

Diss. ETH No. 15182

Effects of elevated atmospheric pCO₂ on net ecosystem CO₂ exchange in managed grassland

A dissertation submitted to the
SWISS FEDERAL INSTITUTE OF TECHNOLOGY
ZÜRICH

for the degree of
DOCTOR OF NATURAL SCIENCES

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2003

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1 Summary

Combustion of fossil fuels and land-use change are responsible for the increase in the partial pressure of atmospheric CO₂ (pCO₂). As photosynthesis and plant productivity are directly affected by pCO₂, terrestrial ecosystems have a regulative effect on the global carbon (C) cycle and are assumed to act as C sinks that mitigate the increase in atmospheric pCO₂. Grasslands are of special interest because they cover large land areas and their soils have a high C storage capacity.

In previous work, elevated pCO₂ stimulated photosynthesis in individual leaves of *Lolium perenne* by more than 35%. However, this resulted in only a relatively weak response of harvestable biomass, indicating that measurements at the leaf level do not sufficiently reflect processes acting at the level of the ecosystem. Leaf level measurements do not take account of canopy structure and carbon losses from the soil, but these factors strongly affect the CO₂ response of ecosystem CO₂ exchange. With regards to C sequestration, particularly the long-term effects of elevated pCO₂ on the ecosystem CO₂ exchange are crucial.

I investigated net ecosystem CO₂ exchange with special focus on: I) the effects of long-term CO₂ enrichment on net CO₂ uptake at midday and on respiration at night, II) effects of the canopy on the CO₂ response, and III) the net ecosystem C input after long-term exposure to elevated pCO₂.

The Swiss FACE (Free Air CO₂ Enrichment) experiment on managed grassland on fertile soil was started in 1993. The experimental set-up with two grassland species, perennial ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.) each grown in monoculture, two levels of pCO₂ (36 and 60 Pa) and two levels of nitrogen (N) supply (14 and 56 g N m⁻² a⁻¹) allowed the interactions of pCO₂ with N availability and species to be tested. In the present study, midday net ecosystem CO₂ exchange (mNEE) and night-time ecosystem respiration (NER) were measured in the field during the growing seasons in 2000, 2001 and 2002 using an

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open-flow chamber system. The data obtained were used to assess the ecosystem C balance. ^{14}C pulse labeling in combination with stratified clipping was used to investigate the CO₂ effect on assimilation in respect to the canopy structure.

Elevated pCO₂ increased mNEE by 12 to 24% in *L. perenne* and by 10 to 32% in *T. repens*. These results are consistent with photosynthesis measurements at leaf level at the same experimental site and indicate, that photosynthetic acclimation to elevated pCO₂ remained small also after nine growing seasons of pCO₂ enrichment. Nevertheless, the net CO₂ uptake at ecosystem level responded less to elevated pCO₂ than photosynthesis of individual leaves. This difference was attributed to an effect of the leaf position on the photosynthetic CO₂ response. Elevated pCO₂ greatly increased ^{14}C assimilation of leaves at the top of the canopy (+ 82%), whereas ^{14}C assimilation of leaves low in the canopy did not respond to elevated pCO₂.

Mean NER was 0.185 and 0.219 g C m⁻² h⁻¹ in *L. perenne* and *T. repens*, respectively. The higher NER in *T. repens* may be caused by the high energy costs of symbiotic N₂-fixation. Elevated pCO₂ increased NER strongly (up to 39%) and this CO₂ response was not significantly altered throughout the growing season. The greater NER at elevated pCO₂ was most probably a result of higher plant and microbial biomass. The stimulatory effect on NER of high N supply (up to 39%) in *L. perenne* may have been related to higher turnover rates of biomass.

The net ecosystem C input in the growing season 2001 ranged between 205 and 615 g C m⁻² and was mainly affected by N supply. Because night-time respiration but not daytime net CO₂ uptake was increased at high N supply, the net ecosystem C input was larger at low N supply than at high N supply. Elevated pCO₂ tended to increase net ecosystem C input but this small effect was not statistically significant. The increased net CO₂ uptake at elevated pCO₂ during day-time was mostly compensated for by a higher night-time respiration.

It is concluded that in a managed grassland ecosystem elevated pCO₂ clearly increased the C fluxes (CO₂ uptake and release) but had little effect on the C balance, which was primarily affected by the amount of N supplied.

2 Zusammenfassung

Der enorme Verbrauch fossiler Brennstoffe und grossflächige Änderungen in der Landnutzung sind verantwortlich für die Zunahme des CO₂-Partialdruckes (pCO₂) in der Atmosphäre. Die Photosynthese und Produktivität von Pflanzen werden durch pCO₂ direkt beeinflusst. Folglich haben terrestrische Ökosysteme einen regulativen Effekt auf den globalen Kohlenstoffzyklus und es wird angenommen, dass sie als Kohlenstoffsenken wirken und somit die CO₂-Zunahme in der Atmosphäre abschwächen. Grasland ist in diesem Zusammenhang von besonderem Interesse, weil es grosse Landflächen bedeckt und seine Böden eine hohe Kohlenstoffspeicherkapazität aufweisen.

Ein erhöhtes CO₂-Angebot steigert die Photosynthese in Einzelblättern von *Lolium perenne* um mehr als 35%. Es resultiert jedoch nur eine verhältnismässig schwache Zunahme der erntbaren Biomasse. Dies deutet darauf hin, dass Messungen auf Blattniveau Prozesse auf Ökosystemebene nur ungenügend reflektieren. Messungen auf Blattebene berücksichtigen die Bestandesstruktur und die Bodenatmung nicht. Diese haben aber einen grossen Einfluss auf die CO₂-Antwort des CO₂-Austausches des Ökosystems. Für die Kohlenstoffeinlagerung sind besonders die langfristigen Effekte eines erhöhten pCO₂ auf den CO₂-Austausch des Ökosystems von Bedeutung.

Ich untersuchte den Netto-CO₂-Austausch des Ökosystems mit speziellem Fokus auf: I) die Effekte einer langfristigen CO₂-Anreicherung auf die Netto-CO₂-Aufnahme über Mittag und auf die Atmung während der Nacht, II) die Effekte der Bestandesstruktur auf die CO₂-Antwort und III) die Kohlenstoffbilanz des Ökosystems nach langjähriger CO₂-Anreicherung.

Im schweizerischen FACE (Free Air CO₂ Enrichment) Experiment wurde bewirtschaftetes Grasland auf fruchtbarem Boden seit 1993 unter Freiluft-Bedingungen erhöhtem pCO₂ ausgesetzt. Das Experiment mit zwei Wiesenpflanzen in Monokultur, Englisches Raygras (*Lolium perenne* L.) und

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Weissklee (*Trifolium repens* L.), zwei pCO₂-Niveaus (36 und 60 Pa) und zwei Stickstoff (N) Niveaus (14 und 56 g N m⁻² a⁻¹) ermöglichte es Interaktionen von pCO₂ mit der N-Verfügbarkeit und den Arten zu untersuchen. Während den Vegetationsperioden 2000, 2001 und 2002 wurde im Feld mittels eines Open-Flow Kammersystems der Netto-CO₂-Austausch des Oekosystems über Mittag (mNEE) und die Oekosystematmung während der Nacht (NER) gemessen. Die erhobenen Daten dienten als Grundlage für die Schätzung der Kohlenstoffbilanz des Oekosystems. Zur Untersuchung des CO₂-Effektes auf die Bestandesphotosynthese in Abhängigkeit von der Bestandesstruktur wurde ¹⁴C Pulsmarkierung in Verbindung mit schichtweisem Schnitt verwendet.

Erhöhter pCO₂ steigerte mNEE in *L. perenne* um 12 bis 24% und in *T. repens* um 10 bis 32%. Diese Resultate zeigen, übereinstimmend mit Blattphotosynthesemessungen im FACE-Experiment, dass sich das photosynthetische Potential auch nach neun Vegetationsperioden kontinuierlicher CO₂-Anreicherung nur in geringem Masse dem erhöhten pCO₂ anpasste. Dennoch reagierte die Netto-CO₂-Aufnahme auf Oekosystemniveau weniger auf erhöhten pCO₂ als die Einzelblattphotosynthese. Dieser Unterschied wurde einem Effekt der Blattposition auf die photosynthetische CO₂-Antwort zugeschrieben. In den oberen Bestandesschichten steigerte erhöhter pCO₂ die ¹⁴C-Assimilation stark (82%), während die ¹⁴C-Assimilation in unteren Bestandesschichten nicht auf erhöhten pCO₂ reagierte.

Die mittlere NER war 0.185 g C m⁻² h⁻¹ in *L. perenne* und 0.219 g C m⁻² h⁻¹ in *T. repens*. Die höhere NER in *T. repens* wurde vermutlich durch die hohen Energiekosten der symbiotischen N₂-Fixierung verursacht. Erhöhter pCO₂ steigerte NER stark (bis 39%) und diese CO₂-Antwort war während der ganzen Vegetationsperiode gleich bleibend. Die gesteigerte NER unter erhöhtem pCO₂ resultierte vermutlich aus höherer Pflanzen- und Mikrobenbiomasse. In *L. perenne* hatte eine hohe N-Düngung einen stimulierenden Effekt (bis 39%) auf NER, dies wurde möglicherweise durch einen erhöhten Biomasse-Umsatz mitverursacht. Der Netto-C-Eintrag ins Oekosystem während der Vegetationsperiode 2001 variierte von 205 bis 615 g C m⁻² und wurde vor allem durch die N-Verfügbarkeit bestimmt.

Da die Atmung während der Nacht, nicht aber die Netto-CO₂-Aufnahme während des Tages durch hohe N-Düngung gesteigert wurde, war der Netto-C-Eintrag ins Ökosystem unter geringer N-Düngung höher als unter hoher N-Düngung. Erhöhter pCO₂ steigerte tendenziell, aber nicht statistisch signifikant, den Netto-C-Eintrag. Die gesteigerte Netto-CO₂-Aufnahme unter erhöhtem pCO₂ während des Tages wurde grösstenteils durch eine höhere Atmung während der Nacht kompensiert.

Es wird gefolgert, dass erhöhter pCO₂ die C-Flüsse (CO₂-Assimilation und -Abgabe) in einem bewirtschafteten Graslandökosystem klar steigerte aber auf die C-Bilanz, die hauptsächlich durch die N-Verfügbarkeit beeinflusst wurde, geringe Auswirkungen hatte.

3 General introduction

3.1 Increase of pCO₂ and the global carbon cycle

In the late 18th century the partial pressure of atmospheric CO₂ (pCO₂) started to increase from the post-glacial level of about 28 Pa to the present level (Oeschger & Siegenthaler 1988). Average pCO₂ reached 36.7 Pa in 1999 (Houghton *et al.* 2001), this is 5.2 Pa more than in 1957, when systematic measurements of pCO₂ began. Information about past pCO₂ is gained from enclosed air in ice-cores from Antarctica and Greenland; further the ¹³C:¹²C and ¹⁴C:¹²C isotopic ratios in this air provide information about the sources of the additional carbon. Living and fossil plant material have a lower ¹³C:¹²C isotopic ratio than the atmospheric air. Thus, the decrease in the atmospheric ¹³C:¹²C ratio since the beginning of the industrial revolution can be attributed to combustion of fossil fuels and widespread deforestation. These human activities have been identified as the main causes for the increase in pCO₂ (Oeschger & Siegenthaler 1988). The current release of CO₂ results in a yearly increase of pCO₂ of 0.15 Pa and pCO₂ may exceed 60 Pa in the second half of this century.

