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Mobilisation of soil N and turnover rates of residual biomass: key factors in the response of *Lolium perenne* L. swards to CO_2 enrichment

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Ἐπεὶ δὲ τὸ ἔκ τινος σύνθετον οὕτως ὥστε ἓν εἶναι τὸ πᾶν, ἂν μὴ ὡς σωρὸς ἀλλ' ὡς (...) ἡ δὲ συλλαβὴ οὐκ ἔστι τὰ στοιχεῖα, οὐδὲ τῷ <βα> ταὐτὸ τὸ <β> καὶ <α> ...

That which is composed of something in such a way that the whole is a unity, is not as a heap is a unity, but (*is more than the sum of its parts*) as a syllable is not the letters, nor is BA the same as B and A ...

Aristotle, *Metaphysics* 1041b

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Abbreviations

^{13}C SSL	^{13}C steady-state labelling
^{14}C MPL	^{14}C multiple-pulse labelling
curCO$_2$	current pCO$_2$ [Pa]
curN	current N supply [g N m^{-2} a^{-1}]
$\delta^{13}C$	concentration of ^{13}C related to Vienna-Pee Dee Belemnite [‰]
DM	dry matter
ETS	effective temperature sum [°C]; sum of daily mean temperatures exceeding a threshold temperature of 0 °C
FACE	*F*ree-*A*ir *C*O$_2$ *E*nrichment
NPP	net primary production [g DM m^{-2} a^{-1}]
%N$_s$	proportion of N in harvestable biomass derived from soil including remobilised N from fertiliser [%]
%N$_{som}$	proportion of N in harvestable biomass derived from unlabelled SOM excluding remobilised N from fertiliser [%]
pCO$_2$	partial pressure of carbon dioxide [Pa]
Pg	1 petagram = 10^{15} g
prevCO$_2$	previous pCO$_2$ [Pa]
prevN	previous N supply [g N m^{-2} a^{-1}]
SLA	specific leaf area [cm^2 g^{-1} DM]
SOM	soil organic matter

Summary

Carbon (C) is increasingly available for plant photosynthesis through the rising partial pressure of carbon dioxide (pCO_2) in the atmosphere, caused by the burning of fossil fuels and large-scale changes in land use. The shift in the relation of C to other resources, in particular to nitrogen (N), alters plant growth, the allocation of biomass and inputs of biomass into the soil. Changed C assimilation in response to elevated pCO_2 is affecting the global C cycle. Altered inputs of biomass may change N dynamics in the soil and feed back on the plant's response to elevated pCO_2.

In swards of *Lolium perenne* L. (perennial ryegrass), elevated pCO_2 stimulated the uptake of C by photosynthesis, but this response was reflected to a lesser extent in harvestable biomass, particularly at low N fertilisation. At high N, the response of harvested biomass to elevated pCO_2 became stronger over the years, suggesting changes in ecosystem processes over time.

To understand the effects of elevated pCO_2 on managed permanent grassland, I investigated the production of residual biomass (stubble and roots) and feedback mechanisms through N sources in the soil as affected by N supply. The investigation focused on (i) the turnover rates of stubble and roots and (ii) the mobilisation of N from soil in swards of *L. perenne* at ambient and 60 Pa pCO_2 combined with a supply of 14 or 56 g N m^{-2} a^{-1}. Elevated pCO_2 was achieved using Free-Air pCO_2 Enrichment (FACE). Turnover rates were determined by ^{14}C multiple-pulse labelling and ^{13}C steady-state labelling of swards growing in tubes inserted into the field. Using ^{15}N-labelled fertiliser, I studied the effects of elevated pCO_2 and N supply on N derived from unlabelled soil organic matter

(SOM) for ten years (1993 to 2002) and effects of long-term pCO_2 and N supply on N derived from soil (including remobilised fertiliser N).

Average turnover rates of stubble and roots were 2.7 a^{-1} and 1.2 a^{-1}, respectively. The two methods of labelling gave estimates of turnover rates of stubble within around 10% indicating that C isotopes are valuable tools for assessing turnover rates of stubble. Elevated pCO_2 increased harvestable biomass by 9% at low N and by 21% at high N. However, harvestable biomass accounted for only 29 to 49% of the net primary production (NPP) and was not a good indicator of the plant's response to elevated pCO_2. Elevated CO_2 increased the turnover rates of stubble by 33% at low N but reduced the turnover rates by 12% at high N. At elevated pCO_2, the production of stubble was stimulated by 70% at low N, but did not change at high N. Elevated pCO_2 stimulated the mass of residual leaf lamina by 50% at low N and by 26% at high N. Turnover rates and production of roots were unaffected by pCO_2. The results suggest a preferential allocation of biomass to stubble when the N supply is limited, which is driven by source-sink relations rather than by optimum resource capture. Production of residual plant biomass linked, thus, the strong stimulation of photosynthesis and the weak response of harvestable biomass in N-limited ecosystems at elevated pCO_2. The response of NPP to elevated pCO_2 was 34% at low N and 9% at high N. Under elevated pCO_2, NPP was unaffected by N supply, probably due to a higher N use efficiency. NPP would have been underestimated by 20 to 40% if the standing biomass alone had been assessed, i.e., a turnover rate for stubble and roots of 1 a^{-1} had been assumed. Hence, it is essential to measure turnover rates of residual biomass when estimating NPP of permanent grassland.

In the long term, the response of harvestable biomass to elevated pCO_2 at high N increased from 7% in 1993 to 32% in 2002, but remained low at low N. At high N, increasingly more N was mobilised from unlabelled SOM at elevated than at ambient pCO_2. In contrast, at low N, the amounts of N derived from unlabelled

SOM were smaller at elevated than at ambient pCO_2. A higher proportion of N from soil was found following long-term elevated pCO_2 and high N supply. N availability in soil may have limited initially the response of harvestable biomass to elevated pCO_2. At high N, altered composition and activity of microbial and fungal communities in the soil as well as adjustments of the plants may have increased the mobilisation of N from soil, especially from unlabelled SOM. Consequently, the response of harvestable biomass to elevated pCO_2 was influenced by a greater N availability. Hence, there are feedback mechanisms in the soil, which may be revealed only in long-term experiments.

A changed availability of C and N, caused by the increasing pCO_2 in the atmosphere, altered the allocation of biomass in favour of stubble. Greater inputs of biomass to the soil under elevated pCO_2 and at high N resulted in feedback mechanisms, which have rarely been observed so far, but may have important consequences for the long-term response of ecosystems to elevated pCO_2 and N supply.

Zusammenfassung

Das Verbrennen fossiler Energiequellen und weiträumig veränderte Landnutzung lassen den Partialdruck von Kohlendioxid (pCO_2) in der Atmosphäre ansteigen und machen Kohlenstoff (C) besser verfügbar für die Photosynthese. Das geänderte Verhältnis von C zu anderen Wachstumsressourcen, insbesondere Stickstoff (N), verändert das Pflanzenwachstum, die Verteilung von Biomasse in der Pflanze und die Biomasseeinträge in den Boden. Veränderte C-Aufnahme in Abhängigkeit von erhöhtem pCO_2 beeinflusst den globalen C-Kreislauf. Veränderte Biomasseeinträge können den N-Kreislauf im Boden und via N-Verfügbarkeit die Pflanzenreaktion auf einen erhöhten pCO_2 beeinflussen.

Ein erhöhter pCO_2 steigerte die C-Aufnahme in Beständen von *Lolium perenne* L. (Englisches Raygras), aber diese Reaktion schlug sich in geringerem Ausmass im Schnittertrag nieder, insbesondere bei geringer N-Düngung. Der Ertrag reagierte bei hoher N-Düngung über die Jahre stärker auf einen erhöhten pCO_2, was Veränderungen in Ökosystemprozessen nahelegt.

Um die Reaktion von bewirtschaftetem Dauergrünland auf ein erhöhtes CO_2-Angebot zu verstehen, untersuchte ich die Produktion von Restbiomasse (Stoppeln und Wurzeln) und Rückkoppelungsprozesse via N-Quellen im Boden bei unterschiedlicher N-Düngung. Schwerpunkte waren erstens die Umsatzraten von Stoppeln und Wurzeln und zweitens die Freisetzung von N aus dem Boden in Beständen von *Lolium perenne* unter natürlichem und auf 60 Pa erhöhtem pCO_2 bei Düngung mit 14 oder 56 g N m^{-2} a^{-1}. Der pCO_2 wurde durch Freiluft-CO_2-Anreicherung (FACE) erhöht. Umsatzraten wurden mittels mehrfacher ^{14}C-Pulsmarkierung und ^{13}C-Dauermarkierung von Beständen in Töpfen bestimmt, die im Feld bodeneben eingelassen waren. Mit ^{15}N-

markiertem Dünger wurden Effekte von erhöhtem pCO_2 und N-Düngung auf die N-Mobilisierung aus unmarkierter organischer Bodensubstanz (OBS) während zehn Jahren (1993 bis 2002) untersucht und Effekte von mehrjährigem pCO_2 und N-Düngung auf die N-Menge aus dem Boden gemessen (einschliesslich remobilisiertem Dünger-N).

Die durchschnittlichen Umsatzraten von Stoppeln und Wurzeln betrugen 2.7 a^{-1} resp. 1.2 a^{-1}. Durch die beiden Markierungsmethoden konnten Umsatzraten der Stoppeln mit einer Abweichung von etwa 10% bestimmt werden, was zeigt, dass Umsatzraten von Stoppeln mittels C-Isotopen erfolgreich geschätzt werden können. Erhöhter pCO_2 steigerte den Schnittertrag um 9% bei tiefer und 21% bei hoher N Gabe. Allerdings machte der Schnittertrag nur 29 bis 49% der Netto-Primärproduktion (NPP) aus. Ein erhöhter pCO_2 vergrösserte die Umsatzrate der Stoppeln um 33% bei geringer N-Düngung, verringerte sie aber um 12% bei hohem N. Unter erhöhtem pCO_2 nahm die Stoppelproduktion um 70% bei tiefem N zu, blieb aber bei hohem N unverändert. Die Restblattmasse vergrösserte sich um 50% bei tiefem und 26% bei hohem N. Umsatzraten und Produktion der Wurzeln wurden vom pCO_2 nicht beeinflusst. Diese Resultate zeigen, dass bei N-Limitierung Biomasse vor allem in die Stoppeln verlagert wird und dass dieser Effekt eher auf Quell-Senke-Verhältnisse als auf eine optimierte Ressourcenaufnahme zurückzuführen ist. Die Reaktion der Restbiomasse erklärte in N-limitierten Ökosystemen unter erhöhtem pCO_2 deshalb die starke Steigerung der C-Aufnahme mit der fehlenden Reaktion des Schnittertrages. Die Reaktion der NPP auf erhöhten pCO_2 betrug 34% bei tiefem und 9% bei hohem N. Unter erhöhtem pCO_2 war die NPP unabhängig von der N-Düngung, möglicherweise aufgrund höherer N-Effizienz. Die NPP wäre um 20 bis 40% unterschätzt worden, wenn nur die stehende Biomasse gemessen, d.h. die Umsatzraten von Stoppeln und Wurzel als 1 a^{-1} angenommen worden

wären. Umsatzraten von Restbiomasse sind deshalb bei Schätzungen der NPP zu berücksichtigen.

Die Reaktion des Schnittertrages auf erhöhten pCO_2 stieg unter hohem N von 1993 7% auf 2002 32% an, blieb bei tiefem N aber niedrig. Unter erhöhtem pCO_2 wurde bei hoher N-Düngung zunehmend mehr N aus der unmarkierten OBS freigesetzt als unter natürlichem pCO_2. Bei tiefem N waren die N-Mengen aus der unmarkierten OBS dagegen kleiner unter erhöhtem als unter natürlichem pCO_2. Bei hoher N-Düngung stammte nach mehrjähriger CO_2-Erhöhung ein grösserer Anteil an N aus dem Boden als unter natürlichem pCO_2. Anfänglich dürfte das N-Angebot im Boden die pCO_2-Reaktion des Ertrages beschränkt haben. Mit der Zeit könnten unter hohem N veränderte Populationen von Bodenorganismen oder Anpassungen der Pflanzen eine vermehrte Mobilisierung von N aus dem Boden, besonders aus unmarkierter OBS, bewirkt haben. In der Folge reagierte der Ertrag auf die zunehmende N-Verfügbarkeit. Es gibt also Rückkoppelungsmechanismen im Boden, die nur in Langzeitversuchen beobachtet werden können.

Ich schliesse daraus, dass die veränderte Verfügbarkeit von C und N durch einen erhöhten pCO_2 zu einer vermehrten Verlagerung von Biomasse in die Stoppeln führte. Grössere Biomasseeinträge in den Boden unter erhöhtem pCO_2 riefen bei hoher N-Düngung Rückkoppelungsprozesse hervor, die bisher selten beobachtet wurden. Für die langfristige Reaktion von Ökosystemen auf einen steigenden pCO_2 und N-Düngung kann dies wichtige Folgen haben.

1
General introduction

1.1 Estimating the imbalance in the global C cycle requires the quantification of net primary production

1.1.1 *Anthropogenic emissions and land-use changes alter the global C cycle*

The photosynthetic uptake of carbon (C) from the atmosphere and oceans provides the fuel for most biotic processes which, in turn, respire C back into the atmosphere. Of the 760 Pg C in the atmosphere, about 60 Pg are taken up annually in net primary production (NPP) of terrestrial vegetation (Saugier et al. 2001). NPP is defined as the total photosynthetic gain minus the respiratory losses from the vegetation per unit ground area. The amounts of C in vegetation are more or less stable at about 650 Pg C (Saugier et al. 2001) and in equilibrium all of the C fixed in NPP is returned to the atmosphere primarily by decomposition. Dead biomass enters the huge pool of detritus and organic matter in the soil (1550 Pg C; Lal 2003), from where it is respired at a rate, which depends on its chemical composition and on environmental conditions.

Human activities make a significant contribution to the global C cycle and are mainly responsible for an imbalance in the C cycle, which causes an increase in the partial pressure of atmospheric CO_2 (pCO_2). Measurements of air enclosed in ice cores revealed that pCO_2 has been increasing steadily since about 1770 from the post-glacial level of about 28 Pa (Siegenthaler et al. 1988). Since 1957, pCO_2 has risen globally from 31.5 Pa to 36.7 Pa in 1999 (Prentice et al. 2001). The burning of fossil fuels and the deforestation of large areas are the main causes of this rise in pCO_2. The anthropogenic influence was revealed by a decrease in

atmospheric O_2 and by studies of $^{13}C/^{12}C$ and $^{14}C/^{12}C$ isotopic ratios in CO_2, because fossil fuels are depleted in ^{13}C and ^{14}C (Siegenthaler et al. 1988; Prentice et al. 2001). Emissions through the combustion of fossil fuels and cement manufacture were estimated to be 6.3 ± 0.4 Pg C a^{-1} in the 1990s,; emissions due to land-use changes range from 1.6 to 2.2 Pg C a^{-1} (Prentice et al. 2001; Houghton 2003). The increase in pCO_2 occurred at a rate of 3.2 ± 0.1 Pg C a^{-1} (Prentice, 2001). The additional absorption of CO_2 by the oceans was 1.7 to 2.4 Pg C a^{-1} (Plattner et al. 2002). Although these estimates are uncertain and vary during the year and between years (Houghton 2003), it is obvious that human-induced changes are large enough to have a significant impact on the global C equilibrium.

1.1.2 *The importance of grasslands in the global C cycle*

The imbalance or residual terrestrial sink in the global carbon budget is estimated to be between 2.3 and 2.9 Pg C a^{-1} and is assumed to be related to a greater C uptake in terrestrial ecosystems (Ciais et al. 1995; Houghton 2003). Enhanced growth at the global level may contribute significantly to the terrestrial C sink (Joos et al. 2002; Houghton 2003). Quantifying responses of NPP and C fluxes in ecosystems is, therefore, of major importance.

Traditionally, forests have been considered the main terrestrial sinks of C, since about 50% of the global C in vegetation and soil is in forests (Prentice et al. 2001). Forests contain 5 to 10 times more C per surface area in biomass and soil than other biomes (Schlesinger 1997). However, 36 to 74% of the net C sink in the USA are in non-forests (Houghton et al. 1999; Pacala et al. 2001). Grasslands, which cover approximately 25% of the earth's land surface, may play an important role in the terrestrial sink, because they are not tilled and usually have a high content of organic matter in the soil (Parton et al. 1993; Scurlock & Hall 1998; Chapin et al. 2002). In Switzerland, grasslands (including mountain

grazing land) amount for 28% of the land area and 70% of the agricultural land (BFS 2001).

The C stocks in grassland soils are about 78% of the C in Swiss agricultural soils and 34% of the total C stocks in Swiss soils (Leifeld et al. 2003). Quantifying the response of NPP in grasslands is important for the estimation of potential inputs of C into the soil. The extent to which soils act as sinks under increasing levels of pCO_2, depends on their capacity to store C and on the degree of saturation (Six et al. 2002).

1.2 Plant responses to the altered availability of growth resources at elevated pCO_2

The increase in pCO_2 alters the availability of C to the plants in relation to other resources for growth, which are light, water and about 15 nutrients. As a consequence, plants adjust growth and the allocation of biomass to the availability of these resources. Since plants drive many processes in the ecosystem through growth, the uptake of resources and the production of biomass, the whole ecosystem responds to rising pCO_2. Some processes, such as enzyme activation, are direct, but indirect effects due to an altered availability of resources may be more numerous.

1.2.1 *C uptake in photosynthesis and interactions with N*

While assimilated C provides energy and substrate for metabolic processes, the metabolism itself is built on enzymes, which are N compounds. For example, enzymes of the photosynthetic apparatus account for about half of the N in the leaves. Rubisco, which captures CO_2 into C skeletons, accounts for about 25% of leaf N. Elevated pCO_2 increases the photosynthetic carbon gain per quantum of light (Long 1991). The content of Rubisco in leaves and its activity are decreased at elevated pCO_2 (Drake et al. 1997; Rogers et al. 1998). A low concentration of

Rubisco may be related to an increased N use efficiency, which may be especially important when N in the ecosystem is limited (Drake et al. 1997).

In *Lolium perenne* L. (perennial ryegrass, a main grass of managed temperate grasslands), measurements at the leaf level showed a 30 to 40% increase in net CO_2 uptake per unit leaf area under elevated pCO_2, which was more or less independent of N fertilisation and persisted for ten years (Rogers et al. 1998; Isopp et al. 2000; Ainsworth et al. 2003). This is in contrast to plants in pots, whose rates of photosynthesis acclimated to a prolonged increase in pCO_2 (Drake et al. 1997). It is suggested that adjustments in the root system and a higher N use efficiency may determine the plant's response in the field.

1.2.2 *Allocation of biomass in response to elevated pCO_2*

In contrast to the rates of C uptake, the pCO_2 response of harvestable product (yield) was weak in a number of field experiments (Kimball et al. 2002). For example, swards of *L. perenne* were exposed to elevated pCO_2 in the Swiss FACE (Free-Air CO_2 Enrichment) experiment. The FACE technique does not make use of enclosures and, therefore, the natural microclimate is not altered. At the beginning of the experiment in 1993, the response of harvestable biomass to elevated pCO_2 was only about 10%, independent of N fertilisation (Hebeisen et al. 1997). Daepp et al. (2000) found that after six years of elevated pCO_2 the production of harvestable biomass at high N fertilisation increased by up to 25%. At low N, elevated pCO_2 even reduced the production of harvestable biomass in some years. Thus, increased C assimilation in photosynthesis was not reflected in harvestable biomass; this discrepancy was much more pronounced at low N. However, only 12 to 37% of the total plant biomass was accounted for in harvestable biomass (Daepp et al. 2001). The large fraction of residual plant biomass (roots and stubble) may, thus, be a key for understanding these phenomena. Roots and stubble are functionally heterogeneous fractions and

have vital functions in many perennial grassland plants. A main characteristic of these plants is their ability to restore foliage after defoliation by grazing or cutting due to (i) the protection of meristematic tissues from defoliation and (ii) the storage of nutrients and energy. Stubble is important for storing carbohydrates (Prud'homme et al. 1992; Fischer et al. 1997; Fulkerson & Donaghy 2001). Stocks are replenished when the photosynthetic rates (*source activity*) exceed the actual demand in growing tissue (*sink activity*). After defoliation, the carbohydrates are mobilised to maintain the production and elongation of new leaves. After a few days, the plant prefers new photosynthates before reserves for building new leaves, the latter may serve as a source of energy supply to an extent which is still unknown (Schnyder & de Visser 1999). N is stored in stubble, too, as amino acids and proteins (Ourry et al. 1988).

It is clear that an increase in the availability of C will change the relationship between storage and remobilisation. Elevated pCO_2 increased the concentration of carbohydrates in stubble of *L. perenne* by about 35% in average (Fischer et al. 1997). Elevated pCO_2 stimulated the allocation of biomass to the residual biomass in many studies, which showed a greater mass of roots (Gorissen 1996; van Ginkel et al. 1997; Cotrufo & Gorissen 1997; Loiseau & Soussana 1999a; Daepp et al. 2001). These findings are in line with investigations of other plants, which showed higher root/shoot ratios (as reviewed by Rogers et al. 1996). Much less is known about stubble.

