₂ concentratie in het Zwitserse FACE experiment gering. Een verhoging van de atmosferische concentratie van CO₂ vergrootte daar de vastlegging van nieuwe N in enkele bodemfracties, maar dit gebeurde uitsluitend bij zeer hoge bemesting. Negen jaar na aanvang van het Zwitserse FACE experiment had een verhoging van de atmosferische CO₂ concentratie geen significant effect op het totale hoeveelheid nieuwe N die werd teruggevonden in de bodem (Hoofdstuk 3). Door de ruimtelijke variabiliteit binnen het Zwitserse FACE experiment gingen deze resultaten echter gepaard met een grote mate van onzekerheid.

Ruimtelijke variabiliteit maakt het vaak lastig definitieve conclusies te trekken over het effect van verhoogde CO₂ concentraties op bodemprocessen. Dit probleem kan wellicht worden verholpen door resultaten van meerdere studies quantitatief te integreren. Het tweede deel van deze dissertatie (Hoofdstuk 6 en 7) richt zich daarom op het samenvatten van beschikbare data uit CO₂ verrijgings experimenten. Ik paste hiervoor meta-analyse toe, een statistische techniek die experimentele observaties van onafhankelijke studies combineert, en daaruit een gemiddeld effect van verhoogde CO₂ concentraties berekent.

In Hoofdstuk 6 toon ik via deze techniek aan dat een verhoogde CO₂ concentratie de mineralisatie van N niet significant stimuleert. Aan de andere kant vergroot het wél de hoeveelheid door microben geïmmobiliseerde N in langdurige veldexperimenten. In een tweede meta-analyse laat ik zien dat stikstoffixatie, de voornaamste natuurlijke bron van N in ecosystemen, niet reageert op hogere CO₂ concentraties tenzij andere essentiële nutriënten (bijv. P, Mo, K) worden toegevoegd (Hoofdstuk 7). Samen suggereren deze bevindingen dat onder verhoogde CO₂ concentraties de hoeveelheid beschikbare nutriënten gelijdelijk afneemt.

Om direct het effect van de beschikbaarheid van N op koolstofopslag in de bodem te testen, vatte ik alle beschikbare metingen aan bodemkoolstof onder verhoogde CO₂ samen. In Hoofdstuk 7 toon ik aan dat gemiddeld over alle studies, verhoogde CO₂ concentraties het gehalte aan C in de bodem alleen doen toenemen als meer N aan een ecosysteem wordt toegevoegd dan dat er normaal via atmosferische depositie binnenkomt. Deze resultaten tonen aan dat koolstofopslag onder verhoogde

CO₂ concentraties op de korte termijn wordt beperkt door de mineralenvoorziening. In de Algemene discussie (General discussion and conclusions) tracht ik de resultaten van alle hoofdstukken uit deze dissertatie met elkaar te integreren. Tevens vergelijk ik de resultaten van de meta-analyse aan bodemkoolstof met voorspellingen van een aantal vegetatie modellen, en probeer ik te verklaren waarom deze twee van elkaar verschillen.

Curriculum vitae

Cornelis-Jan van Groenigen (clamour name Kees-Jan) was born on the 6th of November 1977 on the island of Texel, the Netherlands. From September 1989 to June 1995 he followed secondary education at OSG de Hooze Bergh in Den Burg. In September 1995, Kees-Jan started his study in Soil, Water and Atmosphere at Wageningen University. After obtaining the propaedeutic exam (cum laude), he continued his study in the specialization of soil science. In 1999 and 2000, he worked on two MSc projects. The first MSc project was on SOM dynamics and soil formation under kauri, an endemic evergreen conifer from Northland, New Zealand. For his second MSc project, Kees-Jan stayed in the United States for a year. At the University of California, Davis, he studied the effect of elevated CO₂ on SOM dynamics under fertilized grassland. He thoroughly enjoyed his stay, and when offered the opportunity to extend his research into a Ph. D. project, he gladly accepted. He received his MSc degree in June 2002. Soon after that, Kees-Jan started his Ph. D. research, which was conducted at the Department of Plant Sciences at the University of California in Davis and at the Laboratory of Soil Science and Geology at Wageningen University. This research started in August 2002 and was finished in December 2006.



The SENSE Research School declares that Mr. Cornelis Jan van Groenigen has successfully fulfilled all requirements of the Educational PhD Programme of SENSE with a work load of 34 ECTS, including the following activities:

SENSE PhD courses:

- Environmental Research in Context
- Research Context Activity: "Research essay on: Soils as carbon sinks"
- Basic and Advanced Statistics
- Career Orientation

Other PhD courses and activities:

- Revising Scientific Prose, UC Davis, USA, 2005
- Advanced Soil Microbiology, UC Davis, USA, 2006
- Safe Handling of Radioactive Materials, Larenstein University of Professional Education, The Netherlands, 2002
- Training Amino Sugar Extraction, University of New Hampshire, USA, 2003
- Reviewing Proposals for the National Institute for Climate Change Research, UC Davis, USA, 2006

Oral Presentations:

- Annual ASA Meeting, Denver, USA, November 2003
- Annual ASA Meeting, Seattle, USA, November, 2004
- SENSE PhD Day, Kyoto and Beyond, Ede, The Netherlands, June, 2005

Poster Presentations:

- Swiss FACE Symposium, Ascona, Switzerland, March 2004
- Annual AGU Meeting, San Francisco, USA, December 2005
- WCSS, Philadelphia, USA, July 2006

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Cover illustration:

Atmospheric CO₂ concentration at Mauna Loa (HI)

Source: C.D. Keeling, T.P. Whorf, and the Carbon Dioxide Research Group

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