The average emission of carbon from fossil fuel combustion in the 1980s was calculated to be 5 to 6 Gt C per year. Changes in land use are estimated to release $1.6 \pm 0.7 \text{ Gt C a}^{-1}$ to the atmosphere (Houghton *et al.* 1998). Most of the carbon is emitted as CO₂ but only part of it ($3.3 \pm 0.2 \text{ Gt C a}^{-1}$) remains in the atmospheric C pool, which is about 750 Gt C. About 90 Gt C a^{-1} (Siegenthaler & Sarmiento 1993) are exchanged between the atmospheric and the oceanic C pools, the latter being as large as 35000 Gt C. Since the difference in pCO₂ between the atmosphere and the water surface is small, the oceanic net C uptake is not larger than $2 \pm 0.8 \text{ Gt C a}^{-1}$ (Houghton *et al.* 1998). The terrestrial carbon pool is estimated to contain 2000 to 2200 Gt C. Photosynthesis and respiration drive the carbon exchange of about 60

Gt C a⁻¹ between terrestrial ecosystems and the atmosphere. Temperate and boreal forests are considered to be a carbon sink (0.5 to 1.9 Gt a⁻¹). Regrowth of harvested areas, succession in abandoned agricultural land and direct effects of pCO₂ on plant growth are factors that are driving this sink activity. However, the fate of around 1.4 Gt C a⁻¹ is not known and cannot be attributed to any particular sink.

3.2 Importance of grassland

Studies dealing with carbon sequestration put special emphasis on forests as they have a several times higher carbon content per surface area than grassland. However, the significance of grasslands on the global carbon cycle had probably been underestimated. Grasslands cover 24% of the global land surface (Sims & Risser 2000) and more than 70% of agricultural land area. In Switzerland 18% of the surface is covered by grasslands and they account for 70% of the agricultural area (Bundesamt für Statistik 2001). Most grasslands are rarely tilled and the soil is covered by vegetation during the whole year. Therefore, these soils have a larger C storage capacity than tilled soils under annual crops at the same site. In grassland, litter is not removed and therefore acts as a source of carbon that can potentially be sequestered in the soil. In temperate climates, the C pool of grassland soils can be similar to or even larger than that of forest soils (Goudriaan 1992).

A large proportion of grasslands are not natural climax vegetation but a result of agricultural activities. In mild and humid regions, these semi-natural grasslands on fertile soils are often dominated by perennial ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.). *L. perenne* is very competitive under high cutting frequencies or intensive grazing. Its yield of digestible matter is very high and the nutritional value is not exceeded by any other grass species (Holmes 1980). *L. perenne* has the capability to respond strongly to high soil fertility. The legume *T. repens* is highly palatable and has a high digestibility. Leguminous forages have a lower content of cell walls but a higher pectin and lignin content than grasses. Relative to grasses, legumes contain a higher proportion of protein, organic acids

and mineral elements but a lower proportion of water soluble carbohydrates (Holmes 1980). The high protein content of *T. repens* contributes to its high nutritive value. Thanks to symbiotic N₂-fixation *T. repens* is independent of N fertilization and it can increase the available N in the root medium also to other non-fixing species.

3.3 Plant responses to elevated pCO₂ in interaction with N

3.3.1 Photosynthesis

When investigating plant responses to changes in pCO₂, photosynthesis is the most obvious process to look at, because it is the unique way in which plants take up CO₂ and it is directly affected by pCO₂. A vast majority of the studies have found leaf photosynthesis to be increased at elevated pCO₂. Drake *et al.* (1997) found among 60 studies an average increase of leaf photosynthesis of 58%. Leaf photosynthesis of *Lolium perenne* under FACE conditions showed a highly positive (35 to 43%) response to elevated pCO₂ (Rogers *et al.* 1998; Isopp *et al.* 2000b; Ainsworth *et al.* 2003).

CO₂ has the potential to regulate different mechanisms within the photosynthetic apparatus, but all except one process are saturated at current pCO₂. Rubisco has a low affinity for CO₂ on carboxylation, and this reaction is not saturated at current pCO₂. Consequently, the carboxylation of Rubisco responds to elevation of pCO₂, leading to an increase in the net rate of CO₂ uptake. In addition, Rubisco catalyzes the oxygenation of Ribulose-1,5-bisphosphate (RubP). This reaction is the first step of the photorespiratory pathway that decreases the net efficiency of photosynthesis by 20 to 50%. CO₂ is a competitive inhibitor of the oxygenation reaction, such that doubling of concentration at Rubisco will roughly half the rate of oxygenation (Long 1991). The increase in CO₂ uptake resulting from suppression of the photorespiratory pathway requires no additional light, water, or nitrogen, making the leaf more efficient with respect to each.

Many studies have shown that photosynthesis acclimated to elevated pCO₂; this means that plants adapted their physiology to a higher level of pCO₂. Assimilation was decreased in leaves grown at elevated pCO₂ compared to leaves grown at ambient pCO₂ when both were measured at ambient pCO₂ (Ryle *et al.* 1992). Photosynthetic acclimation is accompanied by a higher carbohydrate concentration, a lower Rubisco concentration (Bowes 1991) and a reduction of photosynthetic capacity (photosynthetic rate per unit leaf mass under non-limiting conditions). One reason for acclimation is the plant's inability to use all the additional carbohydrate provided by photosynthesis at elevated pCO₂, resulting in a reduction of source activity. Another reason is a reduced Rubisco demand at elevated pCO₂. Acclimation has mostly been found in experiments where the root volume was very limited, leading to a reduction of the sink capacity for photosynthates (Arp 1991). The way nutrients are supplied and their availability to plants can also determine whether acclimation occurs or not. When nitrogen was supplied in proportion to plant growth, no acclimation was observed in *Triticum aestivum* even at low N supply. In contrast when nitrogen was supplied at a fixed rate, plants showed photosynthetic acclimation at low N supply (Farage *et al.* 1998). If photosynthetic acclimation occurs in the field, it could be of major ecological importance as it would reduce or even hinder additional carbon sequestration. Rogers *et al.* (1998) and Ainsworth *et al.* (2003) showed that in the long-term acclimation in field grown *L. perenne* can occur at low N supply due to low sink activity, but acclimation was of no importance when N supply was high. Acclimation at low N supply occurred towards the end of a regrowth period but was absent when the sink activity was restored just after cutting (Bryant *et al.* 1998; Rogers *et al.* 1998). Despite of acclimation, *in situ* photosynthetic CO₂ uptake usually remains higher at elevated pCO₂.

The photosynthetic CO₂ response can depend on the environmental conditions. In a *L. perenne* sward, the positive CO₂ effect on canopy assimilation was higher during summer than in spring and autumn (Casella & Soussana 1997). Schapendonk *et al.* (1997) found that the CO₂ effect of photosynthesis increased along a gradient from low to high irradiances. An inverse response to irradiation was found by Idso *et al.* (1994), with increasing photosynthetic CO₂ response under low light conditions.

There is also strong indication that the stage of plant development affects the photosynthetic response to elevated pCO₂. In *Triticum aestivum*, Rubisco content was not affected by pCO₂ in the early growth stages but was reduced at elevated pCO₂ at later stages when leaves became shaded (Nie *et al.* 1995). After completion of grain filling, Rubisco as well as other proteins and pigments were reduced in the flag leaf at elevated pCO₂, suggesting that senescence was faster under these conditions. In *Rumex obtusifolius*, elevated pCO₂ was found to accelerate the natural ontogenetic decline of photosynthesis (Pearson & Brooks 1995). Elevated pCO₂ increased photosynthesis in newly emerged leaves but reduced it in leaves older than 20 days. Osborne *et al.* (1998) found no effects of pCO₂ on carboxylation capacity in recently expanded *Triticum aestivum* leaves, whereas the carboxylation capacity in lower, shaded leaves was reduced at elevated pCO₂ during grain development. All these findings show that the stage of plant and leaf development and light quality and quantity have the potential to affect the photosynthetic CO₂ response. Therefore, if CO₂ induces changes in canopy structure, canopy photosynthesis will respond asymmetrically more to elevated CO₂ than leaf photosynthesis.

When photosynthesis is measured at the ecosystem level, what is actually measured is usually the net ecosystem CO₂ exchange, which is the difference between plant photosynthesis and respiration of plants and microbes. Net ecosystem CO₂ exchange in open-top chambers was increased in *L. perenne* (Ham *et al.* 1995), *Scirpus olneyi* (Drake *et al.* 1996), and in an alpine grassland (Diemer 1997). Assimilation of *L. perenne* at elevated pCO₂ was also found to be increased at canopy level (Casella & Soussana 1997) and there was less photosynthetic acclimation than suggested by leaf photosynthesis studies. Hileman *et al.* (1994) found that in a cotton canopy the CO₂ effect on leaf photosynthesis was largest in July but the effect on canopy photosynthesis was highest in June. This difference was explained by increased mutual shading of leaves and by an increase of non-photosynthetic biomass after June.

There are sufficient results that clearly show that the ecosystem response is not simply the sum of leaf responses. This conclusion demonstrates the need for an ecosystem level approach to the study of the effects of elevated pCO₂.

3.3.2 Respiration

With regards to ecosystem CO₂ exchange and carbon balance, it is not only of interest how much carbon is assimilated, but also how much CO₂ leaves the ecosystem through respiration. Respiration involves mitochondrial oxidation of carbohydrates to produce ATP that is used for nutrient acquisition and production and maintenance of biomass. Effects of elevated pCO₂ on respiration are mainly indirect (Amthor 1997). Nevertheless, there seem to be some direct effects on the activities of the respiratory enzymes cytochrome C oxidase and succinate dehydrogenase that were inhibited by elevated pCO₂ (Gonzalez-Meler *et al.* 1996). Growth is a major consumer of respiratory products, leading to a close link of assimilation and respiration (Amthor 1997). Plant respiration per unit biomass is often found to be decreased at elevated pCO₂ (Drake *et al.* 1996; Schapendonk *et al.* 1997). This phenomenon may be related to a decrease in the N-to-C ratio, resulting in a lower protein concentration in plant tissue at elevated pCO₂. At canopy level and on a ground area basis, respiration is commonly increased at elevated pCO₂ due to increased biomass (Navas *et al.* 1995; Schapendonk *et al.* 1997). Casella & Soussana (1997) found an increase in cumulated above-ground and below-ground respiration in *L. perenne*. Below-ground respiration in a California grassland was increased (Luo *et al.* 1996) whereas in a wetland, ecosystem respiration was reduced (Drake *et al.* 1996). These contrasting results show that the direction in which ecosystem respiration responds to elevated pCO₂ may greatly depend on the environmental conditions and on the habitat.

Because leaves, stubble and roots differ in their specific respiration, ecosystem respiration is affected by how carbon is allocated within the plants.

Beside shoot- and root respiration of plants, ecosystem respiration includes also the respiration of the soil microbes. Microbial biomass was found to be increased at elevated pCO₂ (Sowerby *et al.* 2000) and the population structure of microbes was changed (Montealegre *et al.* 2002). Consequently, heterotrophic respiration, that can account for an important fraction of below-ground respiration, may greatly affect the CO₂ response of ecosystem respiration.

3.3.3 Biomass and its allocation

Since photosynthesis increases at elevated pCO₂ one might expect an increased harvestable plant biomass (> 5 cm above-ground) and total plant biomass. However, in some experiments harvestable biomass did not increase by the amount expected. In the Swiss FACE experiment, effects of elevated pCO₂ on harvestable biomass were strongly dependent on N supply. Harvestable biomass of *L. perenne* increased only when N supply was high and particularly during the first years of the experiment this increase was relatively small (Hebeisen *et al.* 1997b; Daapp *et al.* 2000). The CO₂ response of harvestable biomass increased throughout the experimental period of ten years, indicating that there were long-term changes in nitrogen availability (Daapp *et al.* 2000; Schneider 2003). Legumes such as *T. repens* increased their harvestable biomass much more than grasses and irrespective of levels of N supply level. A decreasing importance of N-fixation at elevated pCO₂ during the course of the experiment showed that N-availability also changed in the long-term for *T. repens* (Richter 2003).

Unlike harvestable biomass, total biomass responded much more to elevated pCO₂ (Daapp *et al.* 2001). This discrepancy is explained by a high increase in root biomass at elevated pCO₂, leading to an increased root-to-shoot ratio. Furthermore, at elevated pCO₂ more biomass was allocated to the residual above-ground plant parts below the cutting height (Schneider 2003). Suter *et al.* (2002) showed that carbon allocation in *L. perenne* was only affected by pCO₂ under field conditions but not under controlled environmental conditions. It was suggested that this contrasting response was a result of differences in the N availability and in the sink activity of the shoots. At elevated pCO₂ tillering was only found to increase in the first weeks after harvest when light transmission to the tiller bases was high (Suter *et al.* 2001). Later, when the leaf area index increased and tiller bases became shaded the number of tillers declined and no CO₂ effect was observed. Obviously, effects of CO₂ on allocation can be dynamic and depend on the stage of plant development.