The size of standing biomass pools does not reveal much about changes in plant growth. For example, they may merely indicate an accumulation of material with a slower turnover. In order to estimate the flux of C into residual plant parts and NPP, not only the size of these pools must be quantified, but their turnover rates must also be determined. Therefore, the quantification of

turnover rates of stubble and roots reveals changes in the allocation of biomass in response to a changed availability of resources.

1.2.3 *C losses of the plant: the other side of the plant's C budget*

There are several pathways by which assimilated C is lost from the plant. Roughly half of the C assimilated in plant photosynthesis is respired in growth and maintenance (Amthor 1997). Elevated pCO_2 directly inhibits the cytochrom oxidase (Gonzàlez-Meler et al. 1996). More importantly, elevated pCO_2 affects respiration indirectly through changes in growth and protein content of the biomass (Amthor 1997). Elevated pCO_2 did not affect clearly the respiration of leaves nor of whole *L. perenne* plants (Ryle et al. 1992; Schapendonk et al. 1997). In an other study, respiration of the whole plant of *L. perenne* was reduced (Bunce & Caulfield 1991). In swards, however, dark respiration and also total below-ground respiration were stimulated by elevated pCO_2 (Casella & Soussana 1997). At the level of the ecosystem, elevated pCO_2 increased ecosystem night-time respiration (Aeschlimann 2003).

Furthermore, senescence plays an important role in the loss of C from the plant. The decay of tillers and roots is affected by defoliation since reproductive tillers lift their apex above cutting height, and these flowering tillers die after defoliation. The production of stubble and roots is also likely affected by elevated pCO_2 due to changes in the allocation of photosynthates (Fitter et al. 1996; Fitter et al. 1997; Loiseau & Soussana 1999b). However, other studies did not detect such effects (Arnone et al. 2000; Higgins et al. 2002). This may be due to methodological difficulties as well as to differences in the grassland systems studied.

An important but poorly understood aspect of senescence is nutrient resorption. Grasses resorb, on average, 60% of N and 70% of phosphorus (P) from senescing leaves (Aerts 1996), and there are indications that resorption efficiency is a

function of sink activity. However, a review of 20 publications did not indicate a clear effect of elevated pCO_2 on the resorption of N (Norby et al. 2001).

Other sinks for C are root exudation, transfer of photosynthates to symbionts and losses through herbivores and pathogens. Root exudation of organic acids, carbohydrates and amino acids and the sloughing of polysaccharides from growing root tips usually accounts for less that 5% of C assimilation; when the availability of nutrients, especially of P, is low, root exudation may increase up to 20% (Hütsch et al. 2002). Little is known about effects of elevated pCO_2 on root exudation. The transfer of C to symbionts such as mycorrhiza may also be higher at elevated than at ambient pCO_2 due to the greater demand for nutrients (Staddon & Fitter 1998). Interactions of plants with herbivores, pathogens and endophytes have received relatively little attention with regard to elevated pCO_2. Alone the fact that elevated pCO_2 almost always increases the C/N ratio in leaves (Cotrufo et al. 1998) suggests that these interactions may change (Mitchell et al. 2003).

1.2.4 *Growth resources other than C and N*

The increased availability of C at elevated pCO_2 also affects other growth resources such as water, light and nutrients. Many studies showed an enhanced water use efficiency due to decreased stomatal aperture, a factor, which may be prevalent in ecosystems in which water is the limiting resource for growth (Pataki et al. 2000; Volk et al. 2000). The complex factor of light use efficiency (biomass produced per unit of light) has been measured only rarely, even though it is a core parameter in many models. Constraints of the response to elevated pCO_2, which are exhibited by nutrients other than N, may be important in tropical ecosystems. In temperate grassland, N is the main limiting factor of plant growth and is, therefore, the focus of this study.

1.3 Ecosystem responses to plant growth under elevated pCO_2

1.3.1 *Elevated pCO_2 changes the balance between C and N in the ecosystem*

Recent investigations of canopy photosynthesis showed a 20 to 30% increase in net C uptake of the ecosystem at midday (Aeschlimann 2003), which indicates a greater C uptake by the ecosystem. Since a concomitant higher N input of 20 to 30% is unlikely to occur, the C/N ratio in the ecosystem may be altered under elevated pCO_2. The results of this top-down approach are corroborated by observations on plants, which showed an increase in units of C per unit of nutrients, especially of N (reviewed in Cotrufo et al. 1998). The C/N ratio is an important factor in determining the decomposition of plant residues entering the soil. For many biotic processes the C/N ratio must be within a certain range. For example, microbes grow best within a narrow range of C/N and adjust quickly to changes in the availability of these resources (Killham 1994; de Nobili et al. 2001). Changes in atmospheric pCO_2 will, therefore, trigger a number of adjustments in the ecosystem. These adjustments are occurring continuously in the real world, but stepwise in most experiments, in which pCO_2 is elevated in one step at the start. This means that, in most experiments (such as in the one outlined hereafter), one observes responses to a sudden change in the availability of resources and the adjustments thereafter. A step enrichment of pCO_2 may disturb processes in the ecosystem (Luo 2001). As a consequence, experimental results under enriched pCO_2 have to be extrapolated with caution, especially when experiments were running only for a short time. Several investigators found varying responses of plants and ecosystems over time (Daepp et al. 2000; Luo et al. 2001) or adaptive changes of plants when growing near natural sources of CO_2 (Jones et al. 1995; Fordham et al. 1997). Hence, long-term experiments are crucial for understanding the response of ecosystems to elevated pCO_2.

1.3.2 *Responses of ecosystems to elevated pCO_2: a question of litter quality?*

Decomposition of plant residues is the main flux of C into the soil. It is a sequence of interacting physical and chemical processes inside and outside of living organisms. Decomposition is initiated by the leaching of soluble nutrients and carbohydrates, followed by the physical fragmentation by soil organisms and the chemical alternation by microbes. The energetic and nutritional demand of the decomposing organisms determines the mineralisation and transformation of litter into CO_2, mineral nutrients, water and complex organic compounds, which are resistant to further microbial breakdown.

Elevated pCO_2 consistently reduced the concentration of N in plant tissue (Cotrufo et al. 1998) and may result in a substrate of lower quality for decomposing organisms. As a consequence, the mineralisation of N in the soil may slow. However, this so-called *litter-quality hypothesis* was not supported by various studies (e.g. Sowerby et al. 2000; M.-A. de Graaff, writ. comm.) and there is no consistent proof of a negative effect of elevated pCO_2 on rates of decomposition (Norby et al. 2001). However, the concentration of N is not the only factor affected by elevated pCO_2, which may interact with decomposition. The relative importance of chemical properties such as the content of lignin, polyphenols and aromatic compounds is not well understood (Arp et al. 1997; Chapin et al. 2002). Moreover, the rates of decomposition are regulated by environmental factors such as temperature, moisture, available nutrients as well as by the presence of plant roots (van der Krift et al. 2002).

Decomposition works at various time scales. Detritus and microbial biomass have a short turnover time of less than ten years. Modified soil organic carbon has decadal to centennial turnover time (Gaudinski et al. 2000). Thus, the complete break-down of litter and its humification into recalcitrant SOM takes much longer than the duration of experiments on CO_2 enrichment.

1.3.3 *Feedbacks in the soil in response to altered plant growth at elevated pCO_2*

A change in decomposition due to changes in biomass inputs likely alters the functioning of decomposer communities in the soil and feed back on plant growth through the availability of nutrients in the soil. There is evidence that such feedbacks are also important in swards of *L. perenne* in the Swiss FACE experiment: (i) Elevated pCO_2 altered the mass, composition and structure of microbial communities (Marilley et al. 1999; Sowerby et al. 2000; Montealegre et al. 2002). (ii) The relative response of harvestable biomass to elevated pCO_2 increased with time at high N supply most probably due to a decreasing N limitation in the system (Daepp et al. 2000). (iii) The symbiotic N_2 fixation of the legume *Trifolium repens* (white clover) declined over the years (Richter 2003). (iv) Gaseous losses of N were higher at elevated pCO_2 and amounted to as much as 17% of the applied fertiliser N (Ineson et al. 1998; Baggs et al. 2003a; Baggs et al. 2003b). However, short-time measurements in 1997 and 2000 did not reveal clear changes in the availability of N in the soil (Gloser et al. 2000; Richter et al. 2003), indicating that a direct determination of these feedback mechanisms may be difficult.

1.4 Objectives

This project investigates the long-term effects of elevated pCO_2, in combination with high and low N supply, on swards of *Lolium perenne*. Specifically, it aims at clarifying the following points and questions:

I. Little is known about the turnover rates of residual biomass, especially not about the turnover rates of stubble. However, turnover rates may be essential in the estimation of NPP with regard to changes in the global cycles of C and N.

How can turnover rates of residual biomass (stubble and roots) be determined and are they a crucial factor in the investigation of NPP in managed grassland under increasing levels of pCO_2?

II. At high N, the production of harvestable biomass increased under elevated pCO_2. At low N, however, there was a striking discrepancy between photosynthesis and the production of harvestable yield.

How were turnover rates and the production of stubble and roots affected by elevated pCO_2 and N supply? What happened to the carbon that was additionally assimilated by the stimulated photosynthesis at elevated pCO_2?

III. Elevated pCO_2 and N supply alter the relationships of resources available to plants. This change induces adjustments of the plant and, indirectly, in the soil, which feed back on plant growth through the availability of nutrients in the soil.

How do changes in plant growth brought about by long-term elevated pCO_2 and N supply affect the mobilisation of N from soil?

The results obtained in this study will help us to understand the long-term responses of plants and plant-mediated changes in the soil in relation to changes in the availability of resources at elevated pCO_2.

2

Quantifying turnover rates of stubble in permanent grasslands: an evaluation of two approaches using carbon isotopes

2.1 Abstract

The determination of net primary production in permanent grassland requires reliable estimates of the production of biomass below cutting height. I evaluated, therefore, two approaches for measuring turnover rates of stubble. First, swards of *Lolium perenne* (perennial ryegrass) were labelled by ^{14}C multiple-pulse labelling and turnover rates of stubble were calculated from the decay of ^{14}C. Second, ^{13}C steady-state labelling was achieved by establishing plants in the ^{13}C-depleted atmosphere of a free-air CO_2 enrichment (FACE) system and then placing them in the non-depleted ambient atmosphere and *vice versa*. Turnover rates of stubble were estimated by both methods within a discrepancy of around 10%. The good agreement of the two methods indicated that the assumption of a homogenous labelling with ^{14}C was valid. The results of this study demonstrate that C isotopes are valuable tools for assessing turnover rates of stubble.

2.2 Introduction

Net primary production (NPP) is a driving force in the global carbon (C) cycle and may mitigate the increase in the atmospheric partial pressure of CO_2 (pCO_2) as a result of the burning of fossil fuels and changes in land use. In estimating future trends of pCO_2 the response of NPP to elevated pCO_2 is critical.

Quantifying this response requires reliable methods to estimate NPP. NPP is estimated from carbon exchange studies or from changes in the above- and below-ground standing biomass for a given period of measurement plus the turnover of this biomass (Davies 1993; Scurlock et al. 2002). Accounting for turnover of biomass increased estimates of NPP in natural grasslands using peak standing biomass by a factor of two to five (Scurlock et al. 2002).

In managed grassland, the production of above-ground biomass is commonly determined by sequential harvesting at a given height. However, these estimates often do not include biomass production of the remaining stubble. Stubble consists of expanding and defoliated tillers and the leaves attached to them. The tillerbase is the location of meristems, from which new tillers are initiated. The growth and decay of tillers, their turnover, are dynamic over the period of regrowth (Davies 1988). Furthermore, the turnover of stubble is an important component of litter production in managed grassland. Since necrotic plant parts above defoliation height are removed to some extent by frequent defoliation, the turnover of stubble has, thus, an important impact on nutrient cycling in the grassland ecosystem.

Basically, turnover rates of stubble can be estimated as follows: (i) by measuring the rates of formation of tissue per population unit (e.g. tiller) combined with estimates of changes in the number of population units (Davies 1993; Bullock et al. 1994; Schippers & Olff 2000) and (ii) by labelling biomass pools and estimating the rate at which the label disappears (Loiseau & Soussana 1999b). These methods have rarely been used to estimate the production of stubble and NPP in grassland. This may be due to the difficulty in determining rates of tiller appearance and the limited applicability of isotopic methods in non-destructive plant sampling techniques. Isotopic methods are not conclusive as far as the biomass is not homogenously labelled, which is of primary importance for the reliability of the results.

In order to evaluate the use of isotopic methods for assessing the turnover of stubble, for the first time, ^{14}C multiple-pulse labelling (^{14}C MPL) and ^{13}C steady-state labelling (^{13}C SSL) were compared. Swards of *Lolium perenne* L. (perennial ryegrass) were labelled by three pulses of ^{14}C during establishment, and the turnover rates of stubble were calculated from the decay of ^{14}C. ^{13}C SSL was achieved by the establishment of swards in the ^{13}C-depleted atmosphere of a FACE (Free-Air CO_2 Enrichment) system and thereafter placing them in the non-depleted ambient atmosphere and *vice versa*. The aim was to determine the homogeneity of ^{14}C MPL, using ^{13}C SSL as a reference, and to investigate whether the methods would reveal changes in turnover rates in response to pCO_2 and N supply.

2.3 Material and Methods

2.3.1 *Experimental setup*

A field experiment with *L. perenne* was conducted on an eutric cambisol in the Swiss FACE array (Hebeisen et al. 1997) at Eschikon (8°41'E, 47°27'N and 550 m above sea level). The experimental field consisted of three blocks, each containing one fumigated circular area (18 m diameter) and a control area of the same size, which were at least 100 m apart to prevent an increase in pCO_2 in the control. The partial pressure of CO_2 was ~36 Pa at ambient and 60 Pa at elevated pCO_2 achieved by means of the FACE technique (Lewin et al. 1992). CO_2 enrichment took place from dawn to dusk throughout the growing season (March to November) when the air temperature was above 5 °C.

Stubble biomass was assessed by five destructive harvests in October 1999, March, June and October 2000 and in March 2001. For this purpose, swards were established in PVC tubes, 0.2 m in diameter and 0.6 m in depth, which were, closely put together, inserted into the soil. The tubes in each experimental area were divided into two groups of ten, receiving 14 or 56 g N m^{-2} a^{-1} as well as 5.5

g P m^{-2} a^{-1} and 24.1 g K m^{-2} a^{-1}. The swards were defoliated at a height of 0.05 m five times per year.

2.3.2 *C isotope labelling and analysis*

In ^{14}C MPL, the biomass of *L. perenne* was labelled by three pulses of ^{14}C at intervals of four weeks from August to October 1999. Per pulse, 74 MBq m^{-2} $^{14}CO_2$ were released into a 0.4-m^3 Plexiglas chamber, which was placed over the ensemble of tubes in each experimental area. The activity of ^{14}C in stubble was determined by digesting 20-mg samples in a suspension of 4 mg cellulase (from *Trichoderma viride*) and 4 mg maceroenzyme (from *Rhizopus sp.*) for 24 h at 45 °C followed by 1 ml of Soluene-350 (Tissue solubiliser, Packard Instrument Company) for 24 h at 45 °C (Suter et al. 2002). Thereafter, 15 ml Hionic-Fluor (Packard Instrument Company) were added and ^{14}C was analysed by liquid scintillation counting (Packard 2500TR, Packard Instrument Company). Internal standards with a known amount of ^{14}C were used to correct for quenching.

^{13}C SSL was achieved by establishing swards during five months (June to November) in two atmospheres with a different ^{13}C signal and by switching them between these atmospheres in March 2000. As the CO_2 gas used for FACE originated from a fossil source, elevated CO_2 had a lower ^{13}C signal than ambient CO_2 (Nitschelm et al. 1997). The exchange between elevated and ambient pCO_2 resulted in a ^{13}C label of new biomass, which differed from the biomass produced before. A group of five tubes was attributed to each combination pCO_2×N. In early March 2000, the tubes which established under ambient pCO_2 were moved to elevated pCO_2 and the tubes which established under elevated pCO_2 were exchanged to ambient pCO_2. Turnover rates of stubble were estimated from the substitution of old C by new C. ^{13}C in 2-mg samples of harvestable biomass and stubble was measured in a continuous-flow mass spectrometer (PDZ Europa, Northwich, UK).

2.3.3 *Calculations*

In an evaluation of models describing the change of isotopic label over time, it was observed that the label decayed exponentially when time was accounted for as cumulative effective temperature sum (ETS; sum of the daily mean temperatures exceeding a threshold temperature of 0 °C), suggesting first-order kinetics. Using single-pool models assumed for stubble and roots two pools of homogenously labelled biomass (*assumption 1*) and a negligible translocation of label from stubble to harvestable biomass through the expansion of leaves and the translocation of reserves after the start of measurements (*assumption 2*). Since ^{13}C SSL guaranteed a homogenous labelling of stubble and roots, the achievement of a homogenous labelling by ^{14}C MPL (*assumption 1*) was checked by comparison with ^{13}C SSL. *Assumption 2* was tested by analysing ^{14}C in harvestable biomass of the first subsequent regrowth in spring.

Given this, the change in ^{14}C activity (A_{14C}) in each pool over ETS is

$$dA_{14C} / dETS = -k \cdot A_0 \qquad [2.1]$$

where A_0 is the total ^{14}C activity [Bq m^{-2}] at ETS = 0 and k is a constant. Total ^{14}C activities were used since the pool size was not constant (Milchunas & Lauenroth, 1992).

$$A_{14C} = A_0 \cdot \exp(-k \cdot ETS) \qquad [2.2].$$

This single-pool logarithmic decay model was fitted to $\log_e$-transformed data as

$$\log_e (A_{14C}) = \log_e (A_0) - k \cdot ETS \qquad [2.3]$$

using the REG procedure in SAS (SAS Institute, 1999) for each combination of factors and block (Fig. 2.1). The turnover rates (TR [a^{-1}]) were calculated using aETS (annual ETS during the experimental period of 3435 °C):

$$TR = k \cdot aETS \qquad [2.4].$$

Annual production (P) of stubble and roots was calculated as

$$P = B \cdot TR \quad [2.5]$$

where B is the average standing biomass of stubble and roots (pool size).

Results of ^{13}C analysis are expressed in δ units [‰]:

$$\delta^{13}C = 1000 \cdot (R_b/R_s - 1) \quad [2.6]$$

where $R_b = {}^{13}C/{}^{12}C$ of biomass and $R_s = {}^{13}C/{}^{12}C$ of the standard Vienna-Pee Dee Belemnite.

The fraction of old C (FOC) was:

$$FOC = 1 - (\delta_b - \delta_0) / (\delta_i - \delta_0) \quad [2.7]$$

where δ_0 and δ_b are the $\delta^{13}C$ in the biomass at ETS = 0 and ETS > 0, respectively. δ_i is the $\delta^{13}C$ of the input source. As δ_i, the average $\delta^{13}C$ in harvested biomass above cutting height prior to the sampling of stubble was used, for each block and treatment. The single-pool model was fitted to $\log_e$-transformed FOC using the REG procedure in SAS (SAS Institute, 1999) in analogy to eq. [2.3]:

$$\log_e (FOC) = - k \cdot ETS \quad [2.8]$$

Turnover rates per year were calculated using eq. [2.4].

2.3.4 *Statistical analysis*

The experimental design was a split-plot with pCO_2 as the main-plot factor, N fertilisation as the sub-plot factor and with three blocks. Differences in turnover rates were analysed as differences in the slopes of the $\log_e$-transformed ^{14}C activity and FOC, respectively, against ETS as the covariate using the MIXED procedure in SAS (SAS Institute 1999). A significant interaction between ETS and a treatment factor indicated a significant difference in the slopes of the regression lines and, thus, a significant effect of the factor on the turnover rate. In order to account for the split-plot design, the main factor pCO_2 and pCO_2×block were stated as RANDOM (Milliken & Johnson 2002).

2.4 Results

In swards at ambient pCO_2, which had been established at elevated pCO_2, the mass of stubble did not change over time (Fig. 2.1). In contrast, in swards at elevated pCO_2 the mass of stubble increased after the switch at ETS = 330 °C. At the last sampling at ETS = 3890 °C, the mass of stubble was increased by 50% at elevated pCO_2 compared to ambient pCO_2, independent of N supply (Fig. 2.1).

Shifting swards from elevated to ambient pCO_2 increased the $\delta^{13}C$ from around -39‰ to -30‰. The increase was slightly faster at high N than at low N (Fig. 2.2). The opposite treatment decreased the $\delta^{13}C$ from around -29‰ to -36‰. The

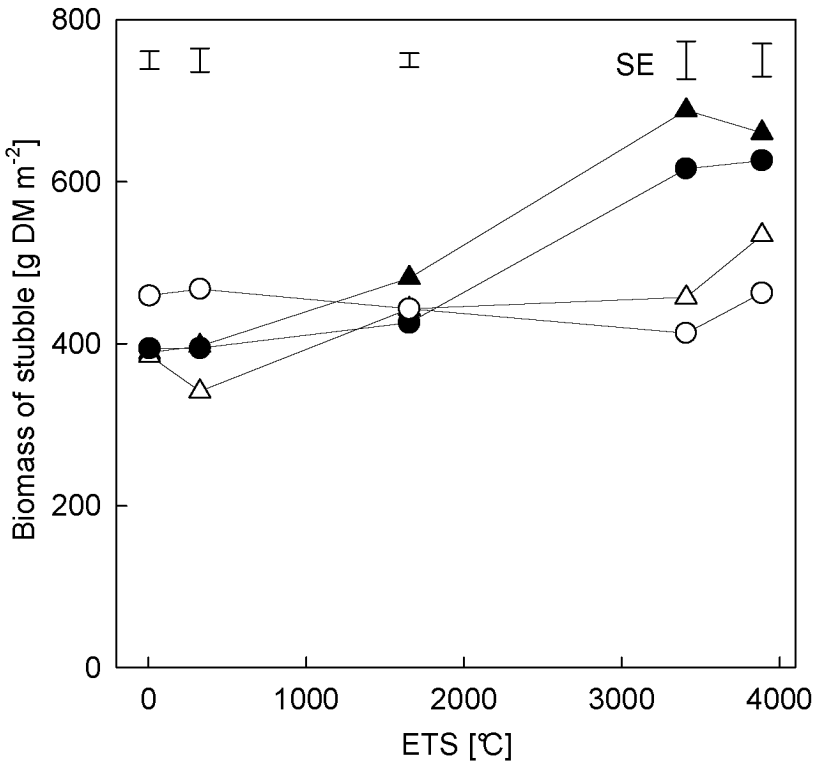

Figure 2.1: Biomass of stubble in swards of *Lolium perenne* over cumulative effective temperature sum (ETS) above 0°C at two levels of N supply (○●: 14 g N m^{-2} a^{-1}; △▲: 56 g N m^{-2} a^{-1}). Swards at ambient pCO_2 (○△; ~36 Pa) were established at elevated pCO_2 until ETS = 0 °C and swards at elevated pCO_2 (●▲; 60 Pa) were established at ambient pCO_2 until ETS = 330 °C.
Symbols show means of three replicates; error bars are standard errors of the means (*SE*).

fitted exponential decay was faster a low N than at high N. At the last sampling at ETS = 3890 °C, the $\delta^{13}C$ increased slightly due to the stop of fumigation during winter. The $\delta^{13}C$ in harvestable biomass showed considerable fluctuations throughout the year. It was lowest in early spring (ETS=1035°C) and in late autumn (ETS=3394°C). The $\delta^{13}C$ increased during summer (Fig. 2.2), likely due to a decreased discrimination caused by water stress and high temperatures.

Turnover rates were estimated from the slopes of regressions fitted to the $\log_e$-

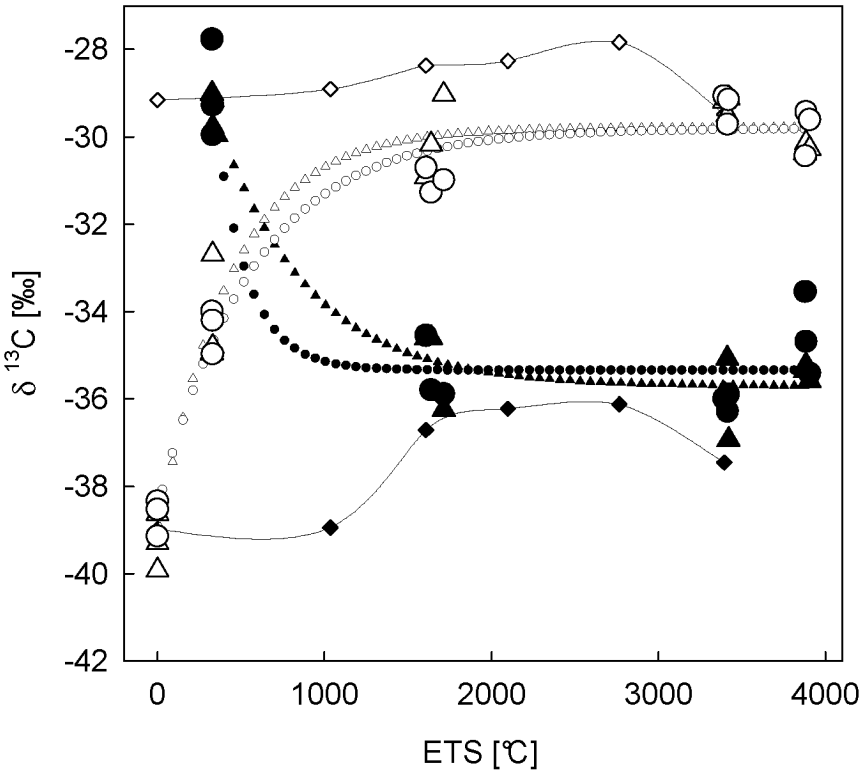

Figure 2.2: Evolution of $\delta^{13}C$ in biomass of stubble of *Lolium perenne* over cumulative effective temperature sum (ETS) above 0°C as affected by ambient pCO_2 (○△; ~36 Pa) and elevated pCO_2 (●▲; 60 Pa) in combination with two levels of N supply (○●: 14 g N m^{-2} a^{-1}; △▲: 56 g N m^{-2} a^{-1}).

Big symbols show measured values and small symbols fitted regression lines:

○: $\delta^{13}C$=-38.5+8.72·(1-exp(-0.00175·ETS)); △: $\delta^{13}C$=-39.3+9.49·(1-exp(-0.00235·ETS));

●: $\delta^{13}C$=-35.3+38.8·exp(-0.00051·ETS); ▲: $\delta^{13}C$=-35.7+11.6·exp(-0.00182·ETS).

Small diamonds show means of $\delta^{13}C$ in harvestable biomass above cutting height, which were used as δ_i to calculate fractions of old C in Fig. 2.3b.

transformed total ^{14}C activity (Fig. 2.3a) and FOC (Fig. 2.3b). Table 2.1 lists the results. All regressions were significant at $P<0.001$. The lowest turnover rates were measured by both methods for swards at high N and ambient pCO_2. The discrepancy between the estimates obtained by the two methods was below 1%. At low N and elevated pCO_2, turnover rates were the highest of all treatments with a discrepancy below 3%. Turnover rates of stubble in swards at high N and elevated pCO_2 and at low N and ambient pCO_2 were intermediate and the differences between the two methods were 12% and 16%, respectively. None of the factors had a significant effect due to the few degrees of freedom in the analysis of only three replicates (*cf.* Tab. 3.3).

Table 2.1: Turnover rates of stubble at previous and current ambient and 60 Pa pCO_2 combined with 14 and 56 g N m^{-2} a^{-1} as measured by ^{14}C multiple-pulse labelling (^{14}C MPL) and ^{13}C steady-state labelling (^{13}C SSL).

N	Previous	Current	^{14}C MPL		^{13}C SSL	
[g N m^{-2} a^{-1}]	pCO_2 [Pa]	pCO_2 [Pa]	[a^{-1}]	r^2	[a^{-1}]	r^2
14	60	36	2.69	0.93	2.38	0.94
	36	60	3.03	0.80	2.96	0.86
56	60	36	2.25	0.87	2.27	0.85
	36	60	2.87	0.80	2.40	0.93
ANCOVA:	$curCO_2$		ns		+	
	N		ns		ns	
	$curCO_2 \times N$		ns		ns	
n			*15*		*12 / 15* [a]	
SE			*0.38*		*0.23*	

Coefficients of determination (r^2) of the regressions, *P*-values (+, $P<0.1$; ns, not significant) of analysis of covariance (ANCOVA), number of measurements (*n*) and average standard errors (*SE*) of the estimates are shown. All regressions were significant at $P<0.001$.

[a] The number of measurements of current elevated pCO_2 (60 Pa) was 12 due to the absence of FACE in winter (see discussion).

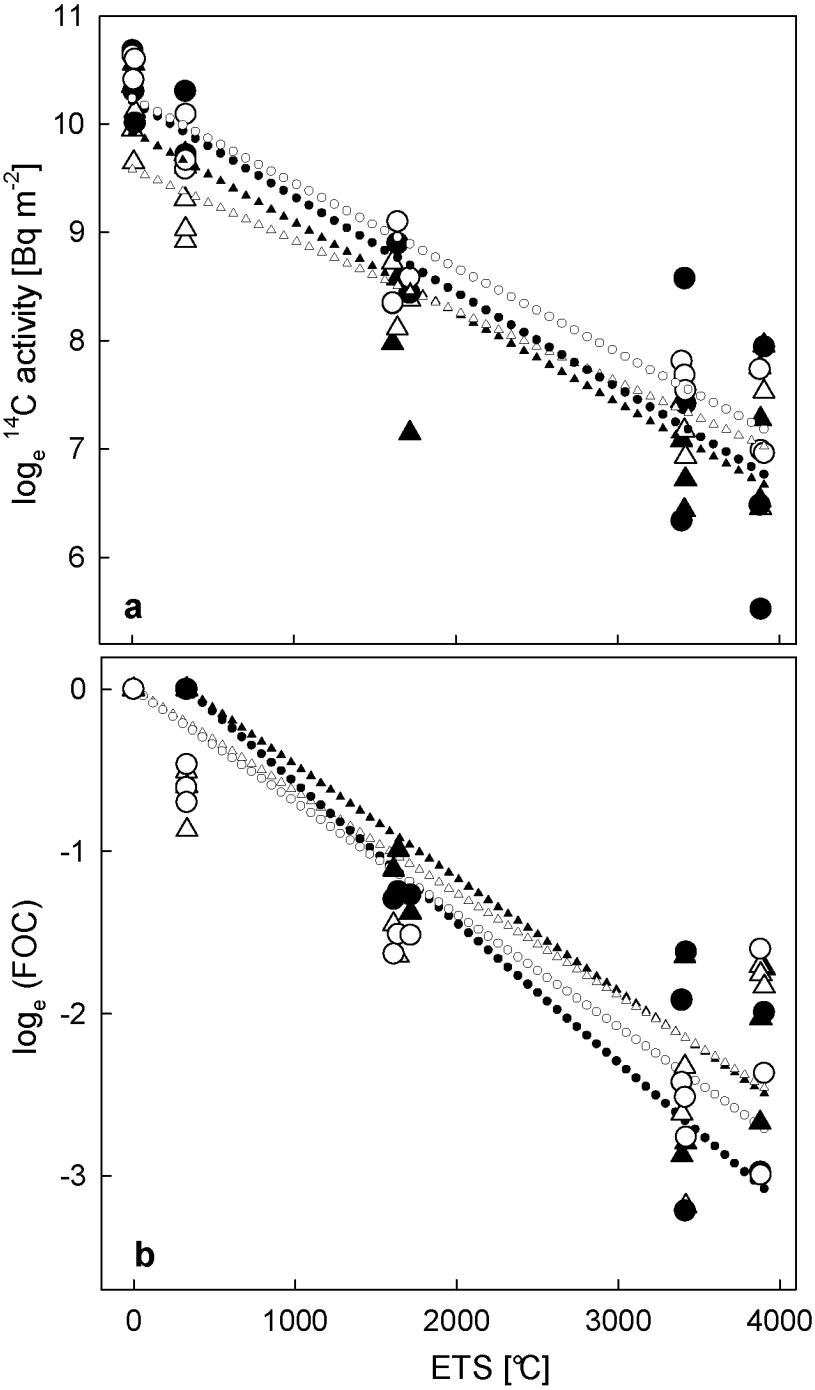

Figure 2.3 a,b: (a) ^{14}C *multiple-pulse labelling*: decay of ^{14}C activity and (b) ^{13}C *steady-state labelling*: decay of the fraction of old C (FOC) in stubble biomass of *Lolium perenne* over cumulative effective sum of temperature (ETS) as affected by ambient pCO_2 (○△; ~36 Pa) and elevated pCO_2 (●▲; 60 Pa) in combination with two levels of N supply (○●: 14 g N m^{-2} a^{-1}, △▲: 56 g N m^{-2} a^{-1}). Big symbols show measured values; small symbols show fitted regression lines (slopes and r^2 are presented in Table 2.1).

2.5 Discussion

Turnover rates of stubble were estimated using ^{14}C MPL and ^{13}C SSL with a discrepancy of around 10% (Tab. 2.1) indicating that *assumption 1* of a homogenous labelling after ^{14}C MPL was valid. Harvestable biomass of the first subsequent regrowth contained only 1.4% of the initial label of ^{14}C confirming *assumption 2* that the translocation of label from stubble to harvestable biomass through the expansion of leaves and the translocation of reserves after the start of measurements was negligible. Similar results were found in spring wheat, where the ^{14}C respired from soil and roots peaked at three days after labeling and was stable from day 19 onwards (Swinnen et al. 1994). This indicated a complete allocation of the label.

Our experiment shows that the two approaches, ^{14}C MPL and ^{13}C SSL, indicated similar effects of CO_2 enrichment and N fertilisation (Tab. 2.1). Turnover rates, as estimated by ^{14}C MPL, were generally higher compared to the estimates obtained with ^{13}C SSL, suggesting systematic differences between the two methods. A main difference between the two methods was the isotopic signal in the source of new C, by which old C was substituted. In case of ^{14}C MPL, the new C that entered plant biomass had the natural background, which is zero, since measured ^{14}C activities were corrected for natural background. In case of ^{13}C SSL, the new C entering plant biomass had the label of new photosynthates. I used the $\delta^{13}C$ of harvested biomass in accordance with Loiseau & Soussana (1999b). $\delta^{13}C$ in biomass varied according to the content in the air and the degree of discrimination during photosynthesis (Fig. 2.2). The latter is determined by stomatal resistance and enzymatic discrimination and, therefore, depends on environmental factors, mainly the water status of the plant (Farquhar et al. 1982). However, the water use efficiency interacts with pCO_2 and feeds back on the discrimination of ^{13}C (Beerling 1997). The $\delta^{13}C$ of the new C, thus, interacts

with pCO_2 and the variation over time in $\delta^{13}C$ of new C may not be fully reflected by the $\delta^{13}C$ of harvested biomass.

A second problem is related to ^{13}C SSL, caused by a lack of CO_2 enrichment during winter in order to reduce the cost of fumigation. It was assumed that C assimilation and plant growth during winter (average daily mean temperature 1.6 °C) was slow, since the rates of photosynthesis were low and especially the response to elevated pCO_2 was weak below 5 °C (Long 1994). Consequently, for ambient swards that were shifted to elevated pCO_2, the input of new C started later than for those shifted *vice versa* (March 2000 instead of October 1999; Fig. 2.2). The calculation of the turnover rate was, therefore, based on four instead of five sampling dates. The lack of CO_2 enrichment during winter may be partially responsible for the increase in $\delta^{13}C$ under elevated pCO_2 at the last sampling in March 2001 at ETS = 3890 °C (Fig. 2.2). On this date, the fractions of old C were generally larger than at the previous sampling at ETS = 3410 °C (Fig. 2.3b), indicating that the biomass reached the new ^{13}C signal at ETS = 3410 °C already.

Few studies aimed at quantifying the turnover of stubble in permanent grassland (Bullock et al. 1994; Loiseau & Soussana 1999b; Schippers & Olff 2000). However, production of stubble is an important part of NPP and is sensitive to changes in available resources and environmental conditions. Standing stubble mass was around 400 to 500 g m^{-2} (Fig. 2.1) and was turned over two to three times per year (Tab. 2.1). As a consequence, the production of stubble accounted for 30 to 50% of NPP (Chapter 3).

The results of this study demonstrate that C isotopes are valuable tools for assessing the turnover rates of stubble. The methods were sensitive to changes in the availability of growth resources; however, more than three replicates are necessary to achieve significant differences. Furthermore, isotopic approaches are less time-consuming than other methods for measuring tissue turnover (Davies 1993). ^{14}C MPL in the field requires nothing but a labelling chamber.

However, legal restrictions may hinder the use of ^{14}C. ^{13}C SSL requires a stable ^{13}C signal in the atmosphere, a requirement met, in the field, only in CO_2 experiments.

The use of different methods to quantify NPP can yield significantly different estimates (Scurlock et al. 2002). Milchunas & Lauenroth (1992) found deviations of up to 300% in NPP of shortgrass steppe as estimated by different methods. Systematic experimental comparisons of methods for estimating NPP are scarce and for estimating stubble production, to the best of our knowledge, completely lacking. Isotopic methods should, therefore, be compared experimentally to other methods. Such comparisons may result in further progress in the estimation of NPP.

In conclusion, turnover rates of stubble were estimated using ^{14}C MPL and ^{13}C SSL with a discrepancy of around 10%, which may be due to variations in the $\delta^{13}C$ of the source of new C or a non-homogenous label of ^{14}C. Nevertheless, the good agreement of ^{14}C MPL and ^{13}C SSL indicates that the assumption of homogenous labelling after ^{14}C MPL was valid. The results of this investigation demonstrate that C isotopes are valuable tools for assessing turnover rates of stubble. However, a systematic experimental comparison of isotopic and other methods is necessary.

3

Turnover rates of residual plant biomass: a key to understanding the response of *Lolium perenne* swards to free-air CO_2 enrichment

3.1 Summary

The response of net primary production (NPP) and allocation of biomass to elevated pCO_2 and N supply are essential for understanding the function of grassland ecosystems in the global C cycle.

Turnover rates of residual biomass (stubble and roots) were measured in *Lolium perenne* swards at ambient and 60 Pa pCO_2 combined with 14 or 56 g N m^{-2} a^{-1} using ^{14}C multiple-pulse labelling.

Average turnover rates were 2.7 a^{-1} for stubble and 1.2 a^{-1} for roots. Elevated pCO_2 increased harvestable biomass by 9 and 21%, stubble production by 70 and 0% and residual leaf mass by 50% and 26% at low and high N, respectively. Turnover rates of roots were unaffected by pCO_2. Under elevated pCO_2, NPP was unaffected by N supply and would be underestimated by 20 to 40% if turnover rates of 1 a^{-1} were assumed for stubble and roots.

The results suggest that plants at limited N supply allocated biomass preferentially to stubble; they are discussed in relation to source-sink relations, optimum resource capture and N conservation in the plant.

3.2 Introduction

The burning of fossil fuels and large-scale changes in land use alter the global carbon (C) cycle and lead to a rising partial pressure of atmospheric carbon dioxide (pCO_2) since industrialisation. Terrestrial ecosystems affect atmospheric pCO_2 through feedback mechanisms in C assimilation by vegetation, the production of biomass and its subsequent decomposition by microorganisms. Enhanced growth globally may contribute significantly to the terrestrial C sink (Joos et al. 2002). Quantifying responses of net primary production (NPP) to elevated pCO_2 is, therefore, a prerequisite for estimating changes in the global C cycle and C inputs into the soil.

In grassland on fertile soil in the Swiss FACE (*F*ree-*A*ir CO_2 *E*nrichment) array, elevated pCO_2 stimulated rates of C uptake in leaves of *Lolium perenne* L. (perennial ryegrass) by about 38%, a phenomenon that was persistent for ten years (Ainsworth et al. 2003). Stimulated C uptake at elevated pCO_2 did not dependent on nitrogen (N) supply. However, the response of harvestable biomass above a cutting height of 0.05 m (agricultural yield) to elevated pCO_2 was only -11 to 8% at low N and 8 to 25% at high N supply (Daepp et al. 2000). Especially at low N, harvestable biomass does not seem to be a good indicator of the plant's response to elevated pCO_2. This is not surprising; in grassland on fertile soil, only 30 to 50% of the standing biomass are harvested depending on N supply and pCO_2 (Daepp et al. 2001). It is essential to account for the non-harvested stubble and roots, thereafter called residual plant biomass. Nevertheless, many predictions of plant responses to elevated pCO_2 were based on measurements of harvested biomass only.

It has been suggested that temperate grasslands have a potential to sequester C, because they cover the soil permanently, are not tilled and their soils usually have a high content of organic matter (van Ginkel et al. 1999). In these ecosystems, a prerequisite for the survival of plants is regrowth after cutting or

grazing. After defoliation, grasses expand leaves from stubble and produce new tillers with the associated adventive roots. Tillers which grow above defoliation height are partly harvested, but many tillers start to senesce before they reach this height (Suter et al. 2001). The production of stubble and the generation of new roots are not accounted for when only standing biomass at a specific harvest is monitored. In order to quantify NPP, the production of residual biomass must be determined. Production of residual biomass can be estimated from the average standing mass (the pool size) multiplied by its turnover rate. Many estimates do not include the turnover of biomass and considerably underestimate NPP (Scurlock et al. 2002).