3.3.4 Open questions

A good deal is known about short-term responses to elevated pCO₂ of the CO₂ exchange of single plants or plant organs. Effects of elevated pCO₂ on herbaceous species under controlled conditions have been investigated in numerous studies, but the results are of limited value in predicting responses in field-grown ecosystems (Suter *et al.* 2001). Ecosystems include numerous interactions, which are absent in single plant experiments, such as competition for nutrients between plants or mutual shading as well as nutrient competition between plants and microbes. For this reason, studies at ecosystem level are needed to explain responses of ecosystems to future higher pCO₂ levels.

Ecosystem CO₂ exchange is a key element in the global carbon cycle and therefore its response to elevated pCO₂ affects the global carbon balance. Most studies dealing with carbon balances have focused on forests because they were assumed to be the ecosystems with the highest potential for carbon sequestration. In annual cropping systems, the soil is covered by vegetation only during a limited time of the year and most of the assimilated carbon is exported by harvest and by losses caused by tillage. Consequently, these cropping systems are not of interest regarding to carbon sequestration. On the other hand, the carbon sink potential of grassland ecosystems systems is assumed to be much higher. Several defoliations per growing season increase the sink activity. Rare tillage and a high proportion of residual biomass favour carbon sequestration. Despite their potential importance for the global C cycle, only few studies have focused on the net ecosystem CO₂ exchange of grasslands, and none of them concentrated on the CO₂ exchange of legumes (e.g. *T. repens*).

There have been no experiments in which grassland was subject to continuous pCO₂ enrichment for nine years. As a consequence, it remained unknown how the CO₂ exchange of a managed grassland ecosystem will develop at elevated pCO₂ in the long-term. Daapp *et al.* (2000), Schneider (2003) and Richter (2003) showed that there are ecosystem feedbacks on N availability that established only after several years of CO₂ fumigation. Changes in microbial biomass and population structure are likely to be involved in these processes. These findings suggest that there is

need for long-term studies because short-term experiments may give misleading answers to CO₂ responses of ecosystem processes.

The long-term effect of elevated pCO₂ on photosynthesis of individual leaves has been determined (Ainsworth *et al.* 2003), but the response of CO₂ exchange at ecosystem level has not yet been investigated. In the Swiss FACE experiment, as well as in other studies, the photosynthetic CO₂ response was much higher than that of harvestable biomass. This discrepancy may have resulted, because no account was taken of the effects of the canopy when measuring leaf photosynthesis. Light conditions within a plant canopy change temporally and spatially and the photosynthetic apparatus of leaves is adapted to the prevailing conditions. Thus, effects of CO₂ on leaf photosynthesis may change within the plant canopy. Up to now, no studies have focused on the CO₂ response of assimilation within a canopy.

Furthermore, photosynthesis accounts only for carbon assimilation but carbon losses through respiration have also influence on biomass production and carbon sequestration. Effects of elevated pCO₂ on whole ecosystem respiration and the importance of its sources have been little investigated. Also, ecosystem respiration is affected by plants and by soil microorganisms, and this may cause complex interactions between various components of the ecosystem.

Ecosystem models are an important tool in ecology, and can be useful in predicting ecosystem processes in a changing environment. Data from long-term CO₂ experiments that investigate ecosystem CO₂ uptake and ecosystem respiration are of great value for modeling. These data have the potential to serve as inputs in ecosystem models and they can help to verify them.

3.3.5 Objective of this study

Experiments conducted under controlled conditions and measurements at plant organ level cannot alone explain the effects of elevated pCO₂ on ecosystem carbon fluxes. Furthermore, long-term effects of elevated pCO₂ on grassland ecosystems in the time-scale of many years have not yet been investigated.

General introduction

Based on results from the Swiss FACE experiment and related CO₂ studies, this study aims at improving our understanding of the CO₂ response of photosynthesis of individual leaves, the canopy and the carbon allocation to harvestable biomass.

Special focus is put on:

- I. The effects of long-term CO₂ enrichment on the net ecosystem CO₂ exchange: first, net ecosystem CO₂ exchange during midday, which is representative for total net carbon uptake; second, ecosystem respiration during night-time, which is characteristic for the CO₂ release of the ecosystem.
- II. How canopy structure affects the response of net ecosystem CO₂ exchange to elevated pCO₂.
- III. The effects of elevated pCO₂ on the net ecosystem carbon input, as determined from the net ecosystem CO₂ exchange.

The experimental setup with the two species *Lolium perenne* and *Trifolium repens* both grown in monoculture at two levels of N supply allowed me to investigate interactions between pCO₂, N and species.

3.3.6 Methodology of net ecosystem CO₂ exchange measurements

With the development of portable systems for measuring CO₂ exchange, photosynthesis and respiration measurements are no longer restricted to greenhouses and growth chambers. Going out in the field has also the enormous advantage that measurements can be done not only on individual plants or plant organs but at the ecosystem level. Several techniques for measuring ecosystem CO₂ exchange have been developed, of which eddy covariance flux measurements, open-top chambers, open-flow systems and closed chambers are the most important (Garcia *et al.* 1990). Their usage depends on the experimental area and the vegetation type. To be accurate, micrometeorological methods, such as eddy covariance require large experimental areas in the magnitude of hectares (Baldocchi *et al.* 1988). Gas fluxes are determined by calculating a covariance statistic of the

fluctuations in vertical wind velocity and the gas concentrations from a series of sequential measurements made over time. This technique is obviously not suitable when the patches of vegetations are combined in an area with a diameter of 18 m as in the Swiss FACE experiment. The use of closed chambers is limited to short time measurements (< 5 min) because conditions reach no steady-state. The microclimate can be heavily affected and additionally any leaks reduce the accuracy of closed chamber systems. Open-top chambers were originally designed for CO₂ fumigation of small areas ($\emptyset < 3$ m). To use them for CO₂ exchange measurements, adaptations have to be made in a manner that negatively affects the microclimatic conditions in the chambers (Ham *et al.* 1993).

The most important advantage of the design of FACE (Free Air CO₂ Enrichment) experiments is that they need no enclosures and therefore the natural microclimate remains undisturbed (Hendrey 1992). Therefore, it was important to find a method of CO₂ exchange measurements that does not completely eliminate this advantage. An open-flow chamber system was considered to be the best technique for my purposes. As in every experiment in which plants are enclosed, also the open-flow chamber system affects the microclimate. However, a high rate of air exchange in the chambers and the use of highly transparent Teflon film to construct the chambers kept this effect in an acceptable range.

In open-flow chamber systems, outside air is continuously introduced into the enclosure and the CO₂ flux is calculated from the product of the airflow rate and the difference in pCO₂ of air entering and exiting the enclosure. The exit air is assumed to have the same pCO₂ as air that has been well mixed within the chamber. Since constant exchange of air enables steady-state conditions, open-flow chamber systems are suitable for prolonged monitoring of gas exchange. Chapter 4 gives a more detailed description of the equipment used for measuring net ecosystem CO₂ exchange in this experiment.

Since light quantity and quality change within the plant canopy it is not only of interest to measure the overall ecosystem CO₂ exchange but also the C assimilation at different levels in the canopy. For this purpose, the method of ¹⁴C pulse labeling was used in combination with stratified clipping.

4 Long-term CO₂ enrichment increased midday net ecosystem CO₂ exchange of grassland

4.1 Abstract

The effects of elevated CO₂ on ecosystem CO₂ exchange were investigated in a managed grassland system that had been exposed continuously to elevated pCO₂ (60 Pa) for nine previous growing seasons using Free Air CO₂ Enrichment (FACE) technology. Perennial ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*) monocultures were fertilized at two N levels and were cut five times during the growing season. Midday net ecosystem CO₂ exchange (mNEE) was measured using an open flow chamber system. Effects of leaf position on assimilation were determined using leaf photosynthesis measurements and ¹⁴C pulse labeling.

In the *Lolium perenne* monocultures mNEE was increased at elevated pCO₂ by 12 to 20% at low N supply and by 23 to 24% at high N supply, depending on intercepted photosynthetically active radiation (PAR). Elevated pCO₂ also stimulated mNEE of *Trifolium repens* monocultures: 24 to 30% at low N supply and 10 to 32% at high N supply.

The CO₂ response of assimilation depended on leaf position. ¹⁴C-assimilation of leaves at the top of the canopy increased greatly (82%) at elevated pCO₂, whereas ¹⁴C-assimilation of leaves low in the canopy did not respond to elevated pCO₂. The photosynthetic potential, measured by photosynthesis at light saturation (A_{sat}) and apparent quantum yield (ϕ_{app}), increased at elevated pCO₂ and was not affected by leaf position.

In-situ, only leaves in the upper canopy layers responded to elevated pCO₂, resulting in a slightly lower CO₂ response of mNEE compared to individual leaf photosynthesis. However, mNEE was in the long-term still markedly higher at elevated pCO₂.

4.2 Introduction

Grasslands play an important role in the global carbon cycle because they cover large land areas and have a high capacity to sequester carbon. They must, therefore, be considered when investigating the effects of elevated partial pressure of atmospheric CO₂ (pCO₂) on ecosystem processes (Prentice *et al.* 2001). Managed temperate grasslands are often dominated by perennial ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*), which are well-adapted to regular defoliation and fertilization.

Photosynthesis is the only way that the plant ecosystem takes up CO₂-carbon and this process is directly affected by atmospheric pCO₂ (Drake *et al.* 1997). Therefore, investigating photosynthesis is crucial for an understanding of plant responses to elevated pCO₂.

The effects of elevated pCO₂ on the leaf photosynthesis of grassland species have been investigated in a large number of studies (Stirling *et al.* 1997; Rogers *et al.* 1998; Davey *et al.* 1999; Clark *et al.* 1999; Isopp *et al.* 2000b). In the vast majority of the studies, a large (58%) increase in leaf photosynthesis of C₃ plants was observed at doubled pCO₂ (Drake *et al.* 1997). Under field conditions, the leaf photosynthesis of *L. perenne* increased by 40% (Rogers *et al.* 1998) and this higher rate persisted for several years (Ainsworth *et al.* 2003). Nitrogen availability affected the CO₂ response of leaf photosynthesis. At low nitrogen supply, sink limitation led to an acclimation of photosynthesis, whereas at high nitrogen supply acclimation was not observed (Fischer *et al.* 1997; Rogers *et al.* 1998).

Measurements of the individual leaf photosynthesis reflect only one component of the CO₂ exchange of the ecosystem. However, net ecosystem CO₂ exchange is

affected importantly by factors such as assimilate distribution, canopy structure or above and below-ground respiration. As a result, the influence of elevated pCO₂ upon CO₂ exchange at the ecosystem level can be very different from that at the level of the individual leaf. Osborne *et al.* (1998) showed that leaf position can affect the CO₂ response of photosynthesis. Root biomass (Daupp *et al.* 2000) and root-to-shoot ratio (Suter *et al.* 2002) increased in *L. perenne* at elevated pCO₂. As a consequence, root respiration probably increased, too, thus affecting net ecosystem CO₂ exchange. In contrast to leaf photosynthesis, harvestable biomass showed little response at high N supply and no response at low N supply under elevated pCO₂ (Daupp *et al.* 2000). This is clear evidence that leaf level measurements alone cannot explain the CO₂ response of ecosystems. It is of major importance that the processes of CO₂-uptake and release are considered at the level of the ecosystem when ecosystem responses to elevated pCO₂ and ecosystem carbon sequestration are investigated.

Contrary to the leaf level, the CO₂ response of CO₂ exchange at the ecosystem level is not well understood. Few studies have investigated the CO₂ exchange of grassland vegetation on fertile soil, and these have either been conducted under controlled conditions at the whole plant level (Ryle *et al.* 1992) or under semi-field conditions at the ecosystem level (Casella & Soussana 1997; Schapendonk *et al.* 1997). There have been no long-term investigations under real field conditions. Long-term studies are important to determine whether net ecosystem CO₂ exchange is persistently increased at elevated pCO₂ over many years. In the Swiss FACE experiment, we had for the first time the opportunity to investigate the net CO₂ exchange of a grassland ecosystem on fertile soil and which had been previously exposed for nine years to elevated pCO₂. The aims of the present work were to investigate net ecosystem CO₂ exchange after long-term CO₂ enrichment and to examine the effect of leaf position on the CO₂ response of carbon assimilation. Midday net ecosystem CO₂ exchange (mNEE) was measured in two growing seasons using an open-flow chamber system. ¹⁴C pulse labeling was used to investigate the assimilation of leaves at different positions in the canopy. Measurements of leaf photosynthesis provided information about the maximum

photosynthetic potential, i.e. assimilation at light saturation (A_{sat}) and apparent quantum yield of CO₂ uptake (ϕ_{app}).

4.3 Material & Methods

4.3.1 Experimental site

The experimental site is located at Eschikon (8°41'E, 47°27'N) near Zurich at an altitude of 550 m above sea level. Free Air Carbon dioxide Enrichment (FACE) technology (Hendrey 1992) was used to investigate the long-term effects of elevated pCO₂ on a fertile grassland ecosystem in the field. The experiment was arranged in three blocks, each consisting of two circular areas (18 m diameter), a CO₂-enriched area (60 Pa pCO₂) and an ambient area as control (36 Pa pCO₂). CO₂ fumigation started in May 1993. The period of fumigation lasted for the whole growing season (March-November) and was carried out only in the day-time. CO₂ fumigation stopped when the temperature fell below 5 °C and started when the temperature rose above 6 °C, because, at low temperatures, plant growth is slow and CO₂ effects are weak (Long 1994). P and K fertilizer was applied to all plots in amounts that were considered to be non-limiting for plant growth under the experimental conditions (5.5 g P m⁻² a⁻¹; 24.1 g K m⁻² a⁻¹). Hebeisen *et al.* (1997b) described the experimental set-up in more detail.

4.3.2 Experimental treatments

Lolium perenne cv. Bastion and *Trifolium repens* cv. Milkanova were grown in monocultures of 5.3 m² since 1993. Two levels of nitrogen (N) fertilization were applied to examine the effects of nitrogen availability on the CO₂ response of the plants. The swards were cut five times a year at a height of about 5 cm above-ground. In the years 2001 and 2002 the first regrowth in mid May was cut by stratified clipping. Cutting heights were 25, 15 and 5 cm. Levels of N fertilization

were 14 g m⁻² a⁻¹ in the low-N treatment and 56 g m⁻² a⁻¹ in the high-N treatment. The N fertilizer was applied as liquid NH₄NO₃ at the beginning of each regrowth. The amount of the fertilizer was split into portions of 30, 20, 20, 15 and 15% from the first to the fifth regrowth, which corresponded to the expected yield at the end of each respective regrowth.

4.3.3 Measurements and data collection

Net ecosystem CO₂ exchange was measured during the growing season in 2000 and 2001 using an open-flow chamber system. The system consisted of two identical but independent units, each having two chambers, so that simultaneous measurements could be made of both N treatments and both pCO₂ levels. The chambers were installed for one to five days on a particular vegetation, and were then moved between vegetation types and blocks.

The chambers were 0.6 m high and covered a square area of 0.49 m². They consisted of an aluminium framework of which three sides and the top were covered with Teflon (PTFE) film. The side with the inlet and outlet for gas was made of Plexiglas. The chambers were fixed to a steel frame that was inserted into the soil to achieve secure closure of the chambers. The air that was to be passed over the vegetation was sampled with a vertical tube 7 m above the ground level in order to minimize short-time variability of pCO₂. Fans then blew the air through flexible PVC tubes into the chambers. The air-flow was measured with a mass flow meter (Accu-Flo 600, Sierra Instruments, Monterey, CA USA) placed in the tubes. A small fan in each chamber assured a good mixing of the air, which was exchanged up to four times a minute. In the day-time, in the fumigated areas, the sampled air was enriched with CO₂, so that pCO₂ in the chambers reached about 60 Pa, consistent with the FACE conditions. To measure the CO₂ exchange in the chambers, air was sampled at the inlet and outlet of the chambers and pumped through flexible PE-tubes to an infra-red gas analyzer (Binos 100 4P, Fisher-Rosemount, Hasselroth, Germany) where the difference in the CO₂ concentration was measured. The air samples were pumped in heated PTFE tubes to a dew point sensor (MTR 2.0, IL Metronic, Ilmenau-Unterpörlitz, Germany).

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Incident photosynthetic active radiation (PAR) was measured continuously by a light sensor (BF2, Delta-T devices, Cambridge, UK) in one FACE area; PAR transmission through the canopies above the cutting height was measured about once a week during the growing season using a Sunfleck Ceptometer (SF-40, Decagon, Pullman, WA USA).

A subsample of the harvested plant material was separated into fractions of leaves, stems, necrotic parts and unsown species. The leaf area of this subsample was measured with an electronic leaf area meter (Li-3000, LI-COR, Lincoln, NE USA). All the harvested material was oven-dried for 48 h at 65 °C before determining dry mass. The dried material of *L. perenne* was ground into powder and analyzed for total content of C and N.

4.3.4 Leaf photosynthesis

Leaf photosynthesis of the high-N treatment of *L. perenne* was measured in May 2001 and 2002 before the first harvest. In 2001, the youngest fully expanded leaf and the second and third leaves of a tiller were measured. In 2002, the youngest fully expanded leaf and a leaf from the canopy layer of 5 to 15 cm above the ground was measured. The response of net photosynthesis (A) to irradiation (Q) at a leaf temperature of 25 °C was measured with a portable, steady-state gas-exchange system, incorporating an infra-red gas analyzer (Li-6400, LI-COR) and a red-blue LED light source (6400-02B LED Light Source, LI-COR). Measurements were taken at growth pCO₂ on leaves, that were cut before dawn. Rapid light-response curves were initiated at high light (1500 µmol m⁻² s⁻¹) and light was reduced step-by-step to 250 µmol m⁻² s⁻¹. Nine measurements were taken at light levels below 250 µmol m⁻² s⁻¹ to ensure a good estimate of apparent quantum yield (ϕ_{app}).

The response of A to Q was analyzed by fitting the non-rectangular hyperbolic function:

$$A = (Q \times \phi_{app} + A_{sat} - ((\phi_{app} \times Q + A_{sat})^2 - 4 \times \phi_{app} \times A_{sat} \times \Theta \times Q))^{1/2} / (2 \times \Theta) - R_d$$

using maximum likelihood regression (SigmaPlot, Jandel Scientific, Erkrath, Germany), where A_{sat} is the light-saturated rate of CO₂ uptake, Θ the convexity

coefficient of the photosynthetic light response and R_d the "dark" rate of CO₂ evolution, when $\mathcal{Q} = 0$.

4.3.5 ¹⁴C pulse labeling

The relative CO₂ uptake rates of different canopy layers of *L. perenne* at high N supply were determined using ¹⁴C pulse labeling. Pulses of 3.7 MBq ¹⁴CO₂ (20 µl ¹⁴CNa₂CO₃ solution, 10mM Tris pH 8) were applied to the swards enclosed in the chambers for 20 minutes. A small fan assured a homogeneous mixture of the air in the chamber. Labeling was done prior to stratified clipping at the first harvest in 2001 and 2002.

4.3.6 ¹⁴C analysis

Twenty milligrams of the ground plant samples were put into 20-ml glass vials and a suspension of 4 mg cellulase and 4 mg maceroenzyme in 200 µl phosphate buffer (pH 6) was added. The vials were incubated for 18 hours at 45 °C on a shaker. After adding 1 ml of Soluene-350 (Packard Instrument Company, Meriden, CT USA) to each vial, they were incubated on the shaker for 24 h at 45 °C. Before liquid scintillation counting (Packard 2500TR, Packard Instrument Company), 15 ml Hionic-Fluor (Packard Instrument Company) was added to each sample, and the samples were shaken to homogenize the dissolved plant material. The method is described in more detail elsewhere (Suter *et al.* 2002).

4.3.7 Calculation and statistical analysis

The statistical analyses were carried out using the Mixed procedure of SAS 8.02. The model was a split-plot with pCO₂ as the main plot factor. Thus block and block x pCO₂ were tested as random effects. Since block x pCO₂ has only two degrees of freedom, the split-plot model requires a high F-value for the main plot factor pCO₂ to be significant. Denominator degrees of freedom were adjusted according to the method of Kenward-Rogers (Littell *et al.* 1996). For the analysis of

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the gas exchange data, the Mixed procedure was used with the variable “midday intercepted PAR” as a covariate. The ¹⁴C data were analyzed using the Mixed procedure with variable “cutting height” as a covariant.

For statistical analysis the ecosystem CO₂ exchange data for the growing seasons 2000 and 2001 were combined. The CO₂ exchange rates of the swards in the chambers, averaged for ten-minute intervals, were integrated into a four hour (11 a.m. to 3 p.m.) total. Data of intercepted PAR were Log_e transformed before statistical analysis in order to obtain linear dependencies.

4.4 Results

4.4.1 Midday net ecosystem CO₂ exchange (mNEE)

Midday net ecosystem CO₂ exchange (mNEE) includes plant photosynthesis as well as plant and heterotrophic respiration. mNEE was strongly correlated with the sum of midday intercepted PAR ($p<0.0001$), and assimilation data were therefore plotted against this variable. Intercepted PAR is dependent upon leaf area and incident PAR, thus differences between the fitted curves were not due to differences in these variables. Averaged for the growing season, the four hour integral (11 a.m. to 3 p.m.) accounted for about 50% of total net CO₂ uptake during the light period.

mNEE in *L. perenne* and *T. repens* monocultures which had been exposed for nine growing seasons to continuous CO₂ fumigation was significantly higher than in controls ($p<0.0001$; Fig. 4.1). A lack of CO₂ x N, CO₂ x species and CO₂ x N x species interactions shows that this increase occurred irrespective of nitrogen supply and species (Table 4.2).

The influence of intercepted PAR on the relative CO₂ effect of mNEE can be demonstrated easily by comparing the curves at 5 and 15 mol m⁻² intercepted PAR (Fig. 4.1).