Elevated pCO_2 primarily increases the availability of C to the plant and to the whole ecosystem relative to light, water and nutrients. As a consequence, the allocation of C and N in the plant changes. Elevated pCO_2 increased the storage of carbohydrates in stubble due to an N-limited sink activity in growing shoots (Fischer et al. 1997). Several authors reported that elevated pCO_2 increased the allocation of photosynthates to roots (Gorissen 1996; Cotrufo & Gorissen 1997; Suter et al. 2002) and affected tiller dynamics during regrowth (Suter et al. 2001). Such processes probably affect turnover rates and NPP under elevated pCO_2. Until now, however, very few investigations have focused on turnover rates of residual biomass in permanent grassland in response to elevated pCO_2, and the results are contradictory (Fitter et al. 1997; Loiseau & Soussana 1999b; Arnone et al. 2000). The effects of pCO_2 on turnover rates of residual biomass seem to be influenced by environmental factors and to depend on the availability of resources.

The investigation quantified the effects of elevated pCO_2 and N supply on NPP in swards of *L. perenne* and on the allocation of biomass to harvestable biomass, stubble and roots. The aim was to understand the discrepancy between a strong increase in C assimilation at elevated pCO_2 and the weak response of

harvestable biomass. Using a ^{14}C multiple-pulse labelling technique, I simultaneously measured, for the first time, turnover rates of stubble and roots under FACE conditions.

3.3 Material and Methods

3.3.1 *Experimental setup*

A field experiment with *L. perenne*, the main grass species in managed grasslands on fertile soil in temperate zones, was conducted on an eutric cambisol (Lüscher et al. 1998) in the Swiss FACE array (Hebeisen et al. 1997) at Eschikon near Zurich, 550 m above sea level. The experimental field consisted of three blocks, each containing one fumigated circular area, 18 m in diameter, and a control area of the same size, which was at least 100 m apart to prevent an increase of pCO_2. The partial pressures of CO_2 were ~36 Pa at ambient and 60 Pa at elevated pCO_2, achieved by using the FACE technique (Lewin et al. 1992). The FACE technique did not require an enclosure, so the microclimate of the experimental area was not altered. CO_2 enrichment took place from dawn to dusk during the entire growing season (March to November) when air temperature was above 5 °C.

Residual biomass was assessed by sequential destructive harvests. For this purpose, PVC tubes, 0.2 m in diameter and 0.6 m in depth, were filled with soil and inserted into the field, tightly put together to form a closed sward. The soil was taken from the top 0.2 m of permanent swards of *L. perenne*. Per tube, six seedlings of *L. perenne* were planted and fertilised with 5.5 g P m^{-2} a^{-1} and 24.1 g K m^{-2} a^{-1}, assumed to be non-limiting for plant growth. Monthly average air temperatures and monthly sums of rainfall during the period of measurement are presented in Table 3.1a.

The tubes in each experimental area were divided into two groups of ten; each group received either 14 or 56 g N m^{-2} a^{-1}. The fertiliser was split into six portions according to the expected biomass production of the plants. The fertiliser was applied as follows: 15% at the beginning and 15% in the middle of the first period of regrowth and 20, 20, 15 and 15% at the beginning of each subsequent period of regrowth.

Table 3.1 a,b: (a) Monthly average air temperatures and monthly sums of precipitation during the measurement period at the experimental site in Eschikon, Switzerland (8°41′E, 47°27′N) and (b) cumulative effective temperature sums (ETS) above a threshold of 0 °C and sums of precipitation for the periods between the destructive harvests.

(a) Monthly data

Year	Month	Temp [℃]	Precip [mm]
1999	November	1.3	108
	December	0.9	149
2000	January	-0.7	31
	February	3.5	137
	March	5.2	93
	April	9.3	56
	May	14.4	93
	June	17.3	104
	July	16.2	215
	August	18.1	111
	September	14.1	127
	October	9.4	66
	November	4.8	76
	December	2.8	39
2001	January	0.7	103
	February	2.8	43
	March	6.0	237

(b) Destructive harvests

Number	Date [dd.mm.yy]	ETS [℃]	Precip [mm]
1	02.11.99	2048	428
2	14.03.00	323	453
3	27.06.00	1262	318
4	28.10.00	1800	508
5	15.03.01	470	418

3.3.2 *Data collection and measurements*

The measurements were carried out from November 2, 1999 to March 15, 2001. Harvestable biomass was determined five times in 2000. Stubble (shoot material below defoliation height) and roots were sampled at five destructive harvests. Dates are given in Table 3.1b.

Stubble was cut at the soil surface, washed and separated into green stubble (mainly pseudo-stems), residual leaf lamina, necrotic material and short pieces of roots that were found close to the tiller base. All plant material was dried at 65 °C for 48 h. The destructively harvested tubes were replaced immediately by pre-established swards to form a continuously closed canopy. The soil was sampled to a depth of 15 cm, where 90 to 95% of the total root mass is found (Hebeisen 1997). Roots were sampled by wet sieving the soil at 2 mm and 250 µm. Roots were separated manually from litter, stubble and soil macro-biota. Organic and mineral fractions were separated by flotation in H_2O and dried immediately.

3.3.3 *^{14}C multiple-pulse labelling and analysis*

^{14}C enabled us to distinguish between old biomass and new photosynthates and, thus, to calculate turnover rates. The goal of multiple-pulse labelling was the homogenous labelling of plant biomass. The swards of *L. perenne* were labelled by three pulses of ^{14}C at four-week intervals from August to October 1999. A 0.4-m^3 Plexiglas chamber was placed over the tubes and 74 MBq m^{-2} of $^{14}CO_2$ per pulse were released into the chamber and assimilated by the plants. Two weeks after each labelling, the biomass was removed above cutting height.

^{14}C in aboveground biomass was analysed according to Suter et al. (2002). Briefly, a sample of 20 mg was digested by 4 mg cellulase (from *Trichoderma viride*) and 4 mg maceroenzyme (from *Rhizopus sp.*) in 200 µl phosphate buffer (pH=6) for 24 h at 45 °C and by 1 ml of Soluene-350 (Tissue solubiliser, Packard

Instrument Company) for another 24 h at 45 °C. Thereafter, 15 ml of Hionic-Fluor (Packard Instrument Company) were added and ^{14}C was analysed by liquid scintillation counting (Packard 2500TR, Packard Instrument Company). Internal standards with a known amount of ^{14}C were used to correct for quenching and unlabelled material was used to determine the background of ^{14}C.

Samples of 100 mg roots (>2000 µm) and 50 mg fine roots (250 to 2000 µm) were oxidised in a Harvey Biological Sample Oxidiser OX 400 (R.J. Harvey, Hillsdale, NJ, USA) at 900 °C for 4 min. Emitted pCO_2 was trapped in 15 ml Oxosol scintillation solution (National Diagnostics, Atlanta, GA, USA). Decays per minute were counted by a Wallac 1414 Win Spectral Liquid Scintillation Counter (Wallac Oy, Turku, Finland) for 10 min. Unlabelled material was used to determine the background of ^{14}C.

3.3.4 *Calculations*

For each harvest, the average bulk density of soil was used to calculate the biomass of roots per m^2.

It was assumed that roots and stubble were two homogenously labelled pools of biomass. The homogeneity of labelling was ensured by multiple labelling during establishment and approved by the consistency with ^{13}C steady-state labelling (Chapter 2). Furthermore, it was assumed that the translocation of label from stubble to harvestable biomass or roots was negligible after the start of measurements. This assumption was valid, because ^{14}C in harvestable biomass of the first subsequent regrowth in spring contained only 1.4% of the initial label in stubble.

The change in total ^{14}C activity (A_{14C}) over time was

$$dA_{14C} / dt = -k \cdot A_0 \qquad [3.1]$$

where A_0 is the total ^{14}C activity (Bq m^{-2}) at t=0 and k is a constant. Based on cumulative effective temperature sum (ETS: sum of the daily mean temperatures exceeding a threshold temperature of 0 °C) as the time variable t:

$$A_{14C} = A_0 \cdot \exp(-k \cdot ETS) \quad [3.2]$$

A constant pool size over time is a prerequisite for the use of specific activity. Since this was not the case, the total ^{14}C activity was used for the calculation. The decay model was fitted to $\log_e$-transformed data in the form

$$\log_e A_{14C} = \log_e A_0 - k \cdot ETS \quad [3.3]$$

using the REG procedure in SAS (SAS Institute 1999) for each combination of factors and block (Fig. 3.1). Annual turnover rates (TR [a^{-1}]) during the period of measurement were calculated using aETS (the ETS in 2000 of 3435 °C):

$$TR = k \cdot aETS \quad [3.4]$$

Annual production (P) of stubble and roots was calculated as

$$P = B \cdot TR \quad [3.5]$$

where B is the average standing biomass of stubble and roots (pool size).

3.3.5 *Statistical analyses*

The experimental design was a split-plot with three blocks (Gomez & Gomez 1984). pCO_2 was the main-plot factor and N fertilisation the sub-plot factor. pCO_2 was tested against the interaction pCO_2×block. Since pCO_2×block has only two degrees of freedom, the *F*-test has low power and effects of pCO_2 are more readily detected as interactions with the sub-plot factor.

Biomass data were analysed using the GLM procedure in SAS (SAS Institute 1999). Differences in turnover rates were analysed as differences in the slopes of the $\log_e$-transformed ^{14}C activity against ETS using the MIXED procedure in SAS. ETS was used as the covariate. A significant interaction between ETS and a

factor indicated a significant effect of the factor on turnover rates. In order to account for the split-plot design, pCO_2 and pCO_2×block were stated as RANDOM (Milliken & Johnson 2002). The method of Kenward-Rogers was used to receive the correct denominator degrees of freedom (Littell et al. 1996).

3.4 Results

3.4.1 *Size of biomass pools*

High N resulted in a 60% stimulation of annual harvestable biomass of *Lolium perenne* compared to low N (anova: N, $P<0.0001$; Tab. 3.2). The response of harvestable biomass to elevated pCO_2 was 9% at low N and 21% at high N (anova: pCO_2, $P<0.1$; pCO_2×N, $P<0.05$).

Elevated pCO_2 increased the mass of residual leaf lamina below cutting height by 50% at low and 26% at high N (anova: pCO_2, $P<0.01$; Tab. 3.2); the mass of residual leaf lamina was larger at low than at high N (anova: N, $P<0.001$). The mass of green and necrotic stubble was larger at elevated than at ambient pCO_2; the total mass of stubble was, on average, 21% larger at elevated than at ambient pCO_2 (anova: pCO_2, $P<0.1$; pCO_2×N, ns).

Elevated pCO_2 significantly stimulated the pool of coarse roots by 37% on average (anova: pCO_2, $P<0.05$), independent of N fertilisation (anova: pCO_2×N, ns). The mass of coarse roots was also higher at low N than at high N (anova: N, $P<0.05$). The mass of fine roots was unaffected by elevated pCO_2. The total pool of roots, however, increased by 14% at elevated pCO_2 (anova: pCO_2, $P<0.1$). Hence, elevated pCO_2 increased the total pool of residual biomass by 21% at low N and by 11% at high N (anova: pCO_2, $P<0.05$; pCO_2×N, ns).

Table 3.2: Annual harvestable biomass (DM) and average size of the above and belowground residual plant biomass pools as green stubble, residual leaves (below 0.05 m cutting height), necrotic stubble and coarse and fine roots of *Lolium perenne* swards as affected by atmospheric pCO_2 and N fertilisation.

N	pCO_2	harvestable biomass	residual biomass [g DM m^{-2}]							
			stubble				roots			
[g m^{-2} a^{-1}]	[Pa]	[g m^{-2} a^{-1}]	green	necrotic	residual leaf lamina	**sum**	coarse	fine	**sum**	total
14	36	952.9	136.9	224.4	49.0	**410.4**	159.2	376.6	**536.6**	949.7
	60	1041.4	189.1	259.8	73.5	**522.4**	228.9	399.9	**628.3**	1152.3
56	36	1456.0	189.4	220.1	39.5	**449.0**	148.0	385.3	**533.2**	1000.4
	60	1765.0	199.5	264.2	50.0	**513.7**	193.6	392.7	**591.6**	1107.7
ANOVA:	pCO_2	0.06	ns	ns	0.003	0.07	0.03	ns	0.09	0.04
	N	0.0001	0.002	ns	0.0004	ns	0.03	ns	ns	ns
	pCO_2×N	0.03	0.04	ns	ns	ns	ns	ns	ns	ns
n		*6*	*30*	*30*	*30*	*30*	*30*	*30*	*30*	*30*
SE		*46.3*	*10.3*	*12.2*	*4.5*	*16.9*	*10.2*	*15.3*	*20.0*	*29.9*

P-values of analysis of variance (ANOVA), number of measurements (*n*) and standard errors of the mean (*SE*) are shown.

3.4.2 *Turnover rates of stubble and roots*

Turnover rates were estimated on the basis of the decay of total ^{14}C activity in the biomass of stubble and roots (Fig. 3.1). Turnover rates for stubble were about three per year (Tab. 3.3). Under elevated pCO_2, the turnover rate of stubble was 33% higher at low N and 12% lower at high N compared to ambient pCO_2 (ancova: $pCO_2 \times N$, $P<0.05$). Turnover rates for roots were less than half those of stubble (1 to 1.5 times per year). Neither N supply nor pCO_2 significantly altered the turnover rates of roots.

Table 3.3: Turnover rates of stubble and roots of *Lolium perenne* swards as affected by atmospheric pCO_2 and N fertilisation.

		turnover rates			
N	pCO_2	stubble		roots	
[g N m^{-2} a^{-1}]	[Pa]	[a^{-1}]	r^2	[a^{-1}]	r^2
14	36	2.57	0.84	1.24	0.69
	60	3.43	0.82	1.18	0.71
56	36	2.59	0.87	1.21	0.59
	60	2.27	0.77	1.08	0.52
ANCOVA:	pCO_2	ns		ns	
	N	0.03		ns	
	$pCO_2 \times N$	0.04		ns	
n		*30*		*30*	
SE		*0.30*		*0.21*	

Data was calculated from single-pool decay models fitted to ^{14}C activities over cumulative effective temperature sum. Coefficients of determination (r^2) of the regressions, *P*-values of analysis of covariance (ANCOVA), number of measurements (*n*) and average standard errors (*SE*) of the parameter estimate are shown. All regressions were significant at $P<0.001$.

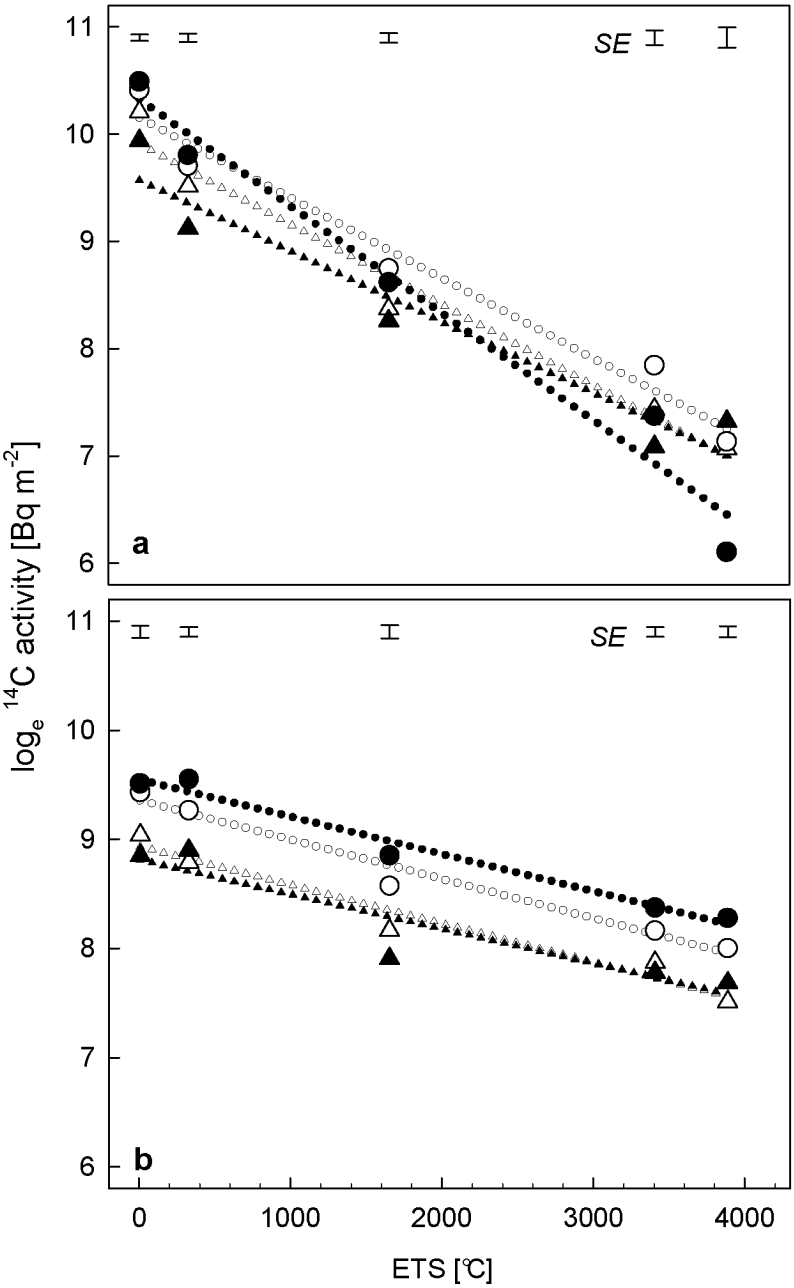

Figure 3.1: Decay of total ^{14}C activity in stubble (a) and roots (b) of *Lolium perenne* swards over cumulative effective temperature sum (ETS) above a threshold temperature of 0 °C as affected by atmospheric pCO_2 (○△: 36 Pa pCO_2, ●▲: 60 Pa pCO_2) and N fertilisation (○●: 14 g N m^{-2} a^{-1}, △▲: 56 g N m^{-2} a^{-1}).

Big symbols show means of 6 replicates; small symbols represent fitted regression lines. Error bars are standard errors of the means. Details about the dates of measurement are given in Tab. 3.1; slopes of the regressions and their coefficients of determination are shown in Tab. 3.3.

3.4.3 *Estimation of net primary production*

Pool size (Tab. 3.2) and turnover rates of residual biomass (Tab. 3.3) were used to estimate the production of residual biomass per year (Fig. 3.2). Elevated pCO_2 increased the annual production of stubble by 70% at low N and by 0% at high N (anova: pCO_2, $P<0.05$; $pCO_2 \times N$, $P<0.0001$).

The annual production of roots was also higher at low N (anova: N, $P<0.1$), but there was no significant effect of elevated pCO_2 on the production of roots. Elevated pCO_2 stimulated the annual production of total residual biomass by 47% at low N but not at high N.

Elevated pCO_2 stimulated NPP by 34% at low N and 9% at high N. At ambient pCO_2, high N resulted in a 22% higher NPP, while at elevated pCO_2 there was no effect of N supply. The harvested biomass was less than half of NPP and was strongly affected by the treatments. At low N supply, the harvest index (proportion of harvestable biomass to NPP) was 36% under ambient pCO_2 and 29% under elevated pCO_2 (Fig. 3.2). At high N, the harvest index was 45% under ambient and 49% under elevated pCO_2.

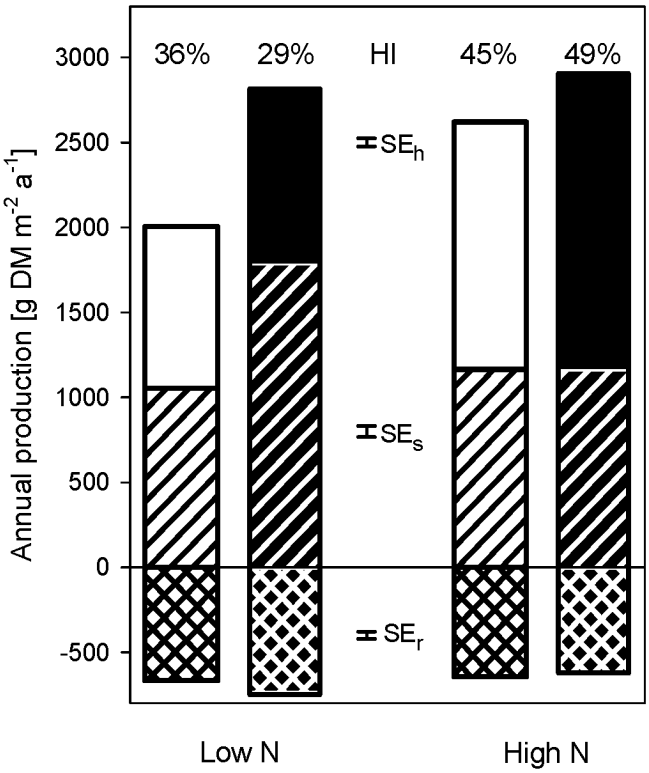

Figure 3.2: Net primary production (NPP) in *Lolium perenne* swards as affected by atmospheric pCO_2 and N fertilisation. NPP is shown as composed of the annual production of harvestable biomass, stubble and roots.
White bars represent 36 Pa pCO_2, black bars 60 Pa pCO_2. Non-hatched bars represent harvestable biomass, hatched bars stubble and chequered bars roots. HI is the harvest index (proportion of harvestable biomass to NPP); SE_h, SE_s and SE_r are standard errors of the mean for harvestable biomass, stubble and roots, respectively.