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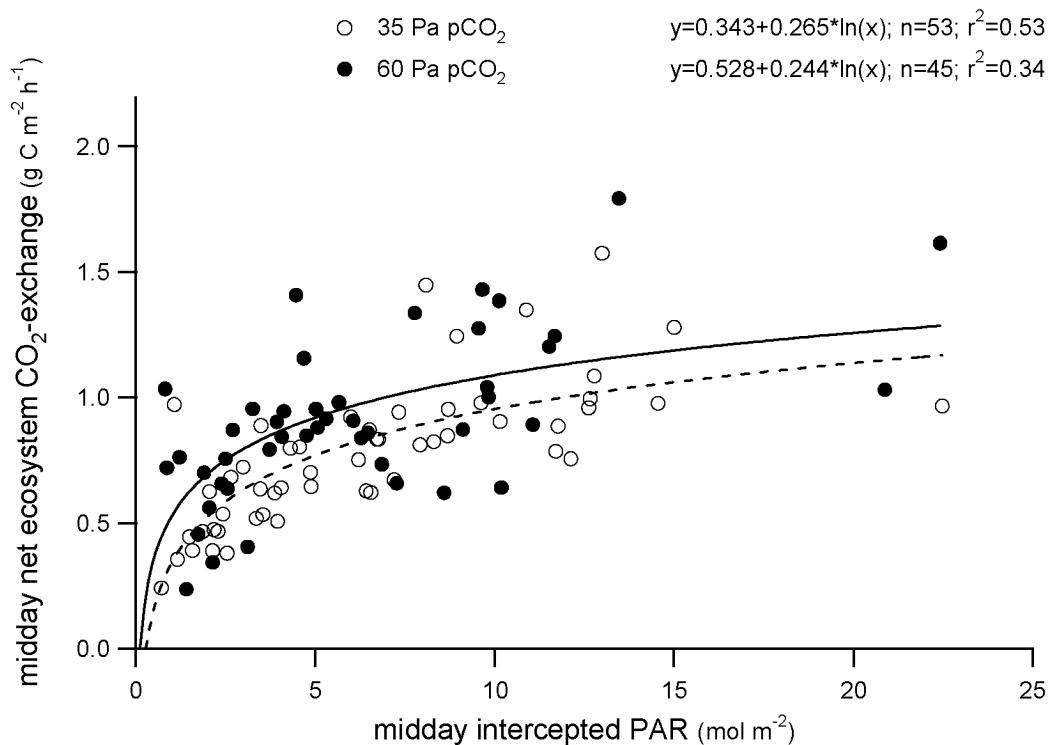


Figure 4.1a

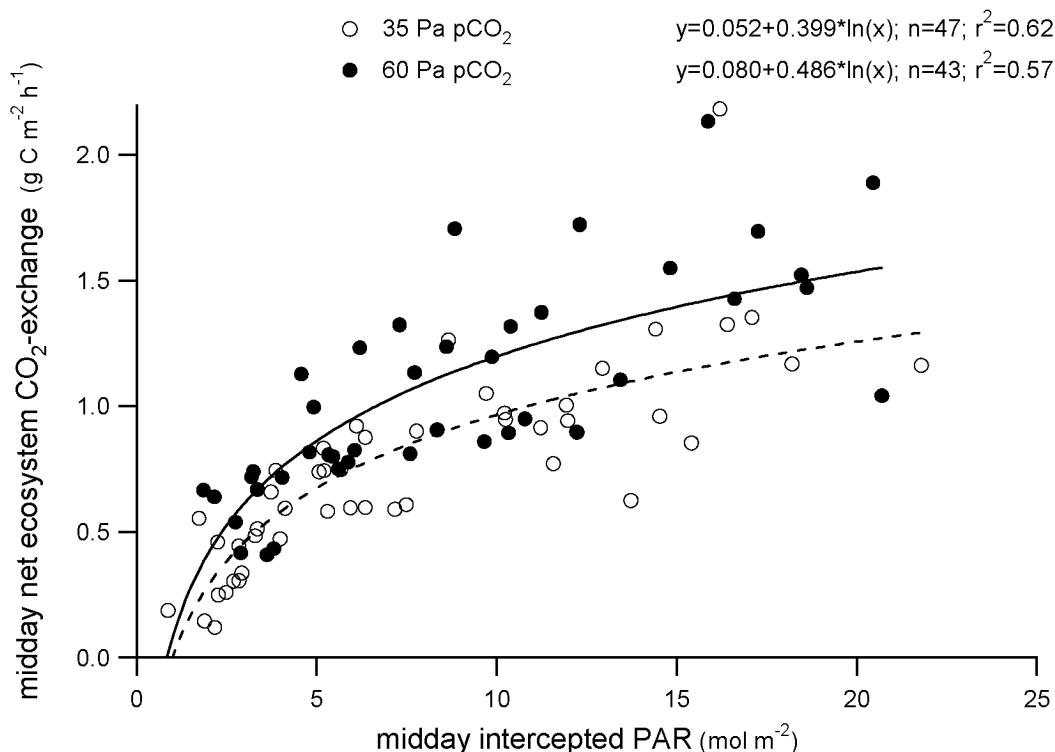


Figure 4.1 b Midday net ecosystem CO₂ exchange (g C m⁻² h⁻¹) with respect to cumulated midday intercepted PAR (mol m⁻²). a) *Lolium perenne* at low N supply (14 g N m⁻² a⁻¹), b) *L. perenne* at high N supply (56 g N m⁻² a⁻¹)

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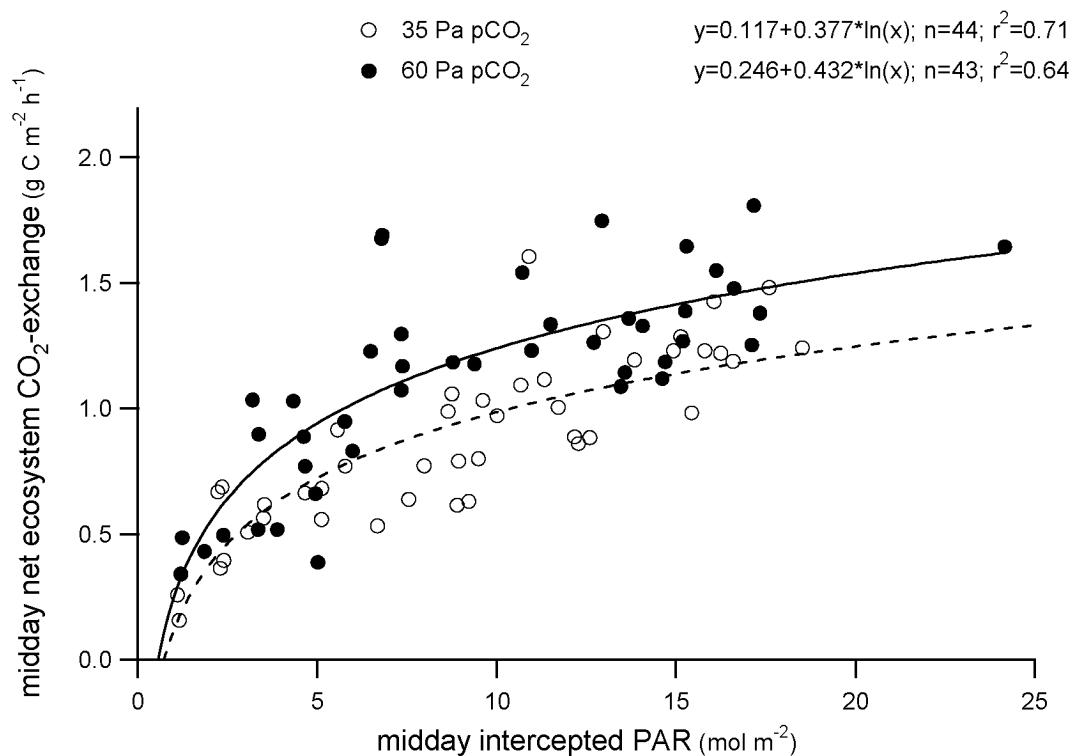


Figure 4.1c

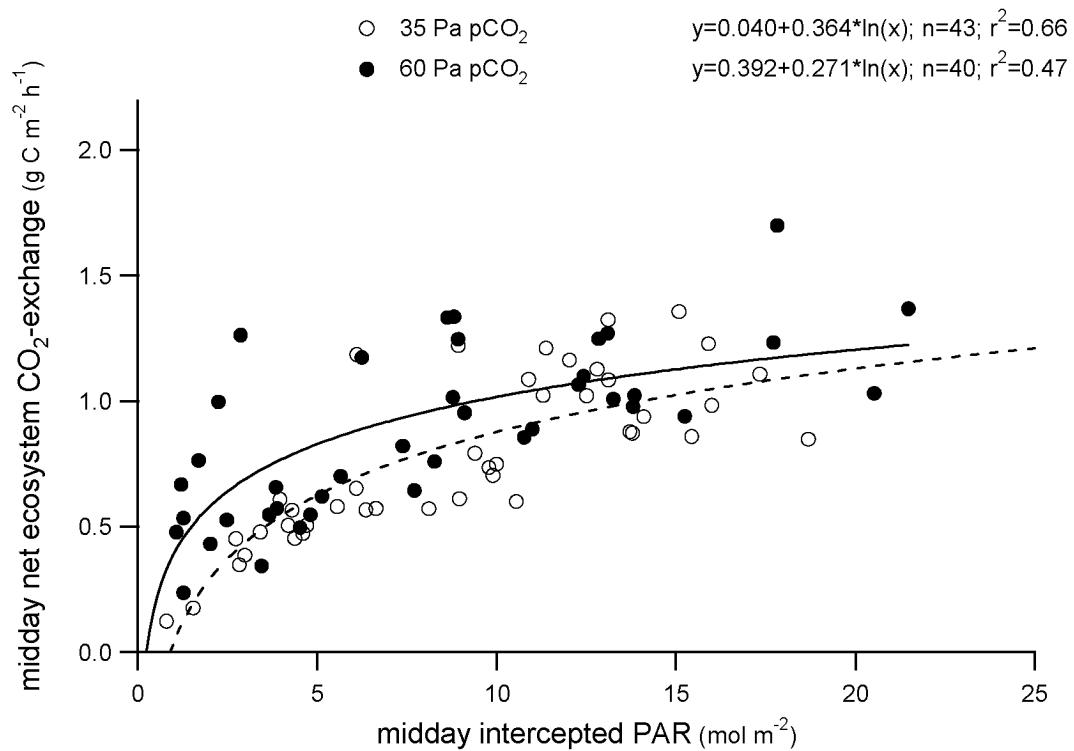


Figure 4.1d Midday net ecosystem CO₂ exchange (g C m⁻² h⁻¹) with respect to cumulated midday intercepted PAR (mol m⁻²). c) *Trifolium repens* at low N supply (14 g N m⁻² a⁻¹), d) *T. repens* at high N supply (56 g N m⁻² a⁻¹).

In *L. perenne*, when intercepted PAR was low (5 mol m⁻²), elevated pCO₂ increased the estimate of mNEE by 20% at low N supply and by 24% at high N supply. However, with increasing interception of PAR the relative differences between the CO₂ treatments decreased, especially at low N supply.

Elevated pCO₂ increased the estimates of mNEE by 12% at low N supply and by 23% at high N supply when intercepted PAR was high (15 mol m⁻²).

In the low-N treatment of *T. repens*, elevated pCO₂ increased the estimate of mNEE at 5 mol m⁻² of intercepted PAR by 30%. This stimulation decreased to 24% at 15 mol m⁻² of intercepted PAR. In the high-N treatment increasing interception of PAR reduced the stimulatory effect of pCO₂ on mNEE from 32 to 10%.

The nitrogen fertilization treatments affected the light response curve of mNEE ($p<0.05$). The species x N interaction ($p<0.01$; Table 4.2) shows that the response of mNEE to nitrogen fertilization differed between *L. perenne* and *T. repens*. In *L. perenne*, high N supply decreased the estimate of mNEE by up to 10% when the interception of PAR was low. On the other hand, when the interception of PAR was high, high N supply increased the estimate of mNEE up to 17% compared to low N supply. In *T. repens*, the N effect on the estimate of mNEE was negative, ranging from -10 to -20%.

4.4.2 Assimilation at different canopy layers

The *Lolium perenne* plants of the high-N treatment were ¹⁴C pulse labeled immediately before the first harvests in the growing seasons 2001 and 2002, and then cut at three heights above ground. At this growth stage, the plant canopy intercepted on average 93% of the incident PAR, irrespective of the pCO₂ level. The light transmission profile and the vertical LAI distribution of the canopy (Table 4.1) were not affected by elevated pCO₂.

¹⁴C-assimilation showed that canopy photosynthesis per unit leaf area and unit intercepted PAR increased in the three layers from near the soil surface to the top of the canopy ($p<0.0001$; Fig. 4.2). At ambient pCO₂, the increase from the layer

Midday net ecosystem CO₂ exchange

below 15 cm to the layer above 25 cm reached 420%; at elevated pCO₂ this increase was as high as 820%.

Elevated pCO₂ did not affect the assimilation per unit intercepted PAR and unit leaf area in the canopy layer below 15 cm. In the canopy layers above 15 cm, the assimilation per unit intercepted PAR and unit leaf area was up to 114% higher at elevated pCO₂ than at ambient pCO₂ ($p<0.05$). At both pCO₂ levels, most of the carbon (> 63%) was assimilated in the canopy layers above 15 cm (Table 4.1), where pCO₂ increased assimilation per unit leaf area and unit intercepted PAR.

Light response curves of individual leaves of *L. perenne* in the high-N treatment were measured before the first harvest in May in 2001 and 2002. A_{sat} and apparent quantum yield (ϕ_{app}) of leaves at the top of the plant canopy and of leaves lower in the canopy were determined. The photosynthetic potential was not affected by leaf position; A_{sat} (Fig. 4.3) and the apparent quantum yield of leaves in the upper and lower layers of the plant canopy were similar. However, elevated pCO₂ increased A_{sat} by 48% in the upper canopy and by 68% in the lower canopy ($p<0.0001$). Apparent quantum yield, measured at leaf level increased at elevated pCO₂ ($p<0.05$) by 33% in the upper canopy and by 50% in the lower canopy.

Table 4.1 Traits of three canopy layers of *Lolium perenne* at high N supply (first harvests in 2001 and 2002). Mean values \pm standard errors.

canopy layer (cm)	ambient pCO ₂			elevated pCO ₂		
	>25	25-15	15-05	>25	25-15	15-05
assimilated ¹⁴ C (% of whole canopy)	20 \pm 5	45 \pm 9	35 \pm 4	23 \pm 6	41 \pm 5	36 \pm 7
dry weight (g)	22 \pm 5	53 \pm 10	116 \pm 16	23 \pm 8	46 \pm 7	126 \pm 25
LAI	0.33 \pm 0.08	0.89 \pm 0.15	1.58 \pm 0.22	0.38 \pm 0.15	0.70 \pm 0.12	1.36 \pm 0.26
SLA (cm ² g ⁻¹)	164 \pm 9	200 \pm 9	229 \pm 7	146 \pm 8	170 \pm 9	188 \pm 14
leaf N (mg g ⁻¹)	35.6 \pm 1.4	37.0 \pm 1.0	36.5 \pm 1.0	29.2 \pm 1.8	31.6 \pm 1.2	32.3 \pm 0.9

Table 4.2 Significance probabilities resulting from analysis of variance of midday net ecosystem CO₂ exchange (mNEE) measurements using the variable “midday intercepted PAR” as a covariate.

	intercept	slope
CO ₂	p < 0.0001	n.s.
N	p < 0.05	n.s.
species	n.s.	n.s.
CO ₂ × N	n.s.	n.s.
CO ₂ × species	n.s.	n.s.
species × N	p < 0.01	p < 0.001
CO ₂ × N × species	n.s.	n.s.

4.4.3 Leaf nitrogen concentration

Lolium perenne leaves from the high-N treatment harvested in May in 2001 and 2002 were analyzed to determine their nitrogen concentration. At elevated pCO₂, leaf nitrogen concentration per unit mass was lower (p<0.05) than at ambient pCO₂ throughout the canopy (Table 4.1). The leaf position affected leaf N concentration only at elevated pCO₂, N concentration in leaves at the top of the canopy being lower than that in leaves low in the canopy (p<0.05).

4.4.4 Harvestable biomass

The harvestable biomass of *L. perenne* was considerably increased in the high-N treatment compared to the low N-treatment (p<0.01) at both pCO₂ levels. In the low-N treatment, harvestable biomass of *L. perenne* did not respond to CO₂ enrichment. The annual harvestable biomass at ambient pCO₂ reached 356 g m⁻² and at elevated pCO₂ it reached 348 g m⁻². In the high-N treatment elevated pCO₂ increased the annual harvestable biomass of *L. perenne* by 28%, but this difference

was not statistically significant. The annual harvestable biomass was 544 g m⁻² at ambient pCO₂ and 698 g m⁻² at elevated pCO₂.

Elevated pCO₂ increased the harvestable biomass of *T. repens* by 11 to 14%, irrespective of the nitrogen treatment; this difference was not statistically significant. The nitrogen treatments had no effect on harvestable biomass of *T. repens*. Annual harvestable biomass at ambient pCO₂ was 533 and 523 g m⁻² in the low-N and high-N treatment, respectively. At elevated pCO₂ annual harvestable biomass was 606 g m⁻² in the low-N treatment and 578 g m⁻² in the high-N treatment.

4.5 Discussion

4.5.1 Effects of elevated pCO₂ on midday net ecosystem CO₂ exchange (mNEE)

For the first time, the effects of elevated pCO₂ on the net CO₂ exchange of fertile grassland were investigated at the ecosystem level in a field-grown sward fumigated for nine growing seasons with CO₂. An important result of this experiment is that there was an increased capacity of carbon uptake in a grassland ecosystem that had previously been exposed to long-term CO₂ enrichment. Our results show that the assimilation of the two species, *Lolium perenne* and *Trifolium repens*, not only increased at elevated pCO₂ in the short term but the ecosystem carbon uptake capacity remained considerably increased for nine years. This finding is in line with results of (Stirling *et al.* 1997), who found no loss or even an increase of photosynthetic capacity among five herbaceous species after long-term exposure to elevated pCO₂. Ainsworth *et al.* (2003) found no evidence of a decrease in photosynthetic stimulation in *L. perenne* leaves over 10 years of CO₂ enrichment. Elevated pCO₂ also stimulated mNEE when the nitrogen supply was low, although nitrogen limitation was reported to promote acclimation (Drake *et al.* 1997). Sink limitation, which was especially severe at low N supply and towards the end of a regrowth

period, can restrict the CO₂ response of carbon assimilation in *L. perenne* (Fischer *et al.* 1997; Isopp *et al.* 2000b). In our experiment, this down-regulation was eliminated after cutting, because the sink potential was restored by harvesting (Rogers *et al.* 1998). As strong down-regulation of mNEE did not occur when the analysis was done over two growing seasons; sink limitation was apparently not important for mNEE in the sward studied.

Increased mNEE of *L. perenne* at elevated pCO₂ (Fig. 4.1a,b) is consistent with the results obtained from leaf photosynthesis investigations in the Swiss FACE experiment. The average rates of midday leaf photosynthesis of *L. perenne* were 35% higher under elevated pCO₂, irrespective of the N supply (Rogers *et al.* 1998; Isopp *et al.* 2000b).

The mNEE of *T. repens* largely increased at elevated pCO₂ (Fig. 4.1c,d), which is consistent with leaf photosynthesis data (Clark *et al.* 1999; E.A. Ainsworth, written communication). *T. repens* has the ability of symbiotic nitrogen fixation and, thus, nitrogen is not limiting and the plant can profit from additional CO₂ (Zanetti *et al.* 1996).

The mNEE per unit leaf area and unit intercepted PAR can be interpreted as being the efficiency of ecosystem assimilation. Considering the biomass data of previous studies in the Swiss FACE experiment (Hebeisen *et al.* 1997b; Daapp *et al.* 2000) the large increase in the efficiency of ecosystem assimilation at elevated pCO₂, led to a considerable increase in the harvestable biomass of *L. perenne* in the high-N treatment. On the other hand, despite the considerable increase in mNEE, elevated pCO₂ did not stimulate the accumulation of harvestable biomass in the low-N treatment. The additionally assimilated carbon obviously had a different fate than being transformed into harvestable biomass, especially when the nitrogen supply was low. The stimulating effect of elevated pCO₂ was more pronounced on total plant biomass than on harvestable biomass (Daapp *et al.* 2001). The root biomass (Soussana *et al.* 1996; Daapp *et al.* 2001) and the root-to-shoot ratio (Suter *et al.* 2002) were largely increased at elevated pCO₂. Above-ground residual biomass also increased markedly at elevated pCO₂ (Schneider 2003). A preferential allocation to the roots and soil of the extra carbon assimilated at elevated pCO₂ was also reported in the semi-field experiment conducted by Schapendonk *et al.* (1997).

Schneider (2003) showed that the pool of residual biomass increased more at elevated pCO₂ when the nitrogen supply was low. Our data support the idea that the CO₂-related changes in allocation are more pronounced at low N supply, because a large increase in leaf photosynthesis and in ecosystem assimilation efficiency did not lead to an increase in harvestable biomass under nutrient poor conditions.

The relative CO₂ effect on mNEE in *L. perenne* was higher when the amount of intercepted PAR was low, especially at low N supply. The measured intercepted PAR could not accurately account for the residual leaf area and its eventual differences between treatments. Above-ground residual biomass (Daupp *et al.* 2001) and residual leaf area (Stadelmann 1993) where increased at elevated pCO₂. Furthermore, the number of tillers increased at elevated pCO₂ in the first weeks after cutting (Suter *et al.* 2001), leading to a higher number of small leaves. Consequently, shortly after a cut, a higher residual leaf area, which was not reflected in intercepted PAR, enabled a higher mNEE at elevated pCO₂.

Multiple linear regressions showed that incident PAR and relative PAR interception were the only non-experimental factors that had a significant impact on mNEE. Precipitation summed for 72 hours before the measurement and the midday air temperature, as well as number of days after cut showed no obvious relation to mNEE. Nevertheless, together with spatial and temporal heterogeneity of the vegetation, these variables contributed to the quite large variation in mNEE that was not explained by intercepted PAR.

Productivity and canopy closure of our poorly N fertilized, managed *L. perenne* swards were comparable to those of unfertilized, permanent or semi-natural grassland. The CO₂ response of harvestable biomass was very small or absent in the managed (Hebeisen *et al.* 1997b) as well as in the natural or semi-natural system (Körner 2000). This indicates that the response of sown, fertilized grassland to elevated pCO₂ is not very different from that of semi-natural or permanent grassland.

4.5.2 N effects on midday net ecosystem CO₂ exchange (mNEE)

The mNEE of *L. perenne* and *T. repens* were affected differently by the nitrogen fertilization treatments (Table 4.2). The effect of nitrogen fertilization on mNEE of *L. perenne* depended on the amount of measured intercepted PAR. At low measured intercepted PAR, the mNEE increased in the low-N treatment when compared with the high-N treatment. When only a small amount of PAR was intercepted, leaf area index was usually low, too; below-ground respiration was, therefore, relatively more important under these conditions. Decreased below ground respiration at low N supply (Van Ginkel *et al.* 1997) has probably favored higher mNEE in the low-N treatment. Furthermore, higher residual leaf area in the low-N treatment (Schneider 2003), which is of importance shortly after the cut when measured interception of PAR was low, enabled higher mNEE.

When incident PAR and leaf area were high and, consequently, much PAR was intercepted, the high N supply had a positive effect on the mNEE of *L. perenne*. Under these conditions, the mNEE was obviously affected by mechanisms other than those which were important at low interception of PAR. Because of the greater above-ground biomass, the residual leaf area and below ground respiration became relatively less important. On the other hand, with increasing leaf area towards the end of a regrowth period, sink limitation may become important in the low-N treatment (Fischer *et al.* 1997; Isopp *et al.* 2000b). Furthermore, at low N supply, the leaf-N concentration was reduced (Zanetti *et al.* 1997). Drecer *et al.* (2000) showed that photosynthesis at light saturation is correlated with leaf-N concentration. Therefore, at low N supply, the photosynthesis of leaves at the top of the canopy was possibly reduced.

As expected, the N-fixing legume *T. repens* responded differently to the nitrogen fertilization treatments. In the low-N treatment, symbiotic nitrogen fixation increases (Zanetti *et al.* 1996) and compensates for the smaller amount of fertilizer nitrogen. Consequently, the production of harvestable biomass of *T. repens* does not respond to different nitrogen fertilization levels (Hebeisen *et al.* 1997b). It is, therefore, not surprising that mNEE of *T. repens* in the low-N treatment is quite similar to that of *L. perenne* in the high N-treatment. The negative response of

mNEE to high N fertilization was observed before (Lee *et al.* 2001). A higher sink for carbon in nodulated, poorly fertilized plants stimulated photosynthesis (Schulze *et al.* 1999); the simultaneous increase in root respiration would not completely compensate for this in mNEE.

4.5.3 Effects of leaf position on assimilation

The stratified clipping of the ¹⁴C-labeled canopy clearly showed, that leaves at the top of the plant canopy can assimilate much more carbon per unit intercepted PAR than leaves lower in the canopy (Fig. 4.2). ¹⁴C-assimilation of leaves at the top of the canopy nearly doubled at elevated pCO₂, whereas shaded leaves low in the canopy did not respond to elevated pCO₂. Therefore, only the upper layers of the canopy contributed to the effect of elevated pCO₂ on mNEE.