3.5 Discussion

3.5.1 *The quantification of NPP requires the assessment of the production of harvestable biomass, stubble and roots*

The most important advantage of the experimental approach was the quantification not only of pool size, but also of turnover rates of biomass below cutting height in a permanent grassland ecosystem under field conditions. The allocation of biomass to harvestable and residual biomass showed that

harvestable biomass was not a good indicator of the plant's response to elevated pCO_2. First, harvestable biomass was only 29 to 49% of NPP (Fig. 3.2). Second, the availability of C and N had different effects on harvestable biomass, stubble and roots (Tab. 3.2). The quantification of turnover rates was a prerequisite for revealing these differences. Measuring the standing biomass alone, i.e., assuming a turnover rate of 1 a^{-1} for residual plant biomass would have resulted in an underestimation of NPP by 20 to 40% (*cf.* Tab. 3.2 and Fig. 3.2). Scurlock et al. (2002) showed that estimates of NPP are two to five times higher when the turnover of biomass is considered. Estimates of turnover rates of residual plant parts, especially stubble, are scarce (Thornley 1998). Most studies did not examine effects of growth conditions or involve management. The few published studies on turnover rates, as affected by elevated pCO_2 in permanent managed grasslands, focused on roots (Fitter et al. 1996; Fitter et al. 1997; Arnone et al. 2000) or treated roots and stubble as one fraction (Loiseau & Soussana 1999b). However, elevated pCO_2 had a stronger effect on turnover rates of stubble than of roots (Fig. 3.1). Since defoliation frequency increases the turnover of roots (Whitehead 1995), management of the sward may be important in explaining some of the differences between the grassland ecosystems studied so far.

Turnover rates of stubble were twice as high as those of roots (Fig. 3.1; Tab. 3.3). These results are comparable to other investigations on *L. perenne*, suggesting average turnover rates of about 1.8 a^{-1} for tillers (Bullock et al. 1994) and of about 1 a^{-1} for roots (Troughton 1981). To the best of our knowledge, this is the first experiment in which turnover rates of roots and stubble, in response to altered availability of resources, were directly compared.

The different turnover rates suggest that roots and stubble, although connected, function differently. The production of stubble is determined primarily by seasonal variations, the generative and vegetative development, management

and weather. In reproductive growth the shoot apex is lifted above the foliage and stubble of these tillers senesce after defoliation; thus, the proportion of reproductive tillers has a strong effect on the turnover of tillers (Fulkerson & Donaghy 2001). Shading of tillers during regrowth regulates the density of tillers and causes senescence of small tillers (Suter et al. 2001). The mass of stubble is also influenced by soluble carbohydrates, which accumulate at the base of the tiller if the supply of photosynthates exceeds the demand by growth. Carbohydrates are mobilised in the first few days after defoliation and stocks are replenished as the leaf area is reinstalled (Fischer et al. 1997).

While each tiller basically supports its own root system, root growth responds to seasonal fluctuations in temperature, precipitation and available nutrients (Matthew et al., 1991). The different factors that influence the growth of roots and tillers may partly account for the observed differences in turnover. Furthermore, the content of carbohydrates in roots of *L. perenne* is three times smaller than in stubble emphasising the primary importance of stubble in the storage of carbohydrates (Prud'homme et al., 1992).

3.5.2 *Contrasting responses of roots and stubble to changed availability of resources*

Elevated pCO_2 enhanced leaf photosynthesis by about 38% (Ainsworth et al. 2003) and, thus, increased the availability of C skeletons in source leaves and the demand for N. Under such conditions, allocation theory suggests that plants at low N invest most of their photosynthates into roots in order to maximise the capture of this most limiting resource (Chapin et al. 2002). In contrast, a strong stimulation of the production of stubble at low N was observed, which might be due to (i) stimulated tillering, (ii) increased tiller size, or (iii) increased storage of carbohydrates. Suter et al. (2001) showed that tillers shorter than 5 cm were increasingly produced in the stubble layer under elevated pCO_2 at the beginning

of regrowth and were reduced towards the end. Concentrations of carbohydrates in stubble increased by about 35% at elevated pCO_2 and low N supply (Fischer et al. 1997). Fischer et al. (1997) suggested that sink activity in expanding leaves was limited by available N in the soil and, thus, more carbohydrates were stored at elevated pCO_2, primarily in stubble. Hence, the allocation of biomass in response to elevated pCO_2 and N supply is driven by source-sink relations rather than by the maximised capture of the most limiting resource. Our data supports the hypothesis of Fulkerson & Donaghy (2001) that restoration of photosynthetic capacity and spreading tillers have a higher priority for the plant than the production of roots.

Changes in the structure of the sward are also indicated by the 50% increase in the mass of leaf lamina remaining below cutting height at low N in response to pCO_2 (Tab. 3.2). A greater mass of residual leaf lamina may be beneficial for canopy photosynthesis and regrowth after defoliation. Schnyder & de Visser (1999) found that *L. perenne* plants preferentially used new photosynthates for growth; reserve-derived C was used to a much smaller extent than current photosynthates for the development of foliage after defoliation. This corroborated our assumption that an important translocation of label from stubble to shoots or roots did not occur during regrowth, which would have led to an overestimation of turnover rates and NPP by ^{14}C multiple-pulse labelling.

3.5.3 *Elevated pCO_2 altered N cycling in the plant and NPP of the sward.*

The effect of elevated pCO_2 on the photosynthesis of *L. perenne* did not depend on the amounts of N available to the plants (Ainsworth et al. 2003), and the content of total leaf protein was significantly reduced (Isopp et al. 2000). These results indicate that smaller amounts of enzymes were required in the photosynthetic apparatus and elevated pCO_2 allowed a higher N use efficiency in assimilation (Drake et al. 1997). Our experiment showed that the proportions

of harvested and residual biomass were strongly affected by pCO_2 and N supply (Fig. 3.2). At both levels of N, the concentration of N in harvestable biomass was reduced by 18% under elevated pCO_2 (data not presented, *cf.* Daepp et al. 2000). At high N, the export of N from the system by harvest did not change as a result of greater amounts of harvestable biomass under elevated pCO_2. In contrast, at low N, less N was exported in harvestable biomass under elevated pCO_2 and consequently more N may be available to the low-N plants. Stubble is the main location for the storage of N and remobilised reserve-derived amino acids may be as important for regrowth as are carbohydrates (Ourry et al. 1990; Schnyder & de Visser 1999). Thus, increased storage and conservation of N in stubble may support regrowth and the production of tillers, resulting in a strong response of NPP to elevated pCO_2 at low N (Fig. 3.2).

3.6 Conclusions

Quantifying NPP by measuring turnover rates of residual biomass has revealed distinct differences in the production of harvestable biomass, stubble and roots in response to a changed availability of resources, as induced by elevated pCO_2 and N supply. Accounting for turnover rates was, thus, essential for estimating responses of NPP to elevated pCO_2 and N supply. The results suggest a preferential allocation of biomass to stubble when N supply is limited. Elevated pCO_2 increased residual leaf lamina and may promote regrowth after defoliation and the production of tillers. Hence, in N-limited ecosystems at elevated pCO_2, the greater production of residual biomass linked the strong stimulation of photosynthesis and the weak response of harvestable biomass.

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4

Ten years of free-air CO_2 enrichment altered the mobilisation of N from soil in *Lolium perenne* swards

4.1 Abstract

Effects of *F*ree-*A*ir *C*arbon dioxide *E*nrichment (FACE, 60 Pa pCO_2) on plant growth as compared with ambient pCO_2 (36 Pa) were studied in swards of *Lolium perenne* L. (perennial ryegrass) at two levels of N fertilisation (14 and 56 g m^{-2} a^{-1}) from 1993 to 2002. The objectives were to determine how plant growth responded to the availability of C and N in the long term and how the two sources of N in the soil, soil organic matter (SOM) and mineral fertiliser, varied over time. In three field experiments, ^{15}N-labelled fertiliser was used to distinguish the sources of available N.

In 1993, harvestable biomass under elevated pCO_2 was 7% higher than under ambient pCO_2. This relative pCO_2 response increased to 32% in 2002 at high N, but remained low at low N. Between 1993 and 2002, the proportions and amounts of N in harvestable biomass derived from SOM (excluding remobilised fertiliser) were, at high N, increasingly higher at elevated pCO_2 than at ambient pCO_2. Two factorial experiments confirmed that at high N, but not at low N, a higher proportion of N in harvestable biomass was derived from soil (including remobilised fertiliser) following seven and nine years of elevated pCO_2, when compared with ambient pCO_2.

It is suggested that N availability in the soil initially limited the pCO_2 response of harvestable biomass. At high N, limitation of plant growth decreased over time as a result of the stimulated mobilisation of N from soil, especially from SOM. Consequently, harvestable biomass increasingly responded to elevated pCO_2. The underlying mechanisms which contributed to the increased mobilisation of N from SOM under elevated pCO_2 are discussed. This study demonstrated that there are feedback mechanisms in the soil which are only revealed during long-term field experiments. Such investigations are thus, a prerequisite for understanding the responses of ecosystems to elevated pCO_2 and N supply.

4.2 Introduction

Carbon (C) is increasingly available for plant photosynthesis through the rising partial pressure of carbon dioxide (pCO_2) in the atmosphere. The alteration in C availability in relation to other resources affects plant growth and nutrient dynamics in terrestrial ecosystems. Under elevated pCO_2, plants use N more efficiently, potentially leaving more N available for growth (Drake et al. 1997).

Elevated pCO_2 has been shown to increase the allocation of biomass below ground (Rogers et al. 1996), which may lead to an enhanced C input into the soil (van Ginkel et al. 2000). Under elevated pCO_2, this C input changes with regards to quantity and composition (Arp et al. 1997). Since the cycles of C and N in the soil are closely linked, altered C inputs will change N dynamics in the soil, which, in turn, will feed back on plant growth through the availability of N. There are two main hypotheses with regards to the short-term outcome of these feedback mechanisms. Zak et al. (1993) suggested that greater C inputs at elevated pCO_2 may stimulate the activity of soil microbes, as well as turnover rates of soil organic matter (SOM) and may increase the availability of N. Alternatively, greater C inputs may reduce the availability of N for plant growth

through the sequestration of N by the increased microbial biomass (Diaz et al. 1993). The amount of N available to the plants depends on the C/N ratios of the biomass input and SOM, as well as on the N supply to the system. Plants can respond quickly to elevated pCO_2, for example, with changes in the rate of photosynthesis within hours, or in the allocation of biomass within days to weeks. In contrast, processes in the soil, such as the decomposition and mineralisation of SOM, operate over longer time periods. Ecosystem responses may, therefore, be dynamic over years.

Since temperate grasslands cover large areas of land and have a capacity to store C (Scurlock & Hall 1998), they are of special interest with regard to rising pCO_2. The Swiss *F*ree-*A*ir CO_2 *E*nrichment (FACE) experiment was established in 1993 and is the longest-running experiment on managed grassland exposed to elevated pCO_2. It provides not only a unique ten-year dataset for studying the long-term effects of elevated pCO_2 on plant growth and N turnover, but also contains soils exposed to elevated pCO_2 for several years. At this site, elevated pCO_2 increased the production of harvestable biomass of *Lolium perenne* L. (perennial ryegrass) by only 7% in the first year (Hebeisen et al. 1997). An analysis of data over the first six years of the experiment showed that the relative pCO_2 response (herein defined as the change at elevated pCO_2 relative to ambient pCO_2) of harvestable biomass production increased with time at high N supply (Daepp et al. 2000). Daepp et al. (2000) suggested that this was due to an increasing N availability in the system. In addition, Daepp et al. (2001) showed that the pCO_2 response of harvestable biomass was a function of N fertilisation and, thus, of available N in the soil. However, it remains uncertain whether this higher N availability was an effect of long-term CO_2 enrichment, of long-term high N supply or a combination of the two.

The objective of this paper is to report to what degree pCO_2 and N fertilisation affected plant growth and the sources of available N in the soil in the long term.

In this system, the N available to plants was considered to be mainly derived either from the decomposition of SOM or from mineral fertiliser. CO_2 enrichment and mineral N fertilisation were considered not to affect atmospheric N deposition, and non-symbiotic N_2 fixation was assumed to be negligible. The two main sources were distinguished using ^{15}N-labelled fertiliser. In *experiment 1*, the effects of elevated pCO_2 and of N supply on the proportions of N derived from SOM ($\%N_{som}$; excluding remobilised fertiliser) in swards of *L. perenne* over a ten-year period were examined. As a result of the long-term ^{15}N labelling, however, effects of the current levels of pCO_2 and N supply on plant growth were confounded with carry-over effects of pCO_2 and N supply from previous years. To overcome these limitations, a factorial experiment (*experiment 2*) was conducted to determine the effects of previous and current pCO_2 on the proportion of N derived from soil ($\%N_s$; including remobilised fertiliser N), by using soils that had been exposed to elevated or ambient pCO_2 for seven years. A second factorial experiment (*experiment 3*) determined the effects of the previous rates of N supply on $\%N_s$ by using soils that had been fertilised at high or low N for nine years. These factorial experiments provided direct evidence on the outcome of feedback processes in the soil affecting N mobilisation in the long term.

4.3 Material and Methods

4.3.1 *Experimental site and CO_2 fumigation*

The three field experiments were conducted on an eutric cambisol (Lüscher et al. 1998) in the Swiss *F*ree-*A*ir CO_2 *E*nrichment (FACE) array (Hebeisen et al. 1997) at Eschikon near Zurich, 550 m above sea level. The partial pressures of CO_2 were ~36 and 60 Pa. Since the FACE technique does not require an enclosure, the microclimate of the experimental area was not altered (Hendrey 1992). The experiment consisted of three blocks, each containing one fumigated circular

area (18 m diameter) and a control area of the same size, which were at least 100 m apart to prevent an increase of pCO_2. The period of CO_2 enrichment covered the entire growing season (March to October) during daytime when air temperatures were above 5 °C. Plant growth is slow below 5 °C and the effects of elevated pCO_2 are weak (Long 1994).

Each year, 5.5 g P m^{-2} and 24.1 g K m^{-2} were applied to all plots. These amounts were considered non-limiting for plant growth. Average monthly temperature and monthly rainfall during the growing season are shown in Tables 4.1a and 1b, respectively.

4.3.2 *Experiment 1: effects of pCO_2 and N supply over ten years*

Swards of *L. perenne cv.* Bastion were established in monoculture in mid-August 1992. The swards were exposed to two N treatments: low N (10 g N m^{-2} in 1993 and 14 g N m^{-2} a^{-1} from 1994 to 2002) and high N (40 g N m^{-2} in 1993 and 56 g N m^{-2} a^{-1} from 1994 to 2002). The fertiliser was applied at the beginning of each period of regrowth in portions of 30, 20, 20, 15 and 15% of the total annual amount. A fixed sampling area (1 m^2) in each plot was fertilised with ^{15}N-enriched NH_4NO_3 (ammonium and nitrate equally enriched, diluted in H_2O) at each fertilisation. In 1993 and 1994, the atom%-^{15}N excess of the fertiliser was 0.4% in the high and 1.6% in the low N treatment. From 1995 to 2002, the ^{15}N excess was 0.3% at high and 1.1% at low N. The remaining area of the plot was fertilised with equal amounts of unlabelled NH_4NO_3 (see Zanetti et al. 1996).

Biomass above 0.05 m cutting height was harvested from the labelled area. Biomass from a central area (0.25 m^2) in the plot was dried at 60 °C for 48 h and the harvested dry mass per m^2 was calculated. From 1993 to 1995, the swards were cut at two frequencies: frequent (six times in 1993 and eight times in 1994 and 1995) and infrequent (four times from 1993 to 1995) to examine effects of management (Hebeisen et al. 1997). The infrequently cut swards were resown in

1996 because of deterioration. From 1996 to 2002 all swards were cut five times per year, resulting in four treatments (2 N levels × 2 pCO_2 levels) with two replicates in three blocks.

Table 4.1 a,b: (a) Monthly averages of air temperature and their means during the main growing season and (b) monthly sums of precipitation and their sums during the main growing season in the FACE experiment at Eschikon, Switzerland (8°41′E, 47°27′N).

(a)	Temperature [℃]								
Year	March	April	May	June	July	Aug	Sept	Oct	Average
1993	3.8	10.0	14.0	15.8	16.0	16.7	12.2	6.9	11.9
1994	8.4	6.6	12.5	16.5	20.8	18.4	12.7	7.9	13.0
1995	3.8	9.3	14.0	14.2	20.1	17.1	11.4	12.1	12.7
1996	1.5	8.0	11.6	16.6	16.5	16.3	9.9	8.6	11.1
1997	6.9	7.0	12.9	15.1	16.3	19.0	14.4	8.3	11.5
1998	4.5	7.7	13.8	16.4	18.6	18.2	13.3	9.7	12.8
1999	5.9	8.5	14.5	15.3	18.5	17.3	15.7	8.5	13.0
2000	5.2	9.3	14.4	17.3	16.2	18.1	14.1	9.4	13.0
2001	6.0	6.6	15.0	14.5	17.9	18.3	10.8	12.3	12.7
2002	6.3	8.1	12.4	18.4	17.5	16.8	12.1	9.0	11.8
(b)	Precipitation [mm]								
Year	March	April	May	June	July	Aug	Sept	Oct	Sum
1993	46	71	82	121	262	105	91	112	889
1994	72	115	233	82	62	147	107	67	884
1995	118	111	182	171	72	165	91	7	917
1996	38	58	133	74	142	138	54	90	728
1997	21	80	63	150	154	69	32	68	637
1998	62	90	57	140	74	69	172	131	795
1999	62	109	238	154	103	110	115	48	939
2000	93	56	93	104	215	111	127	66	865
2001	237	169	71	190	100	132	196	97	1191
2002	54	77	161	87	102	159	160	136	937

4.3.3 *Experiment 2: effects of previous long-term pCO_2*

The effects of previous long-term pCO_2 ($prevCO_2$) were distinguished from current pCO_2 ($curCO_2$) by testing the response of *L. perenne* to $curCO_2$ on soils that had been under elevated or ambient pCO_2 for seven years. The plants were

exposed to the same N treatments as in *experiment 1* (low N: 14 g N m^{-2} a^{-1} and high N: 56 g N m^{-2} a^{-1}), resulting in a factorial experiment with eight treatments (2 prevCO_2 × 2 curCO_2 × 2 N) in three blocks. To achieve this, topsoil (0-20 cm) was collected from both pCO_2 treatments in May 1999 and filled into PVC tubes, 0.2 m in diameter and 0.6 m in depth. Previously, swards of *L. perenne* had been grown at the site and treated like the high-N swards of *experiment 1*. Six seedlings of *L. perenne cv.* Bastion with two or three leaves were transplanted from the glasshouse into each tube. The tubes were placed side by side in their initial respective pCO_2 treatment and inserted into the soil to form a closed sward. P and K were supplied as in *experiment 1*. In March 2000, the tubes in each experimental area and N treatment were divided into two sets of five tubes. One set was left in its respective pCO_2 treatment and the other was exchanged between the pCO_2 treatments.

Between June and October 1999, the swards were cut three times and 20, 15 and 15% of the annual rate of N fertiliser were applied as unlabelled NH_4NO_3 at the beginning of the three regrowth periods. In 2000, harvestable biomass was sampled five times and the swards were fertilised with ^{15}N-enriched NH_4NO_3 (atom%-^{15}N excess 0.3% at high and 1.1% at low N). Labelled fertiliser was applied at 15% of the annual amount at the beginning and the middle of the first regrowth, and at 20, 20, 15 and 15% at the beginning of each consecutive period of regrowth.

4.3.4 *Experiment 3: effects of previous long-term N supply*

This experiment tested the effect of elevated pCO_2 (CO_2) on the growth of *L. perenne* as affected by the previous (prevN) and current rate of N supply (curN). The factorial experiment was carried out in the unlabelled part of the plots investigated in *experiment 1*, which had been exposed for nine years to low or high N and to ambient or elevated pCO_2. The experiment had eight treatments

(2 CO_2 × 2 prevN × 2 curN) with three blocks. In March 2002, PVC-sheets were inserted vertically into the soil to 0.3 m depth, thereby enclosing two areas of 0.9 m × 0.95 m in each plot, in order to prevent lateral translocation of N. Leaves from 20 plants were collected on each plot to determine the ^{15}N background level. Thereafter, the areas were supplied with ^{15}N-enriched NH_4NO_3, either at 14 or 56 g N m^{-2} a^{-1}, in five portions as in *experiment 1* (curN). The atom%-^{15}N excess was as in *experiment 2*. At the harvests in *experiment 1*, harvestable biomass was sampled from a central area (0.12 m^2) to avoid border effects, and dried at 60 °C for 48 h.