Leaf photosynthesis measurements showed that leaf position within the plant canopy did not affect A_{sat} and apparent quantum yield (ϕ_{app} ; Fig. 4.3). The increase in A_{sat} and ϕ_{app} at elevated pCO₂ is in line with Drake *et al.* (1997). Long (1991) showed that quantum yield in a C₃ sedge remained increased at elevated pCO₂ in the long-term and also in leaves adapted to light-limited conditions. As quantum yield is mainly determined by the light compensation point, the positive CO₂ response of apparent quantum yield indicates that the light compensation point was lower at elevated pCO₂.

Measurements of A_{sat} and ϕ_{app} revealed that elevated pCO₂ increased the potential rate of photosynthesis at all leaf positions in the canopy. However, in-situ ¹⁴C-assimilation was not stimulated by elevated pCO₂ low in the canopy when light was the limiting resource. When interception of PAR in the canopy was high, the mNEE measurement were most probably done at light saturation and, therefore, comparable to measurements of A_{sat} .

Under these conditions the positive effect of elevated pCO₂ on mNEE (Fig. 4.1b) was less pronounced than on A_{sat} (Fig. 4.3). Ainsworth *et al.* (2003) observed higher CO₂-induced stimulation of leaf photosynthesis than we found for mNEE. This was due in part to the fact that assimilation of shaded leaves low in the canopy did

not respond to elevated pCO₂ (Fig. 4.2). Additionally, increased root biomass (Soussana *et al.* 1996; Daupp *et al.* 2001) and, consequently, increased total root respiration may have lowered the CO₂ effect on mNEE compared to leaf photosynthesis. Reduced maintenance respiration, as often found at elevated pCO₂ (Drake *et al.* 1996), would not compensate for this.

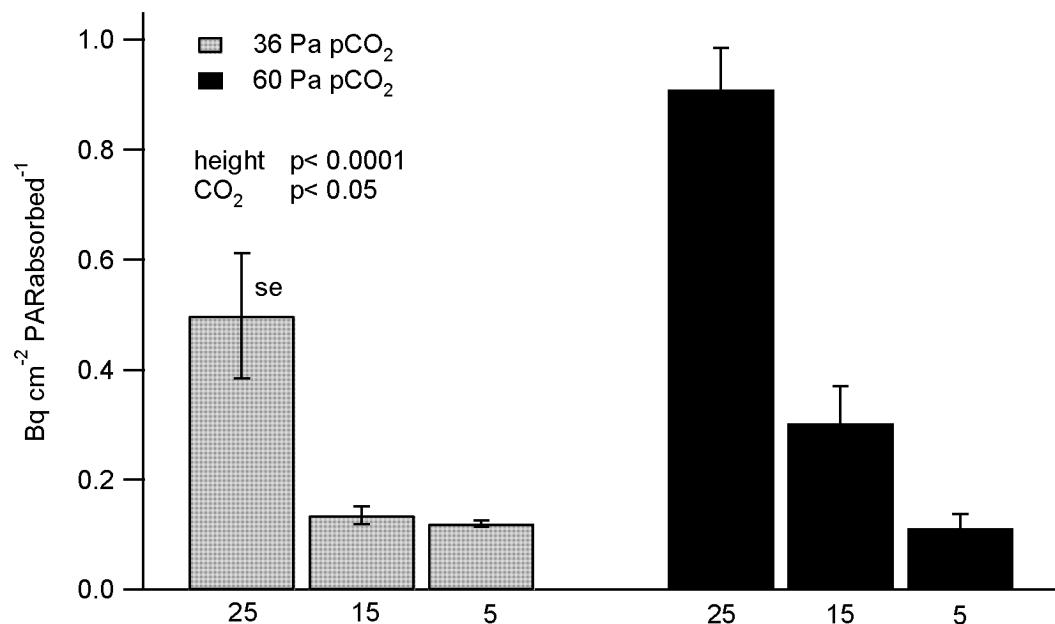


Figure 4.2 ¹⁴C activity (BQ) per leaf area (cm²) and intercepted PAR (µmol m⁻² s⁻¹) at different cutting heights (25, 15 and 5 cm). Means ± standard errors of the high-N treatment of *Lolium perenne* (first harvests in 2001 and 2002).

4.5.4 Effects of leaf N concentration on assimilation

Nitrogen concentration on a mass basis was reduced at elevated pCO₂ at all layers of the canopy, but this did not hinder an increase in photosynthesis. Reduction of the leaf nitrogen concentration at elevated pCO₂ is very common (Yin 2002). C₃ grasses grown at elevated pCO₂ show a greater nitrogen use efficiency (Davey *et al.* 1999), thus enabling them to maintain high rates of photosynthesis, despite of a lower nitrogen concentration.

Midday net ecosystem CO₂ exchange

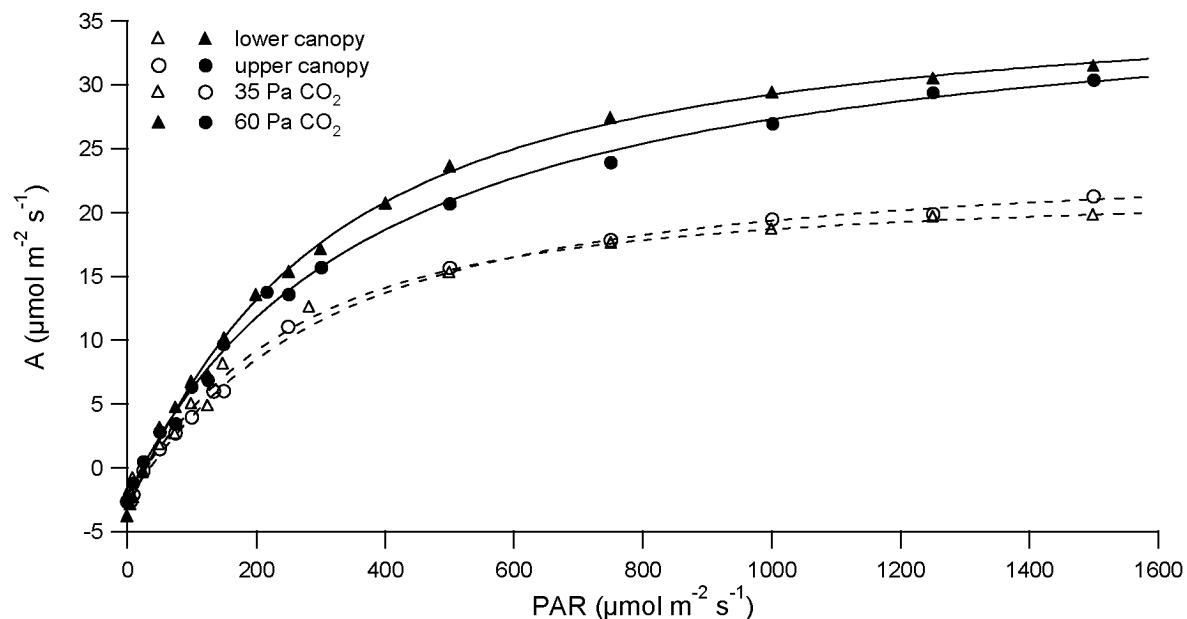


Figure 4.3 Average light response curves of leaf photosynthesis for *Lolium perenne* at high N supply, measured before the first cut in spring 2001 and 2002.

In summary this experiment showed that managed grassland ecosystems on fertile soil maintained increased midday net ecosystem CO₂ exchange at elevated pCO₂ for nine years. This is a prerequisite for an increasing biomass production and carbon sequestration. The CO₂ effect on midday net ecosystem CO₂ exchange was less pronounced than on individual leaf photosynthesis. This was due to the fact that CO₂ response of assimilation depended on leaf position, and only leaves in the upper canopy layers could benefit from elevated pCO₂.

5 Night-time ecosystem respiration (NER) in grassland as affected by long-term CO₂ enrichment and N fertilization

5.1 Abstract

Effects on night-time ecosystem respiration (NER) were investigated in managed *Lolium perenne* (perennial ryegrass) and *Trifolium repens* (white clover) monocultures that had been exposed for nine growing seasons to Free Air CO₂ Enrichment (FACE, 60 Pa pCO₂). Two levels of nitrogen (N) fertilization (14 and 56 g m⁻² a⁻¹) were applied to the swards that were cut five times a growing season. NER was measured in six periods in 2000, 2001 and 2002 using an open-flow chamber system.

Mean night-time ecosystem respiration (NER) was 0.185 g C m⁻² h⁻¹ in *L. perenne* and 0.219 g C m⁻² h⁻¹ in *T. repens*. Compared to low N supply, high N supply increased NER by 36 and 39% in *L. perenne* and 20 and 0% in *T. repens* at ambient and elevated pCO₂, respectively. Elevated pCO₂ increased NER in *L. perenne* by 39 and 31% at low N and high N, respectively. In *T. repens* NER was increased at elevated pCO₂ by 39% at low N and 16% and high N. The CO₂ response of NER was not significantly affected throughout the growing season by factors such as soil temperature or plant canopy closure. However, the effect of N supply and species on NER varied between periods of measurement.

Higher plant and microbial biomass at elevated pCO₂ were most probably the causes for the increased NER. Effects such as decreased tissue protein concentration and inhibition of enzyme activity that may reduce specific respiration appeared to be of minor importance for night-time respiration at ecosystem level.

Since the CO₂ fluxes leaving the ecosystem were higher at elevated pCO₂ the higher net CO₂ uptake during day-time will at least in part be compensated for, resulting in a reduction or dissipation of additional carbon sequestration in grassland ecosystems exposed to elevated pCO₂.

5.2 Introduction

Ecosystem respiration is an integrated measure of autotrophic plant respiration and heterotrophic microbial respiration. Since about half of the C assimilated by photosynthesis of terrestrial plants is released as CO₂ during subsequent plant respiration (Amthor 1997), the rate of autotrophic respiration has a major effect on the carbon balance of ecosystems. Soil respiration is a major carbon source, with about 10% of the atmosphere's CO₂ passing through soils each year (Raich & Potter 1995). In young forests, soil respiration contributed 67 to 70% (Valentini *et al.* 2000; Xu *et al.* 2001) to ecosystem respiration. Maximum rates of ecosystem respiration in forests were not related to the leaf area index but to the age of the stand (Buchmann & Schulze 1999). In grassland systems the effects of clipping and grazing (Bremer *et al.* 1998), ecophysiology (Craine *et al.* 1999) and environmental conditions (Mielnick & Dugas 2000) on soil CO₂ efflux have been investigated in several studies. However, little information is available on respiration at ecosystem level in grasslands, e.g. Franzluebbers *et al.* (2002), and effects of elevated partial pressure of atmospheric CO₂ (pCO₂) on ecosystem respiration have scarcely been investigated in any terrestrial ecosystem.

Plant respiration is closely linked to growth and photosynthesis. A higher carbon availability to plants caused by increasing pCO₂ enables higher rates of photosynthesis at leaf level. After ten years of CO₂ enrichment under field conditions, leaf photosynthesis at elevated pCO₂ in a grassland remained higher at both high and low levels of nitrogen supply (Ainsworth *et al.* 2003), showing that photosynthetic acclimation to elevated pCO₂ was only small. Although N availability has little direct effect on photosynthetic rates, nitrogen is often the

limiting resource for plant growth. Consequently, the CO₂ response of harvestable plant biomass was smaller than that of leaf photosynthesis and depended on nitrogen supply (Daupp *et al.* 2000). Obviously, under low N conditions the additional photosynthates assimilated at elevated pCO₂ are not necessarily transformed into harvestable biomass. Allocation of photosynthates to residual below- and above-ground biomass is increased at elevated pCO₂ (Daupp *et al.* 2001) and higher root biomass (Suter *et al.* 2002; Schneider 2003) and exudation may stimulate carbon input to the soil compartment. On the other hand, higher carbon losses of the ecosystem at elevated pCO₂ caused by higher rates of respiration probably reduce soil carbon input and the CO₂ response of biomass.