4.3.5 *Sample preparation and measurements*

All dried plant material was ground and prepared as described by Zanetti et al. (1996). In *experiment 3*, the material from all tubes in each treatment combination and at each sampling date was pooled for analysis. The samples were analysed for N and ^{15}N by continuous flow mass spectrometry (Europa Scientific, Cambridge, UK). The leaf area of a sub-sample was measured using an electronic area meter (Li-Cor LI-3000, Lincoln, USA).

4.3.6 *Calculations*

In *experiment 1*, mean values were calculated for the two cutting frequencies between 1993 and 1995. This was justified, because there were no significant differences between the cutting frequencies detected (Hebeisen et al. 1997). Since the infrequently cut swards were resown in 1996, these plots were excluded in 1996 and 1997.

Relative pCO_2 responses [%] were calculated as

$$pCO_2 \text{ response} = (1 - V_e / V_a) \cdot 100 \qquad [4.1]$$

where V_e is the value at elevated and V_a the value at ambient pCO_2.

The N in plant biomass (N_p) was derived from unlabelled SOM (N_{som}), remobilised fertiliser (N_{rf}) and current fertiliser (N_{cf}):

$$N_p = N_{som} + N_{rf} + N_{cf} \quad [4.2]$$

In *experiment 1*, N_{rf} and N_{cf} were labelled and N_{som} was unlabelled. Therefore, N from total fertiliser and N from unlabelled SOM were separated and the proportion of N from SOM ($\%N_{som}$) was calculated using the ^{15}N excesses above natural background in harvested plant biomass (E_p) and total fertiliser (E_f):

$$N_f = N_{rf} + N_{cf} \quad [4.3]$$

$$\%N_{som} = (1 - E_p / E_f) \cdot 100 \quad [4.4].$$

After eight years, only 10% of the N in soil originated from fertiliser (van Groenigen et al. 2002). The results of *experiment 3* also suggested that the effect of previously applied fertiliser N on recoverable fertiliser N in subsequent years was small. Therefore, the excess of the fertiliser applied in the year corresponding to the sampling was used as E_f.

In *experiments 2* and *3*, N_{cf} was labelled and N_{som} and N_{rf} were unlabelled prior to the experiments. Therefore, the proportion derived from soil ($\%N_s$) was calculated using the ^{15}N excesses above natural background in harvested biomass (E_p) and in current fertiliser (E_{cf}):

$$N_s = N_{som} + N_{rf} \quad [4.5]$$

$$\%N_s = (1 - E_p / E_{cf}) \cdot 100 \quad [4.6].$$

4.3.7 *Statistical analyses*

The statistical design of *experiment 1* was a split-plot with pCO_2 as the main-plot factor and N as the sub-plot factor (Gomez & Gomez 1984). *Experiment 2* was analysed as a split-split-plot with $curCO_2$ as the main-plot factor, N as the sub-plot factor and $prevCO_2$ as the sub-sub-plot factor. *Experiment 3* was analysed as a split-split-plot with pCO_2 as the main-plot factor, prevN as the sub-plot factor

and curN as the sub-sub-plot factor. In all split-plot analyses, the effect of pCO_2 as the main-plot factor was tested against the interaction pCO_2×block. Since pCO_2×block has only two degrees of freedom, the *F*-test has a low power and effects of pCO_2 are more readily detected as interactions with the sub-plot factors. The sub-plot factors were tested against their interaction with block nested within pCO_2.

4.4 Results

4.4.1 *Harvested biomass and N, %N derived from SOM and SLA over ten years*

In *experiment 1*, the amounts of biomass harvested varied annually and between the N treatments (Fig. 4.1a). At high N, harvestable biomass was largest in 1997 and decreased continuously thereafter. Harvestable biomass was significantly enhanced by elevated pCO_2 and high N supply (anova: pCO_2, $P<0.01$; N, $P<0.0001$). The pCO_2 response of harvestable biomass was higher at high N than at low N (anova: pCO_2×N, $P<0.01$) and increased significantly over ten years at high N (Fig. 4.1b). In 1993, the pCO_2 response of harvested biomass was 7.2%, increasing to 32% in 2002. At low N, the pCO_2 response varied annually.

The amounts of harvested N varied widely over the years (Fig. 4.2a), with the largest amounts found between 1995 and 1997. In these years, the amounts of harvested N were similar to the amounts of applied fertiliser N. Prior to 1995 and after 1997, less N was harvested than applied at high N. At high N, the amounts of harvested N decreased after 1997. The amounts of harvested N were higher in the high-N than in the low-N swards (anova: N, $P<0.0001$). At high N, the response of harvested N to elevated pCO_2 increased significantly over time (Fig. 4.2b). Prior to 1997, more N was harvested in swards under ambient pCO_2 than in those under elevated pCO_2. After 1998, the amounts of harvested N at high N were the same or higher at elevated than at ambient pCO_2. At low N, elevated pCO_2 reduced harvested N in all years.

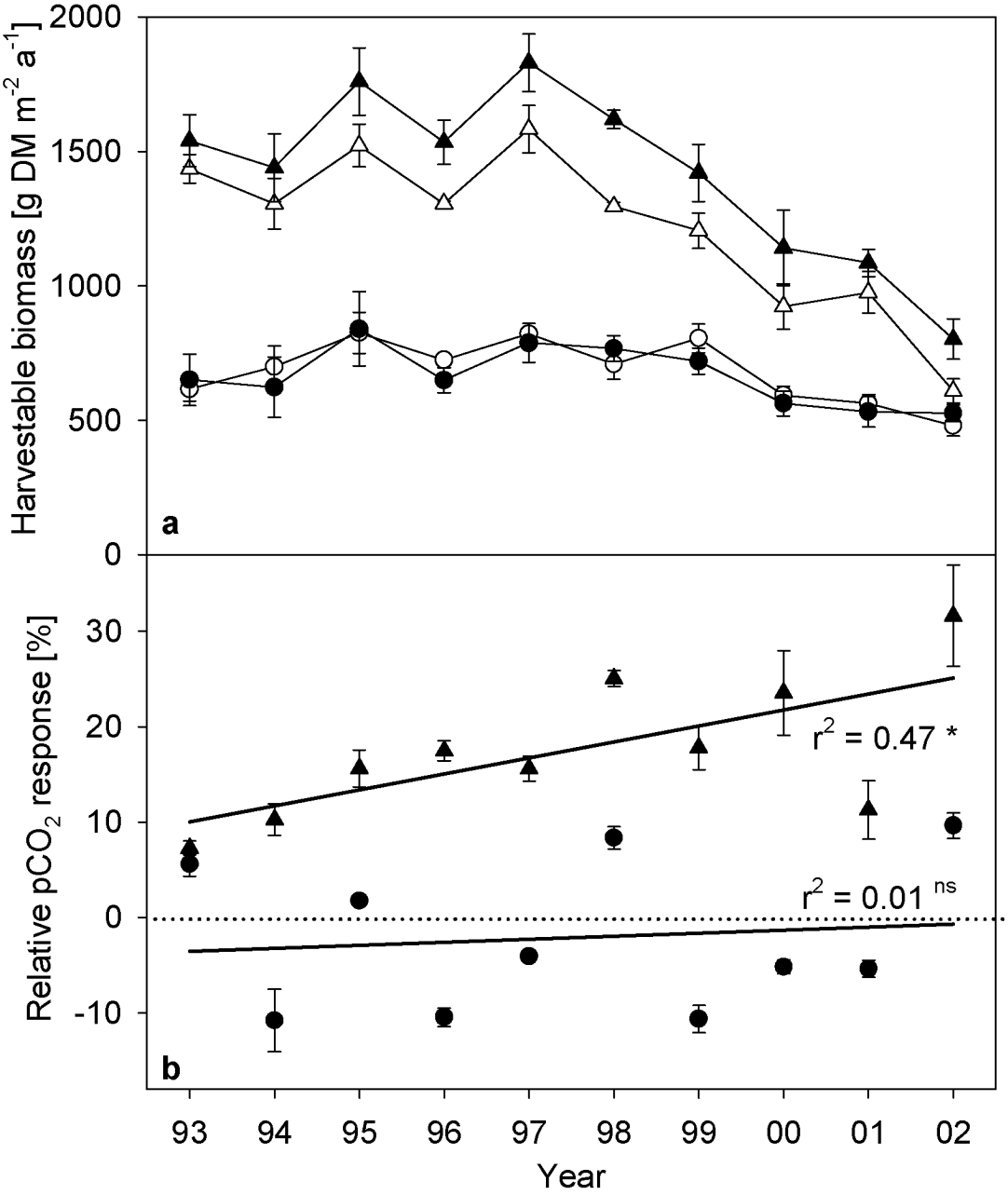

Figure 4.1 a,b: *Experiment 1:* (a) Annual amounts of harvestable biomass in swards of *Lolium perenne* at two levels of pCO_2 (○△: ~36 Pa pCO_2 and ●▲: 60 Pa pCO_2) and at two levels of N supply (○●: 14 g N m^{-2} a^{-1} and △▲: 56 g N m^{-2} a^{-1}) and (b) the pCO_2 response from 1993 to 2002, calculated using eq. [4.1].

Error bars are standard errors of the means (n = 6). Some bars may not be visible because they are smaller than the symbol. Linear regressions are shown with their coefficient of determination (r^2) and the level of significance (* P<0.05; ** P<0.01; ns not significant).

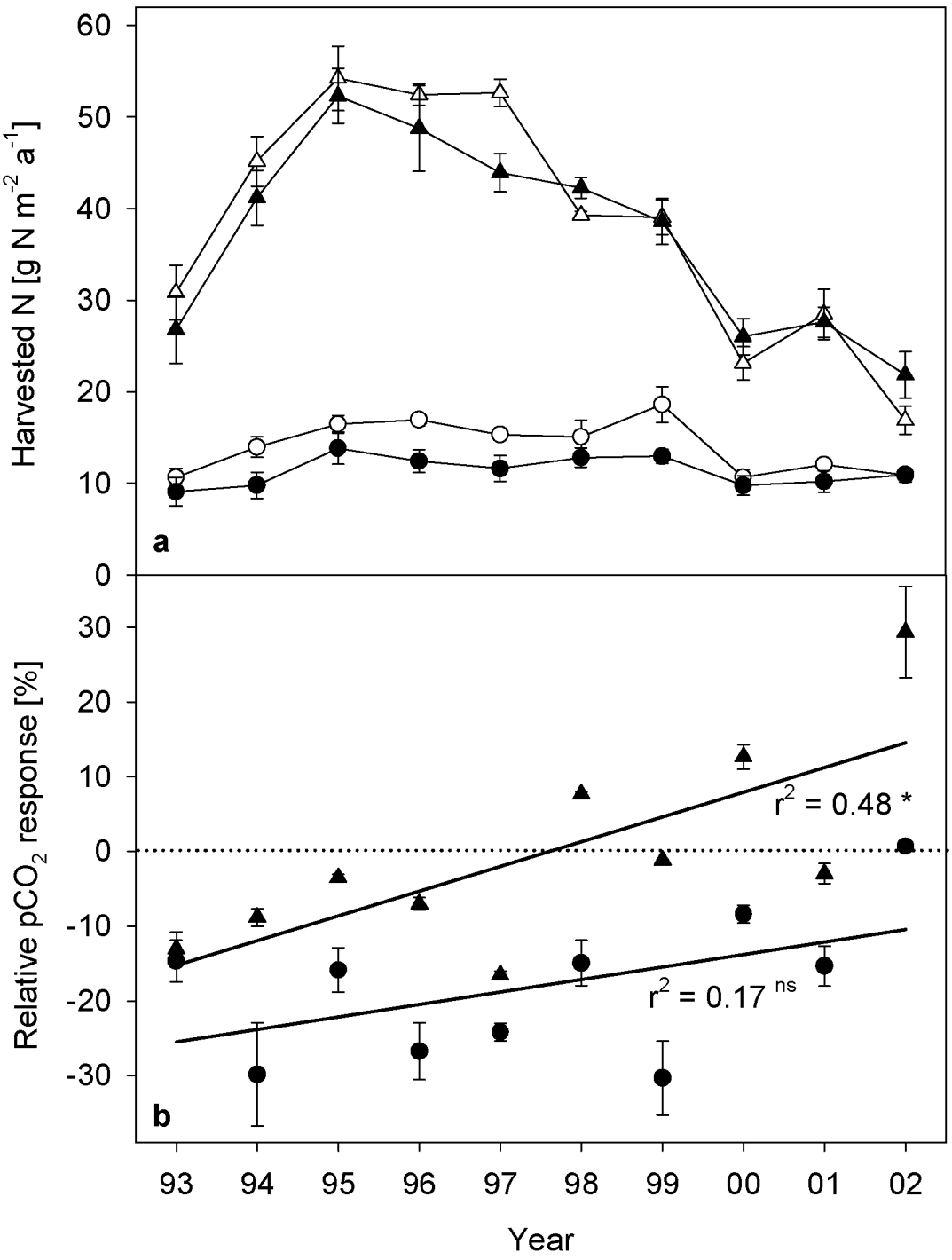

Figure 4.2 a,b: *Experiment 1:* (a) Annual amounts of harvested N in swards of *Lolium perenne* at ambient and elevated pCO_2 in combination with two levels of N supply and (b) the pCO_2 response from 1993 to 2002. See Fig. 4.1 for details.

Throughout the duration of *experiment 1*, the $\%N_{som}$ in harvestable biomass was higher at low N than at high N (anova: N, $P<0.001$; Fig. 4.3a). In both N treatments, the $\%N_{som}$ decreased until 1998 and increased thereafter. At high N, the pCO_2 response of $\%N_{som}$ was initially negative and then strongly increased over the years (Fig. 4.3b). The duration of CO_2 enrichment accounted for 49% of the pCO_2 response of $\%N_{som}$ at high N ($P<0.01$). At low N, pCO_2 did not affect $\%N_{som}$.

The specific leaf area (SLA), taken as an indicator for the status of N nutrition of the plant (van Arendonk et al. 1997), was initially reduced by elevated pCO_2 and low N supply (Fig. 4.4a). The negative effect of low N persisted over all years (anova: N, $P<0.05$), whereas, at high N supply, the initial negative effect of elevated pCO_2 was negligible after two to three years (Fig. 4.4b).

4.4.2 *N derived from soil after previous long-term ambient and elevated pCO_2*

In *experiment 2* (Tab. 4.2), we compared swards grown on soils that had been under ambient or elevated pCO_2 for seven years ($prevCO_2$), under current ambient or elevated pCO_2 ($curCO_2$), and fertilised at two levels of N (N). At low N, $\%N_s$ nearly doubled compared to the swards at high N (anova: N, $P<0.0001$). The $\%N_s$ was significantly higher after previous elevated pCO_2 when compared to previous ambient pCO_2 (anova: $prevCO_2$, $P<0.0001$). There was also a trend for increased $\%N_s$ at current elevated pCO_2 (anova: $curCO_2$, $P<0.1$). High N supply during the experiment strongly increased the amounts of harvested N; current pCO_2, however, had no significant effect on harvested N (anova: N, $P<0.01$; $prevCO_2$, ns; $curCO_2$, ns).

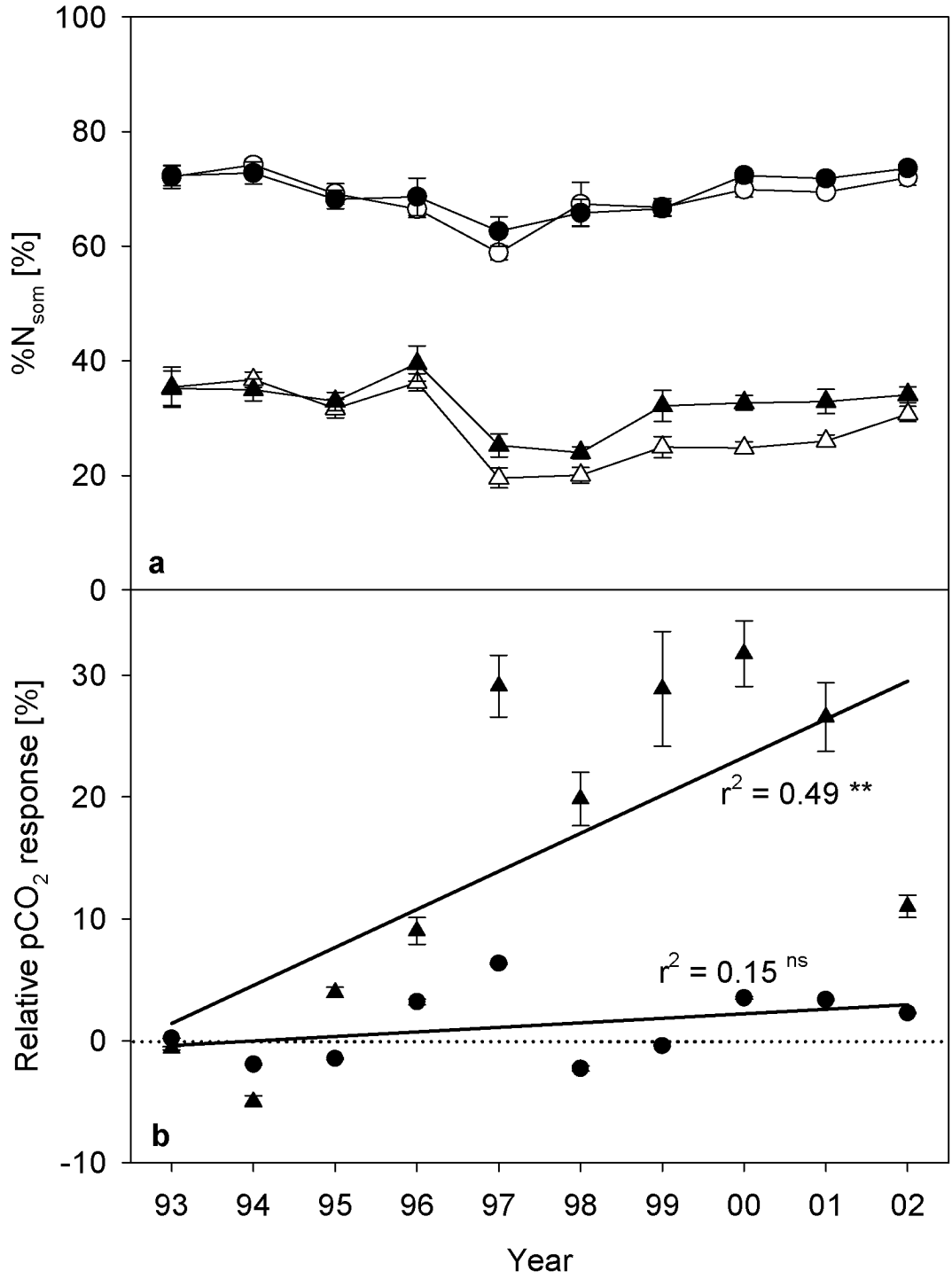

Figure 4.3 a,b: *Experiment 1:* (a) Proportion of N derived from unlabelled soil organic matter ($\%N_{som}$) in swards of *Lolium perenne* at ambient and elevated pCO_2 in combination with two levels of N supply and (b) the pCO_2 response from 1993 to 2002. See Fig. 4.1 for details.

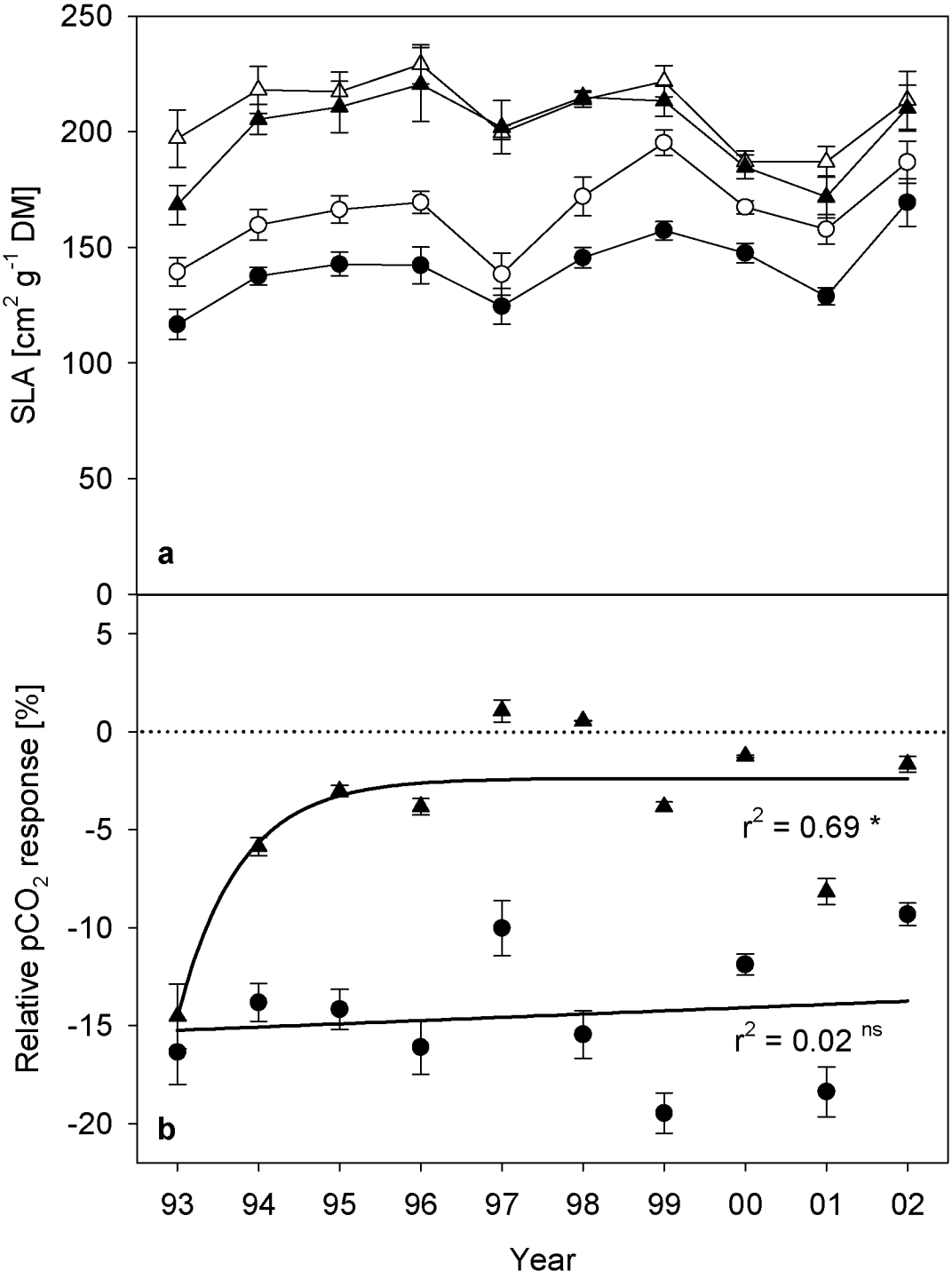

Figure 4.4 a,b: *Experiment 1:* (a) Specific leaf area of *Lolium perenne* at ambient and elevated pCO_2 in combination with two levels of N supply and (b) the pCO_2 response from 1993 to 2002. A single-exponential function rising to a maximum was fitted to the pCO_2 responses at high N. See Fig. 4.1 for further details.

Table 4.2: *Experiment 2*: Effects of previous ambient and elevated pCO_2 ($prevCO_2$) on harvested amounts of N and proportions of N derived from soil ($\%N_s$) in *Lolium perenne* swards at current ambient and elevated pCO_2 ($curCO_2$) and at two levels of N supply (N).

Current pCO_2 [Pa]	N supply [g N m^{-2} a^{-1}]	Previous pCO_2 [Pa]	Harvested N [g N m^{-2} a^{-1}]	$\%N_s$ [%]
36	14	36	17.2	77.5
36	14	60	17.6	81.2
36	56	36	32.6	41.4
36	56	60	32.7	44.2
60	14	36	15.6	79.2
60	14	60	16.5	81.3
60	56	36	31.7	43.3
60	56	60	32.7	45.7
ANOVA:	$curCO_2$		ns	+
	$prevCO_2$		ns	****
	N		**	****
	$curCO_2$×$prevCO_2$		ns	ns
	$curCO_2$×N		ns	ns
	N×$prevCO_2$		ns	ns
	$curCO_2$×N×$prevCO_2$		ns	ns
n			*3*	*15*
SE			*2.04*	*0.65*

Results of analysis of variance (ANOVA) are shown with levels of significance (+ $P<0.1$; * $P<0.05$; ** $P<0.01$; *** $P<0.001$; **** $P<0.0001$; ns not significant), number of measurements (*n*) and standard errors of the mean (*SE*).

4.4.3 *N derived from soil after previous long-term high and low N supply*

In *experiment 3* (Tab. 4.3), we compared swards on soils that had previously been fertilised at high or low N (prevN), at current high or low N (curN) and under ambient or elevated pCO_2 (pCO_2). The current high N supply significantly lowered the $\%N_s$ (anova: curN, $P<0.0001$). The $\%N_s$ on soil at previous high N was higher than at previous low N (anova: prevN, $P<0.1$). The $\%N_s$ was higher under elevated pCO_2 than at ambient pCO_2 only following previous high N fertilisation (pCO_2×prevN, $P<0.1$). There was a significant interaction between

the previous and the current rate of N supply (anova: prevN×curN, $P<0.001$). The amounts of harvested N were higher at current high N than at low N (anova: curN, $P<0.05$), but were not affected by pCO_2 and previous N supply (anova: prevN, ns; pCO_2, ns).

Table 4.3: *Experiment 3*: Effects of the level of fertiliser application from 1993 to 2001 (previous N supply; prevN) and in 2002 (current N supply; curN) on harvested amounts of N and proportions of N derived from soil ($\%N_s$) in *Lolium perenne* swards at ambient and elevated pCO_2 (pCO_2).

Current N supply [g N m^{-2} a^{-1}]	pCO_2 [Pa]	Previous N supply [g N m^{-2} a^{-1}]	Harvested N [g N m^{-2} a^{-1}]	$\%N_s$ [%]
14	36	14	10.7	76.5
14	36	56	11.1	**73.5**
14	60	14	7.9	76.2
14	60	56	11.4	**80.2**
56	36	14	21.0	41.4
56	36	56	17.2	**44.5**
56	60	14	23.7	39.2
56	60	56	21.4	**47.7**
ANOVA:	pCO_2		ns	ns
	prevN		ns	+
	curN		*	****
	pCO_2×prevN		ns	+
	pCO_2×curN		ns	*
	prevN×curN		ns	***
	pCO_2×prevN×curN		ns	ns
n			*3*	*12*
SE			*1.80*	*0.9*

Bold numbers indicate previous high N supply. See Tab. 4.2 for details.

4.5 Discussion

4.5.1 *The effect of pCO_2 and N supply on harvestable biomass and harvested N changed over the years*

The results from *experiment 1* clearly demonstrate the dynamic nature of ecosystem responses to a changed availability of C and N, resulting from elevated pCO_2 and N fertilisation. Following the step increase of pCO_2 in 1993, photosynthetic uptake of C increased immediately by 30 to 40% (Ainsworth et al. 2003). However, there was no significant change in the amount of harvestable biomass (Fig. 4.1a). The increased allocation of biomass below ground (Hebeisen et al. 1997; Daepp et al. 2000) and an increased production of stubble (data not shown) under elevated pCO_2, when compared to ambient pCO_2, may explain the limited change in harvestable biomass during the first years of the experiment. After 1997, the amounts of harvestable biomass and harvested N decreased (Fig. 4.1a). This decline may be a consequence of the long duration of the experiment. It is well recognised that the productivity of monocultures of *L. perenne* is reduced after several years of cultivation (Hoogerkamp 1978; Ennik et al. 1980; Hopkins et al. 1995). Over the years, plant death in the swards may have increased, especially over winter. A high N supply has been shown to cause lifted crowns in *L. perenne* and an increased number of tillers susceptible to frost, due to poor connectivity with the soil (Davies 1980). Large yields at high N supply may lead to poor re-growth and low persistence (e.g. Ennik et al. 1980). Furthermore, increased tiller aggregation by mutual shading resulted in open spaces in the ageing sward at high N (Alberda & Sibma 1982; Lafarge & Loiseau 2002). Despite deterioration, yields of 923 to 1419 g DM m^{-2} a^{-1} in the years 1999 to 2001 still represent a high sward productivity.

Over the years, the pCO_2 response of harvestable biomass steadily increased at high N (Fig. 4.1b). Daepp et al. (2001) demonstrated that the N available to the

plants limited the response of harvestable biomass to elevated pCO_2. Therefore, it is suggested that the observed increase in the pCO_2 response (Fig. 4.1) was related to a decreasing N limitation for plant growth in the high-N swards over the years. N limitation may decrease due to (i) processes in the soil which increase N availability, and/or (ii) a reduced demand for N by a less dense sward between 1999 and 2002 (Fig. 4.2). Thus, a greater share of N may be available to the fewer and stronger individual plants. The crucial role of N availability, with regards to the increasing pCO_2 response of harvestable biomass, is supported by the continuous weak pCO_2 response at low N. The small amounts of harvestable biomass at low N demonstrate that N availability strongly limited growth, even under ambient pCO_2. A decreasing N limitation at high N is corroborated by the vanishing negative effect of elevated pCO_2 on the SLA (Fig. 4.4). A low SLA indicates an accumulation of photosynthates in the leaf, due to N-limited sink activity in the growing shoots (Fischer et al. 1997). Furthermore, the pCO_2 response of harvestable biomass at high N was weak in 2001 and correspondingly, SLA was reduced at elevated pCO_2. This may have been due to excessive rainfall in spring 2001 (Tab. 4.1b), which may have increased N losses through denitrification and leaching and thus, reduced N availability.

In *experiment 1*, indications of a change in ecosystem response to elevated pCO_2 can be gained only by comparing the early and late years of the experiment. As a result of the long-term ^{15}N labelling, the effects of current and previous pCO_2 are confounded and cannot be separated. Thus, a changed pCO_2 response needs not to be explicitly related to changes in N availability, but may, in part, also be due to plant age or climatic conditions. In contrast, *experiment 2* and *3* allowed, for the first time, the comparison of long-term effects of pCO_2 and N independent of the current environmental conditions.

4.5.2 *Elevated pCO_2 altered the mobilisation of N from unlabelled soil organic matter*

The use of ^{15}N allowed the detection of long-term changes in soil processes in the ecosystem, which would not have been revealed, if only harvestable biomass and harvested N were considered. Evidence from the three field experiments indicated that long-term exposure to elevated pCO_2 at high N resulted in an increased mobilisation of N from unlabelled SOM, regardless of current pCO_2. In *experiment 1*, elevated pCO_2 at high N, resulted in increasingly higher $\%N_{som}$ than at ambient pCO_2 (Fig. 4.3b). The same effect of long-term elevated pCO_2 was observed for absolute amounts of N derived from SOM (data not shown, $r^2 = 0.82$, $P<0.001$). *Experiment 2* demonstrated that independent of the current level of pCO_2, the $\%N_s$ was significantly higher following long-term elevated pCO_2 compared to previous ambient pCO_2 (Tab. 4.2). This result is especially important, as elevated pCO_2 has been found to affect the leaf ^{15}N signature through changes in N fractionation between sources in the soil and the leaf (BassiriRad et al. 2003). However, current pCO_2 had no effect on $\%N_s$, thus demonstrating that the observed differences were not an artefact of the altered fractionation of ^{15}N at elevated pCO_2. Moreover, the comparison of soil previously fertilised at high and low rates of N (*experiment 3*) revealed that long-term high N supply tended to increase $\%N_s$. More importantly, the stimulating effect of previous pCO_2 only occurred after a long-term high N supply (bold numbers in Tab. 4.3).

The three experiments took a plant-centred approach to N availability by measuring ^{15}N in the harvestable biomass. Using the ^{15}N signal of the plant rather than measuring the ^{15}N label in the soil pools had the advantages of integrating N uptake over the whole period of regrowth and of reflecting the N sources as they are available to the plant. It is well established that N is continuously cycling in the plant: regrowth relies on N reserves from roots and

stubble (Volenec et al. 1996) and N is resorbed from senescent leaves (Aerts 1996). These cycles are acting in the time scale of hours to days especially after defoliation (Volenec et al. 1996) and are thus short-term compared to our sampling at five-week intervals over a period of ten years. The rapid cycling of N in the plant leads to a homogenous distribution of label within the plant. Therefore, the approach assumed that the ^{15}N signal measured in harvestable biomass was representative of the whole plant.

The basic assumption of the ^{15}N approach is that, despite the relatively rapid mixing of N in the soil, all ^{15}N above natural abundance available to the plants originates from fertiliser regardless of the exact date of its application. Nonetheless, the use of ^{15}N has to be examined carefully for potential artefacts, which may result in an apparent mobilisation of SOM, despite the lack of a biological mechanism. Applied ^{15}N may take the place of unlabelled N which would otherwise have been immobilised from the plant-available pool (Jenkinson et al. 1985). In this case, the greater amount of unlabelled N in the plant would be misinterpreted as a mineralisation of SOM. Such processes would be expected to occur especially at the beginning of the application of ^{15}N, when the pool of plant-available N is completely unlabelled. The fact that the observed effect of pCO_2 needed several years to become detectable suggests a real change in N mobilisation. More importantly, *experiment 2* demonstrated that a positive effect of previous pCO_2 was observed independent of N supply. Since high N supply is known to enhance apparent priming effects (Hart et al. 1986), there is little evidence to assume a significant effect of such an artefact. Finally, the comparison of *experiments 1* and *2* clearly demonstrates that the long-term effect of elevated pCO_2 on $\%N_{som}$ was not an experimental artefact: using an identical method, the effect of pCO_2 on N mobilisation in the soil was determined following one year of ^{15}N labelling. After one year of fumigation in

experiment 1, no effect was detected while after seven years of fumigation in *experiment 2*, a significant effect of long-term pCO_2 ($P<0.0001$) was observed.

Several mechanisms may have contributed to the observed increase of $\%N_{som}$ under elevated pCO_2 at high N. Their outcome may have been amplified over the experimental period by the cycling of N through plant and microbial biomass. Greater inputs of C into the rhizosphere, combined with high N supply, may have stimulated microbial activity and decomposition of SOM. A greater microbial biomass was detected in the experimental soil under elevated pCO_2 (Sowerby et al. 2000). Following a time lag, increased activity of microbial biomass may have resulted in more N available for the competing plant roots (Cheng 1999; Hodge et al. 2000) or for denitrifying bacteria (Baggs et al. 2003a). Similar results have been reported for crops (Cheng & Johnson 1998) and grasslands (Hungate et al. 1997; Ebersberger et al. 2003) but the underlying mechanisms are not well understood.

Alternatively, adaptive shifts in the composition of soil communities or in the abundance of fungi may have altered the competitive relationships in soil under elevated pCO_2. Fungi decompose substrate with a higher C/N ratio than bacteria (Killham 1994). Studies at the Swiss FACE site revealed changes in the composition of microbial communities in the rhizosphere of *L. perenne* (Montealegre et al. 2002). The functioning of these communities may also be altered (Marilley et al. 1999).

In addition, the step increase in pCO_2 in 1993 increased the C/N ratio of biomass. Biomass inputs with a higher C/N ratio may have resulted in an increased immobilisation of available N, which was unlabelled in this initial stage of the experiment. Over time, the C and N dynamics may have equilibrated, and the temporarily immobilised unlabelled N may have been mobilised and interpreted as N from SOM. However, the amounts of mineral N available at a

certain date are small compared to the amounts which were fertilised or mineralised over ten years.

Responses of plant growth to elevated pCO_2 may also have affected the mobilisation of N from SOM. Due to stimulated root growth, plants under elevated pCO_2 may have explored the soil to a greater depth (Jongen et al. 1995). Alteration in the kinetics of plant N uptake may also have increased the plant's competitiveness for N (BassiriRad et al. 2001). Thus, greater amounts of soil N may have been available in the system under elevated pCO_2, and may be interpreted as an increased mobilisation of SOM. However, these changes would have occurred during the first few months following the step increase of pCO_2 and could thus, not explain the observed long-term effect over many years. In addition, Hebeisen (1997) did not find evidence for effects of pCO_2 or N supply on the root distribution or rooting depth in the swards, which would, anyway, affect ^{15}N uptake only if it is inhomogenously distributed in the soil. The analysis of ^{15}N label throughout the soil profile at the site (Zanetti et al. 1996) indicated a homogenous labelling of the soil. Furthermore, plant species with different root morphologies did not differ in their uptake of ^{15}N (Zanetti et al. 1996; Lüscher et al. 2000).

4.5.3 *Elevated pCO_2 affected the cycling and losses of fertiliser N*

At the beginning of *experiment 1*, elevated pCO_2 reduced the amounts of harvested N in both N treatments (Fig. 4.2). This was due to the consistently lower N concentration in the biomass under elevated pCO_2 (reduced by 16%, from 0.021 to 0.017 g N g DM^{-1} at low N and by 15%, from 0.031 to 0.026 g N g DM^{-1} at high N). The reduction of harvested N by elevated pCO_2 persisted at low N. At high N, elevated pCO_2 did not reduce harvested N after 1998 and, between 1995 and 1997, the harvested amounts of N were in the range of the

applied fertiliser. This means that a higher N input into soil under elevated pCO_2 potentially occurred only in the first two years of fumigation.

There was no clear indication of an altered incorporation of fertiliser N into SOM under elevated pCO_2 when compared to ambient pCO_2 during *experiment 1*. In 2000, van Groenigen et al. (2002) found 70% more fertiliser N in soil under elevated pCO_2 than under ambient pCO_2, but this was primarily explained by the presence of fresh organic material, which was highly labelled. In soil samples collected in 2001, the content of fertiliser N was the same at ambient and at elevated pCO_2 (van Groenigen et al. 2003).

Several studies have indicated greater losses of N at elevated pCO_2. In the summer of 1995, Ineson et al. (1998) measured in the high-N treatment of *experiment 1* a 27% increase in N_2O emissions at elevated pCO_2 when compared to ambient pCO_2. The increase was particularly evident after the application of N fertiliser. In 2000, the annual emission of N_2O at high N was 62% higher under elevated pCO_2 than under ambient pCO_2 (Baggs et al. 2003b). Baggs et al. (2003a) found that losses through denitrification (N_2O and N_2), accounted for 6% of the applied fertiliser N at ambient pCO_2 and 17% at elevated pCO_2 in the low-N swards. Furthermore, greater amounts of mineralised N under elevated pCO_2 at high N fertilisation (Gloser et al. 2000) may have enhanced the leaching of N.

Throughout *experiment 1*, the amounts of N derived from fertiliser (N_f) in the harvestable biomass were unaffected by elevated pCO_2 (data not shown). This indicated that there was either no additional remobilisation of fertiliser N or that an increased remobilisation was concealed by higher losses of fertiliser N at elevated pCO_2. However, our approach did not allow the separation of recent additions of fertiliser N and N from remobilised fertiliser. Thus, there was no evidence that the higher $\%N_s$ in *experiments 2* and *3* was derived from remobilised fertiliser N.

Our results suggest that the pools in soil, into which fertiliser N was incorporated, may be different to those pools, from which N was mobilised. Further research is needed to understand the dynamics of N pools in the soil, under varying levels of pCO_2 and N supply. Furthermore, this study has shown that conclusions from short-term experiments (Zak et al. 1993; Diaz et al. 1993) may not be sufficient to describe long-term processes in ecosystems in response to elevated pCO_2.

4.6 Conclusions

Long-term changes in the availability of CO_2 and N induced dynamic feedbacks within the grassland ecosystem, via N sources in the soil. At high N supply, more N was mobilised from the soil following long-term exposure to elevated pCO_2 than following long-term exposure to ambient pCO_2. An important part of this mobilised N was derived from unlabelled SOM. There was no indication of increased sequestration and a subsequent remobilisation of fertiliser N at elevated pCO_2. At high N, feedback processes helped to explain the increasing pCO_2 response of harvestable biomass, due to a reduction in N limitation. In contrast, at low N, the reduced availability of N constantly limited the response of harvestable biomass to elevated pCO_2 throughout the experiment. The experiments reported here convincingly demonstrate that there are feedback mechanisms in the soil which are only revealed after several years of exposure to elevated pCO_2. Long-term experiments are, thus, an essential prerequisite for understanding how ecosystem functioning is affected by elevated atmospheric pCO_2 and N supply.

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5
General discussion

The aim of this study was to improve our understanding of how a grassland ecosystem responds to changes in the availability of the two primary resources C and N, a question, which has gained in actuality as a result of the increase in atmospheric pCO_2 (§ 1.1). To reduce effects of unknown confounding variables, the system was simplified. Thus, investigating *Lolium perenne* as a model grass at two levels of pCO_2 in combination with two levels of N supply, gave the following results:

I. Turnover rates of stubble and roots were 2.7 ± 0.3 a^{-1} and 1.2 ± 0.2 a^{-1}, respectively (Tab. 3.3). Turnover rates of stubble were estimated within a range of 10% when ^{14}C multiple-pulse and ^{13}C steady-state labelling were used (Tab. 2.1). Net primary production (NPP) was between 124 and 163% of the average standing biomass (§ 3.5.1). Quantifying turnover rates and production of stubble and roots is, therefore, crucial for estimating NPP, in particular with regard to elevated pCO_2.

II. Elevated pCO_2 increased turnover rates of stubble by 33% at low N and decreased them by 12% at high N (Fig. 3.1; Tab. 3.3), resulting in an increase in the production of stubble of 70% at low N, but no change was found at high N. Elevated pCO_2 increased the mass of residual leaf lamina by 50% at low N and 26% at high N (Tab. 3.2). Elevated pCO_2 led to a primary allocation of biomass to stubble when the N supply was limited. Elevated pCO_2 increased NPP by 9% at high N and by 34% at low N (Fig. 3.2). At low N, the production of residual biomass linked, thus, the strong

stimulation of photosynthesis at elevated pCO_2 and the weak response of harvestable biomass.

III. Long-term observations over ten years revealed an increase in the relative response of harvestable biomass to elevated pCO_2 at high N from less than 10% in 1993 to 32% in 2002 (Fig. 4.1). This trend was not observed at low N. Investigating the N sources in the soil showed that, at long-term high N supply, the proportions and amounts of N derived from SOM were increasingly higher at elevated pCO_2 than at ambient pCO_2. (Fig. 4.3b). The change in the allocation of biomass in response to elevated pCO_2 induced feedback mechanisms, which may explain, at least in part, the concomitant increase in the response of harvestable biomass to elevated pCO_2 (§ 5.5.1). In the long term, even small changes in soil N dynamics potentially have a strong influence on the cycling and storage of C and N in terrestrial ecosystems.

The measurements made with ^{14}C MPL and ^{13}C SSL confirmed the assumed homogenous labelling of biomass by ^{14}C MPL. This is usually the most critical assumption made in labelling studies (Meharg 1994). Multiple-pulse labelling is, thus, a mean of achieving homogenously labelled biomass in the field, which is otherwise achieved only by continuous labelling in closed chambers or tunnels (Johnen & Sauerbeck 1977; Loiseau & Soussana 1999b) or by FACE (§ 2.2.2). The second assumption, which was made in both labelling approaches, was that the translocation of label between the measured pools was negligible. This assumption was justified by measuring ^{14}C in the harvestable biomass which regrew after the last labelling (§ 3.3.4).

The main advantage of the ^{15}N approach (§ 4.4) was to allow a 'plant's eye view', i.e., to reveal the sources and amounts of N which are available to the plants. This is in contrast to measurements of N in the soil (e.g. van Groenigen et al. 2002), which do only partly reflect the N available to the plants. The three

experiments described in § 4.4, indicated that the swards in the investigated ecosystem relied mainly on the currently applied fertiliser. This surprising conclusion may be related to the large stocks of 1160 ± 70 g N m^{-2} in the soil to 0.5 m depth at the experimental site (M. Richter, pers. comm.). Fertiliser N may likely be immobilised, independent of an additional mobilisation of unlabelled N, which was found to be in the range of 5 g m^{-2} a^{-1}.

5.1 Allocation of biomass in *Lolium perenne* in response to elevated pCO_2: implications for the input of C into soil

5.1.1 *Allocation of biomass is a consequence of altered source-sink relations rather than of maximised capture of resources*

Plants allocate biomass to minimise resource limitation and to maximise resource capture and NPP (Chapin et al. 2002). Maximising resource capture would suggest that plants under elevated pCO_2 invest assimilates primarily into roots in order to increase the acquisition of nutrients. Such changes were revealed in many studies, which showed higher standing root masses (Gorissen 1996; van Ginkel et al. 1997; Cotrufo & Gorissen 1997; Loiseau & Soussana 1999a; Daepp et al. 2001; Suter et al. 2002). However, elevated pCO_2 had the strongest effect on the production of stubble (Fig. 3.2). The limited availability of N in the soil at low N led to a preferential allocation of biomass to stubble under elevated pCO_2 (§ 3.5.2). Larger quantities of stored carbohydrates and a larger mass of residual leaf lamina may have increased the number (Stadelmann 1993) and the production of tillers (Suter et al. 2001). Hence, the responses of NPP and biomass allocation on this fertile soil were driven primarily by source-sink relations rather than by maximised capture of limited nutrients. The measurement of turnover rates of residual biomass was a prerequisite for revealing these changes.

5.1.2 *Effects of elevated pCO_2 on the resorption and allocation of N*

Elevated pCO_2 had a stronger effect on NPP at low N than at high N; N supply had no effect on NPP at elevated pCO_2 (Fig. 3.2). A higher N use efficiency in photosynthesis may have led to a greater production of stubble and may explain, in part, why NPP at elevated pCO_2 was unaffected by N supply (§ 3.5.3). Plants may also reduce losses of N through leaching and exudation as well as increase the resorption of N from senescing biomass. In order to quantify N resorption, Norby et al. (2001) calculated an *N resorption efficiency* as the difference in the N concentration of green leaves and leaf litter divided by the N concentration of green leaves. N resorption efficiency of *L. perenne*, as calculated analogously between harvestable biomass and necrotic stubble, was unaffected by pCO_2 at low N (50% at ambient and 52% at elevated pCO_2), but decreased at high N from 46% at ambient pCO_2 to 39% at elevated pCO_2. Thus, increased N resorption does not explain the response of *L. perenne* to elevated pCO_2. This is in line with a number of experiments (reviewed by Norby et al. 2001). However, N resorption in grasses is difficult to determine and I do not claim that nutrients were completely resorbed from the fraction of necrotic stubble as it was considered in this investigation, because it was not the aim to separate between stubble litter and leaves from which nutrients were still resorbed. Leaching of N from necrotic plant material may also be of importance, and further investigations of the N balance in response to pCO_2 are necessary.

Another important aspect in this context is that elevated pCO_2 did not increase the C/N ratio in roots and necrotic stubble to the same extent as in harvestable biomass. At elevated pCO_2, the C/N ratio of harvestable biomass increased by 22%, from 19 to 23. In contrast, the C/N ratio of necrotic stubble increased on average by 14%, from 33 to 38, and the C/N ratio of roots increased by only 1%, from 31 to 32. This indicated that a greater share of N was allocated to stubble

and roots at elevated pCO_2 and proves that the increased availability of C also affected the allocation of N within the plant.

5.1.3 *Increased C losses may contribute to the weak response of harvestable biomass to elevated pCO_2*

The stimulation of the production of residual biomass helps to explain the discrepancy between a strongly increased C assimilation and a weaker response of harvestable biomass at elevated pCO_2 (§ 3.5.1). Nevertheless, other processes contribute to the C balance of a plant. As reviewed in § 1.2.3 elevated pCO_2 does not affect the respiratory C losses consistently, neither at the level of the plant, nor of the ecosystem. In the experimental system described in § 3.3, below-ground respiration increased significantly ($p<0.05$) by 43% under elevated pCO_2 in the daytime (data not shown). This is in line with an increased respiration of the ecosystem during the night at elevated pCO_2 (Aeschlimann 2003), which may be due to a bigger amount of respiring plant tissue, mainly roots and stubble (Tab. 3.2) or due to more organic material, which is available for microbial decomposition. A stimulated respiration may, thus, help to explain why the response of harvestable biomass to elevated pCO_2 was weaker than the response of photosynthetic C uptake.

Little is known about losses of C through root exudation and the transfer of photosynthates to symbionts. In sterile sand culture or small pots, *L. perenne* released a larger proportion of photosynthates to the rhizosphere at elevated pCO_2 than at ambient pCO_2 (Cotrufo & Gorissen 1997; Paterson et al. 1999); the importance of these changes under field conditions is unknown. Preliminary studies with *L. perenne* plants on monoliths from the Swiss FACE experiment have shown reduced root exudation under elevated pCO_2 (S. Bazot, writ. comm.). These results challenge the usual hypothesis that plants at elevated pCO_2 would lose excess fixed C through their roots. Since the amounts of

exudated C may be as high as 20% of assimilated C, they may be of importance for the C balance of the swards, especially when nutrients are in limited supply (Merbach et al. 1999).

At elevated pCO_2, higher concentrations of carbohydrates in the plants and a greater demand for nutrients would suggest that the transfer of photosynthates to mycorrhizal fungi may be increased. Mycorrhiza may act as a sink for excess fixed C, which may otherwise downregulate photosynthesis. There is a lack of experimental data to evaluate these hypotheses (Treseder & Allen 2000).

5.1.4 *C input to the soil at elevated pCO_2*

The greater production of stubble and roots under elevated pCO_2 (Fig. 3.2) leads to an increased deposition of decaying stubble and roots. Van Kessel et al. (2000b) estimated an input of 1500 g DM m^{-2} a^{-1} at both levels of N supply. This quantity is not so different from the estimated average production of stubble and roots of 1800 g DM m^{-2} a^{-1} at high N in this study (Fig. 3.2). At low N, the estimated input was bigger suggesting that more C was lost during its incorporation into SOM than at high N.

Furthermore, root exudation and the transfer of photosynthates to symbionts contribute to the flux of C into the soil. Especially when nutrients are limited, plants may enhance nutrient acquisition through root exudation and mycorrhizal symbiosis.

5.2 Effects of increased C availability on the mobilisation of N from soil

5.2.1 *Incorporation of biomass C into SOM*

Inputs of biomass into the soil are subject to numerous transformations prior to their incorporation into SOM (§ 1.3.2). The examination of these processes in

ecosystems is methodologically difficult, and in the field one commonly observes the integrated outcome. In the Swiss FACE experiment, Sowerby et al. (2000) found that, at high N, leaf litter of *L. perenne* decomposed significantly faster at elevated pCO_2 than at ambient pCO_2. Contrarily, in an incubation study, the decomposition of residues was unaffected by pCO_2 (M.-A. de Graaff, writ. comm.). In the field, the amount of macro-aggregates doubled at elevated pCO_2 after six years; there was also a 40% increase in the C concentration in these aggregates (Six et al. 2001). Stabilisation of C in the soil may play an important role in protecting C from decomposition and subsequent respiration (Six et al. 2002). Martens (2000) showed that the contents of phenolic acids and carbohydrates in litter significantly affected soil aggregation and microbial decomposition. This suggests that elevated pCO_2 may have affected the composition and protection of organic C in the soil rather than the absolute amount of C.

5.2.2 *Hypotheses of microbial feedback mechanisms in soil*

Growth under elevated pCO_2 induced feedback mechanisms in the soil, which affected plant growth via N sources (§ 4.5.2). Over ten years, the proportion of N derived from unlabelled 'old' SOM significantly increased under elevated pCO_2 compared to ambient pCO_2 at high N (Fig. 4.3). In the long run, even small changes in soil N dynamics had, potentially, a strong effect on the cycling and storage of C and N in terrestrial ecosystems.

Ten years ago, Zak et al. (1993) and Diaz et al. (1993) proposed positive and negative feedbacks of elevated pCO_2 on N availability (see § 4.2 for details). These hypotheses were based on short-term studies and, thus, did not include relevant relationships in the soil, which govern the long-term responses of an ecosystem: (i) substrate preferences and competition between types of microorganisms (Fontaine et al. 2003), (ii) microbial grazing (Kuikman et al.

1990) and (iii) competition between plants and microorganisms (Hodge et al. 2000). A mechanistic understanding of these interactions is lacking. Therefore, several mechanisms were proposed in § 4.5.2 to explain the observed phenomena.

In swards of *L. perenne*, the proportion of new N in various SOM fractions after eight years showed that fertiliser N was sequestered in the soil parallel to new C (van Groenigen et al. 2002). Isotopic evidence presented in § 4.4 raised doubts about the strict hierarchical organisation of N pools in SOM, because it showed that elevated pCO_2 increased the mineralisation of N from unlabelled SOM, whereas fertiliser N may have been sequestered into other pools. The assumption of uncoupled sinks and sources of N in the soil are supported by investigations on C sinks and sources after the conversion of tropical forests to crop land. Shang & Tiessen (2000) showed that most of the forest C was lost from intermediate density silt fractions whereas most of the new crop C entered light density fractions. Hence, several pools with different rates of turnover may co-exist in physical SOM fractions.

5.2.3 *The quantitative contribution of the mobilisation of soil N to the pCO_2 response of harvestable biomass*

The responses to elevated pCO_2 of harvestable biomass, harvested N and proportions of N derived from unlabelled SOM showed a positive trend over time (Figs. 4.1 to 4.3). This suggests that larger amounts of N from SOM may be one reason for the stronger response of harvestable biomass to elevated pCO_2. However, the extra N from SOM at elevated pCO_2 was too little to fully explain the response of harvestable biomass. Daepp et al. (2001) showed that the effect of elevated pCO_2 on harvestable biomass increased from 21 to 28% when the N supply was doubled from 56 to 112 g N m^{-2} a^{-1}. However, the maximum additional amount derived from unlabelled SOM was only 4.4 g N m^{-2} a^{-1}. If the

same efficiency of uptake of N from soil and fertiliser is assumed, the amount of N mineralised from soil (N_{min}) would be

$$N_{min} = N_{fert} \cdot \%N_s / (100 - \%N_s) \quad [5.1]$$

where N_{fert} is the rate of fertiliser supply [g N m^{-2} a^{-1}]. These additional amounts of N mobilised from the long-term fumigated soil at high N would correspond to an increase in fertiliser application of 7.5 to 17.9 g N m^{-2} a^{-1}. Nevertheless, additional factors have to be identified to explain the increase in the effect of elevated pCO_2 on the production of harvestable biomass over the years. In § 4.5.1, it was proposed that (i) a less vigorous sward may have a lower N demand and, consequently, respond more strongly to elevated pCO_2, (ii) a lower sward density may increase the N available to individual plants (Suter et al. 2001) and (iii) the dynamics of N availability may change over time, thus allowing a better synchronisation with the demand of the plants.

5.3 Towards a mechanistic understanding of the response of managed grassland ecosystems to elevated pCO_2

Models may be the only way to predict the probable response of ecosystems to rising pCO_2 in the future. Model calculations have predicted that, after a step change in pCO_2, C and N cycles in the ecosystem may reach a new state of equilibrium after a period of transition, during which a number of responses of the system are possible. The Swiss FACE experiment lasted ten years after a step increase in pCO_2 and showed that the responses of the ecosystem changed over time (§ 4.4). Thornley & Cannell (2000) stated that 'this makes it perilous indeed to extrapolate from even the best-designed 1 to 5 year pCO_2-doubling experiment to the real world'.

It was proposed that the additional C captured by plants under elevated pCO_2 retains more nutrients in the ecosystem (Thornley & Cannell 2000). Ecosystems with large nutrient fluxes are likely to be the most responsive to elevated pCO_2,

because adjustments in their nutrient cycles respond quicker to the increase in C (Rastetter et al. 1997; Kirschbaum et al. 1998). Adjustment of N cycles are possible through (i) the deposition of atmospheric N, (ii) fertilisation (§ 4.5.3), (iii) reduced losses by means of N retention (§ 3.5.3) and (iv) symbiotic N_2 fixation, which was not examined here. Symbiotic N_2 fixation showed an initial strong and positive response to elevated pCO_2 (Zanetti et al. 1997), but the response became weaker with time due to a greater remobilisation of N from soil (Richter 2003). A high-N system may adjust quicker to elevated pCO_2 than a low-N system (cf. Fig. 4.3). On the other hand, N-poor ecosystems may finally even show a stronger response to elevated pCO_2 than N-rich ecosystems (Cannell & Thornley 1998; Rastetter et al. 1997).

5.4 Outlook

5.4.1 *'Summary for policy makers'*

The public may pose two questions with regard to increasing levels of pCO_2 and grasslands: (i) To what extent do permanent managed grassland ecosystems act as C sinks and are accounted for in future C trading? (ii) What are future scenarios for the agricultural yield of permanent managed grasslands as dependent on N supply?

Chapter 3 showed that greater C inputs into the soil through the production of residual biomass are likely to occur at elevated pCO_2. The calculation of a C budget revealed a tendency towards a higher net C input into soil at elevated pCO_2, but effects of N supply and species were more important (Aeschlimann 2003). Monitoring the C content of bulk soil under FACE and ambient pCO_2 for ten years, did not reveal significant changes (van Kessel et al. 2000a; van Kessel et al. 2000b; van Groenigen et al. 2002; van Groenigen et al. 2003). This may be due to the size of the C pool compared to the annual inputs, the high variability in the soil or increased respiration, which offsets the greater C input (§ 5.1.3).

Moreover, the soil in the Swiss FACE experiment has a high content of SOM and may already be saturated with C (Sauerbeck 2001). We can, therefore, not yet answer the first question.

Chapter 4 showed that the agricultural yield of managed grassland may increase considerably by rising pCO_2 if the N supply keeps pace. However, in high-N systems, gaseous losses of N may increase at elevated pCO_2 due to a greater availability of C in the soil and a potentially higher soil moisture (§ 4.5.3; Ineson et al. 1998; Baggs et al. 2003a; Baggs et al. 2003b). N_2O is of concern due to its global warming potential and its involvement in the destruction of stratospheric ozone. Furthermore, losses of gaseous N contribute to the eutrophication of natural ecosystems. Thus, an increased N supply is environmentally hazardous. On the other hand, my results (§ 3.4) suggest that the low-N system responded to elevated pCO_2 as do natural grassland ecosystems. A weak response of harvestable yield and a stimulated allocation of biomass to residual biomass were also observed in natural grasslands (Owensby et al. 1999; Niklaus et al. 2001).

Another important aspect of agricultural yield is its quality, which may also be affected by elevated pCO_2. The N concentration in harvestable biomass of *L. perenne* was significantly decreased (*cf.* § 3.5.3 and 5.1.2) potentially along with a reduction of the protein content. Effects on fibre content and digestibility were usually small and variable (Idso & Idso 2001). However, species composition of mixed swards and N fertilisation may offset a reduced N concentration in biomass at elevated pCO_2. It has therefore been concluded that effects of pCO_2 on forage quality are modest compared to those of other management factors (Campbell & Smith 2000).

5.4.2 *Unanswered questions*

Based on the reported changes in NPP and in the allocation of biomass in response to elevated pCO_2 and N supply (Chapter 3) as well as the observed long-term changes in the mobilisation of soil N (Chapter 4), it would be important to answer the following questions:

1. **How does defoliation affect the C balance in *Lolium perenne*?** Chapter 3 demonstrates that the production of residual biomass provides a link between the responses of photosynthesis and of harvestable biomass to elevated pCO_2. However, little is known about the effects of elevated pCO_2 on key pathways, by which C is getting from the plant to the soil and atmosphere and especially about the mechanisms of respiration and root exudation. Little is known about the fate of unused storage carbohydrates when foliage is restored after defoliation. A considerable amount of carbohydrates will decay in senescent stubble and another part will be respired, potentially by the alternative oxidase pathway, which is activated by a high C availability (Millar et al. 1996; Lambers 1997). The alternative pathway is assumed to be favoured at elevated pCO_2, because the cytochrome pathway is inhibited by high pCO_2 (Wullschleger et al. 1994). Higher respiration later during the period of regrowth has recently been detected through measurements of ecosystem gas exchange, potentially due to an increase in the respiration of storage carbohydrates at elevated pCO_2 (Aeschlimann 2003). Since defoliation may have a strong effect on these processes, special attention should be paid to the spatial and temporal responses of respiration and exudation, as affected by the interaction of defoliation with N supply. The dynamics of non-structural carbohydrates, as well as their mobilisation and respiration over several periods of regrowth should be investigated in-depth.

2. **How does elevated pCO_2 and N supply affect the N balance in the plant, i.e., N use efficiency, N demand, N resorption and N losses?** A higher N use efficiency in photosynthesis does not mean *per se* that plants require less N at elevated pCO_2. More N will be available for growth per unit C, but the N demand increases parallel to the stimulated assimilation of C. The losses of N from the plant and N resorption from senescing organs are probably affected by elevated pCO_2, N supply as well as defoliation of the plants. The interaction of these factors makes it difficult to extrapolate results from investigations in different ecosystems. Quantifying these processes is important for understanding the response of plants and ecosystem to elevated pCO_2.
3. **What are sources and sinks of C and N in the soil, and what is the spatial and temporal dimension of the fluxes between them?** The observations reported in chapter 4 have raised doubts about the hypothetical strict hierarchy of soil fractions (§ 4.5.3), at least of those fractions, which are detectable by physical or chemical methods (*cf.* Shang & Tiessen 2000). Furthermore, the distribution of N in these fractions may not reflect the availability of N to the plant. Little is known about how processes of decomposition change under elevated pCO_2. Incubation studies do often not reflect the natural situation with respect to the residues used, the decomposing community and the spatial heterogeneity of the soil (Norby et al. 2001). The analysis of the effects of elevated pCO_2 and N supply on enzyme activities and on organic compounds produced by microbes may clarify changes in the incorporation of C and N into SOM through the microbial biomass and its consecutive remobilisation.
4. **What do the responses of plants and the ecosystems to a step increase in pCO_2 tell us about their response to the gradually rising pCO_2 in the real world?** The Swiss FACE experiment has investigated the response of a grassland ecosystem to an almost doubled pCO_2 and the acclimation of

the system for ten years. This provided a relatively sound understanding of the plant's response to this situation. Little is known about ecosystem responses to levels of elevated pCO_2 in-between ambient and doubled pCO_2. The responses are probably not linearly dependent on pCO_2, as indicated by experiments with different levels of pCO_2, and with experimental or natural gradients of pCO_2 (Polley et al. 1993; Luo et al. 1998; Hättenschwiler & Körner 2000; Gill et al. 2002). Furthermore, models of the global C cycle rely on the estimated responses of plants and ecosystems to relatively small and gradual increases in pCO_2.

In order to answer the above questions further experiments as well as an in-depth analysis of the numerous results from the Swiss FACE experiment by means of models will be necessary. Future research should evaluate the sensitivity of the ecosystem to the investigated processes and relate the changes in the morphology and physiology of the plant to processes and pools in the soil.

6
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