In theory, pCO₂ can affect respiration directly or indirectly. A direct effect of elevated pCO₂ on respiration may be inhibition of cytochrome-c-oxidase (Gonzalez-Meler *et al.* 1996). More importantly, elevated pCO₂ can affect respiration indirectly through changes in biomass growth and protein content (Amthor 1997). Specific leaf respiration tends to be reduced at elevated pCO₂ (Schapendonk *et al.* 1997) whereas at the whole plant level or on a ground area basis respiration often increases due to higher biomass (Casella & Soussana 1997). Little is known about the interacting effects of pCO₂, N and species as well as about possible effects of environmental conditions and plant development on the respiratory CO₂ response.

Because grasslands cover 24% of the terrestrial surface (Sims & Risser 2000) and have a large carbon storage capacity, they are of special interest with regard to effects of elevated pCO₂. This work aimed at identifying the effects of elevated pCO₂ and N supply on ecosystem respiration, which are determining whether grasslands can act as an additional carbon sink when pCO₂ is rising. For this purpose we investigated the total night-time respiration of a grassland ecosystem that was exposed to elevated (60 Pa) and ambient (36 Pa) pCO₂ for nine years. The interactions between pCO₂ and two levels of N supply (14 and 56 g m⁻² a⁻¹) were studied in monocultures of *Lolium perenne* and *Trifolium repens*. Additionally we investigated how night-time ecosystem respiration and its response to pCO₂ was affected by soil temperature and plant canopy closure during the course of the

growing season. Night-time ecosystem respiration was determined over three different growing seasons using an open-flow chamber system.

5.3 Material & Methods

5.3.1 Experimental site and treatments

The experiment was conducted in the Swiss grassland FACE array at Eschikon ($8^{\circ}41'E$, $47^{\circ}27'N$) near Zurich, at an altitude of 550 m above sea level. Using Free Air Carbon-dioxide Enrichment (FACE) technology (Hendrey 1992) allows the investigation of the long-term effects of elevated pCO_2 on ecosystems in the field without changing the microclimate. CO_2 -fumigation during the daylight hours was begun in May 1993 and lasted each year for the whole growing season (from March to November) at day-time. A more detailed account of the experimental set-up is given by Hebeisen *et al.* (1997b).

The experiment was arranged in three blocks, each consisting of a circular CO_2 -enriched area (60 Pa pCO_2) with a diameter of 18 m and an ambient area as control (36 Pa pCO_2) of the same size.

Lolium perenne cv. Bastion and *Trifolium repens* cv. Milkanova, two important species of managed grassland in temperate and humid climates, were grown as monocultures in 5.3 m^2 plots since 1993. All plots were fertilized each year with $5.5\text{ g P m}^{-2}\text{ a}^{-1}$ and $24.1\text{ g K m}^{-2}\text{ a}^{-1}$. The amount supplied was considered to be non-limiting for plant growth under the experimental conditions (Daupp *et al.* 2000). The swards were cut five times per growing season at a height of about 5 cm above-ground. Two levels of N fertilization (14 and $56\text{ g m}^{-2}\text{ a}^{-1}$) were applied in order to examine the effects of resource availability on the CO_2 response of the ecosystem. The N-fertilizer was applied as liquid NH_4NO_3 at the beginning of each regrowth. The fertilizer was divided between the five successive regrowth periods in the proportions 30, 20, 20, 15 and 15%, these percentages corresponding to the expected yields at the end of each period.

5.3.2 Measurements and data collection

Night-time ecosystem respiration (NER) was measured during the growing seasons in 2000, 2001 and 2002 using an open-flow chamber system. The system consisted of two identical and independent units, each including two chambers. This setup allowed us to make simultaneous measurements under both N treatments and at both pCO₂ levels. The chambers remained installed on the same experimental treatment for one to five days, and were then moved between species and blocks.

The chambers consisted of an aluminium framework which was covered with Teflon (PTFE) film. One side, where the gas inlet and outlet were placed, was made of Plexiglas. The chambers covered a square area of 0.49 m², and were 0.6 m high. To achieve a complete seal of the chamber at ground level, it was fixed to a steel frame that was inserted into the soil. The air that was to be passed over the vegetation was sampled with a vertical tube 7 meters above ground level in order to minimize short term variability of pCO₂. Fans then blew the air through flexible PVC tubes into the chambers. A mass flow meter (Accu-Flo 600, Sierra Instruments, Monterey, CA USA) placed in the tubes measured the air flow. A small fan was placed in each chamber in order to assure a thorough mixture of the air. During night-time, the air within the chambers was exchanged about once in a minute. In the fumigated areas, the sampled air was enriched with CO₂ during the day-time, so that pCO₂ within the chambers reached a level similar to the FACE conditions of about 60 Pa. At night, as in the FACE experiment, the air within the chambers was the same as the ambient CO₂ concentration.

CO₂ exchange within the chambers was measured by sampling air at the inlet and the outlet of the chambers. This air was pumped through flexible PE-tubes to an infra-red gas analyzer (Binos 100 4P, Fisher-Rosemount, Hasselroth, Germany) which measured the difference in CO₂ concentration. In order to determine the air humidity, the air samples were pumped in heated PTFE tubes to a dewpoint sensor (MTR 2.0, IL Metronic, Ilmenau-Unterpörlitz, Germany).

For biomass determination, the harvested plant material was oven-dried for 48 h at 65 °C. In order to get an indication of above-ground standing biomass during regrowth, relative light transmission through the canopies was measured about

weekly during the growing season with a Sunfleck Ceptometer (SF-40, Decagon, Pullman, WA USA).

Meteorological data were obtained from the weather station next to the experimental site at the research station Eschikon.

5.3.3 Calculation and statistical analysis

The ecosystem respiration rates in the chambers, averaged for 10 minutes intervals, were integrated to a seven-hour (10 p.m. to 5 a.m.) total. During this time period respiration in full darkness was relatively constant so that mean values could be used to investigate trends between treatments and during the growing season.

The statistical analysis of the experimental factors was carried out using the Mixed procedure of SAS 8.02. The model was a split-plot with $p\text{CO}_2$ as the main plot factor, thus block and block \times $p\text{CO}_2$ were tested as random effects. As block \times $p\text{CO}_2$ has only two degrees of freedom, the split-plot model requires a high F-value for the main plot factor $p\text{CO}_2$ to be significant. Denominator degrees of freedom were adjusted according to the method of Kenward-Rogers (Littell *et al.* 1996).

Prior to statistical analysis NER data were Log_e -transformed in order to obtain normal distribution. Data of midday net ecosystem CO_2 exchange (mNEE) on the preceding day were only available in a sub-set of the whole data set. Therefore, the effect of mNEE on NER was tested in this sub-set only, using mNEE as a covariate in the Mixed model. A multiple linear regression model was applied to Log_e -NER data, including the explaining variables “soil temperature” and “relative PAR absorption of the canopy”. Those variables which were not statistically significant ($p > 0.05$) were excluded using backward elimination.

5.4 Results

5.4.1 Night-time ecosystem respiration as affected by $p\text{CO}_2$ and N

Mean night-time ecosystem respiration (NER) between 10 p.m. and 5 a.m. measured during six periods (spring 2001 and 2002, summer 2000 and 2001, autumn 2000 and 2001) ranged between 0.062 and 0.459 g C m⁻² h⁻¹. The period of measurement had significant effect on NER ($p<0.0001$), with highest NER in summer 2001 and lowest NER (in 7 out of 8 treatments) measured in autumn 2000 (Table 5.1). NER in *T. repens* was consistently higher than in *L. perenne* (Table 5.1; $p<0.001$). Depending on experimental treatments, this difference ranged from 1% to 30% and was 18% across all treatments. The species response of NER was influenced by the period of measurement ($p<0.01$). The difference in NER between *T. repens* and *L. perenne* fluctuated between 7% in summer 2000 and 45% in spring 2001.

Nitrogen supply strongly affected NER (Table 5.1; $p<0.0001$), leading to increased NER at high N supply. In *L. perenne* high N supply stimulated NER by 36% at ambient $p\text{CO}_2$ and by 29% at elevated $p\text{CO}_2$. In *T. repens* high N supply only increased NER at ambient $p\text{CO}_2$ (by 20%). These results show that NER of the legume and of the grass species differed considerably in their responses to N supply (N x species: $p<0.01$). The period of measurement also affected the N-response of NER (N x period: $p<0.0001$), indicating that it may depend on changes in environmental conditions and in plant physiology (e.g. allocation of photosynthates or reproductive growth). The N-response of NER in *L. perenne* was small or absent (-7 to 12%) in the spring periods, and largest (> 100%) during autumn 2000.

Elevated $p\text{CO}_2$ markedly stimulated NER (Table 5.1; $p<0.01$). The relative CO_2 response of NER was slightly higher at low N supply than at high N supply (CO_2 x N: $p<0.05$). In *L. perenne* elevated $p\text{CO}_2$ led to a mean stimulation of NER of 39% at low N supply and of 31% at high N supply. In *T. repens* elevated $p\text{CO}_2$ increased NER by an average of 39% at low N supply and by 16% at high N supply. These results demonstrate that NER of both species responded comparably to elevated

pCO₂ (CO₂ x species: not significant). Remarkably, the CO₂ response of NER did not vary significantly between the periods of measurement (CO₂ x period: not significant).

5.4.2 Night-time ecosystem respiration as affected by assimilation, soil-temperature and relative light absorption

Night-time ecosystem respiration (NER) was positively correlated with midday net ecosystem CO₂ exchange of the preceding day ($p<0.0001$, Fig. 5.1). NER of *T. repens* responded stronger to changes in mNEE than NER of *L. perenne* ($p<0.0001$). In order to investigate whether factors affecting respiration varied seasonally, a multiple linear regression model was applied to the whole data-set, including the explaining variables “soil temperature”, and “relative PAR absorption” (a measure of canopy closure, Table 5.2). In *L. perenne* NER was positively correlated with soil temperature throughout the growing season with highest regression coefficients in summer. This correlation was stronger at low N supply than at high N supply. In *T. repens*, NER was positively correlated with soil temperature during summer only. In spring, NER in *L. perenne* was positively correlated with the relative PAR absorption of the canopy at high N supply only. In summer, this correlation was negative at both N levels and in autumn it was absent. In *T. repens*, NER was positively correlated with relative PAR absorption in spring and autumn but not in summer.

5.5 Discussion

This experiment was carried out after nine growing seasons of continuous CO₂ enrichment and is therefore indicative of long-term effects of elevated pCO₂ and N supply on ecosystem processes. The results show that elevated pCO₂ as well as high N supply led to a marked stimulation of night-time ecosystem respiration (NER).

Table 5.1 Means of night-time ecosystem respiration (10 p.m. to 5 a.m.) in six periods of measurement, total means, standard errors (s.e.) and number of measurements (n). Significance probabilities of statistical analysis for the total means.

Species	N ($\text{kg m}^{-2} \text{a}^{-1}$)	pCO_2 (Pa)	Respiration ($\text{g C m}^{-2} \text{h}^{-1}$)												
			Spring		Summer		Autumn		Mean		s.e.		n		
			2001	2002	2000	2001	2000	2001	2000	2001	2000	2001	2000		
<i>L. perenne</i>	14	35	0.167	0.169	0.108	0.228	0.062	0.117	0.136	0.009	99				
		60	0.221	0.206	0.142	0.355	0.088	0.152	0.189	0.014	83				
<i>T. repens</i>	56	35	0.187	0.175	0.178	0.311	0.134	0.147	0.185	0.012	100				
		60	0.233	0.192	0.242	0.389	0.179	0.211	0.243	0.012	81				
<i>T. repens</i>	14	35	0.260	0.196	0.093	0.326	0.104	0.158	0.176	0.011	88				
		60	0.370	0.277	0.170	0.516	0.130	0.263	0.245	0.019	82				
CO_2												p < 0.01			
N												p < 0.0001			
species												p < 0.0001			
period												p < 0.0001			
$\text{CO}_2 \times$ species												n.s.			
$\text{CO}_2 \times$ N												p < 0.05			
$\text{CO}_2 \times$ period												n.s.			

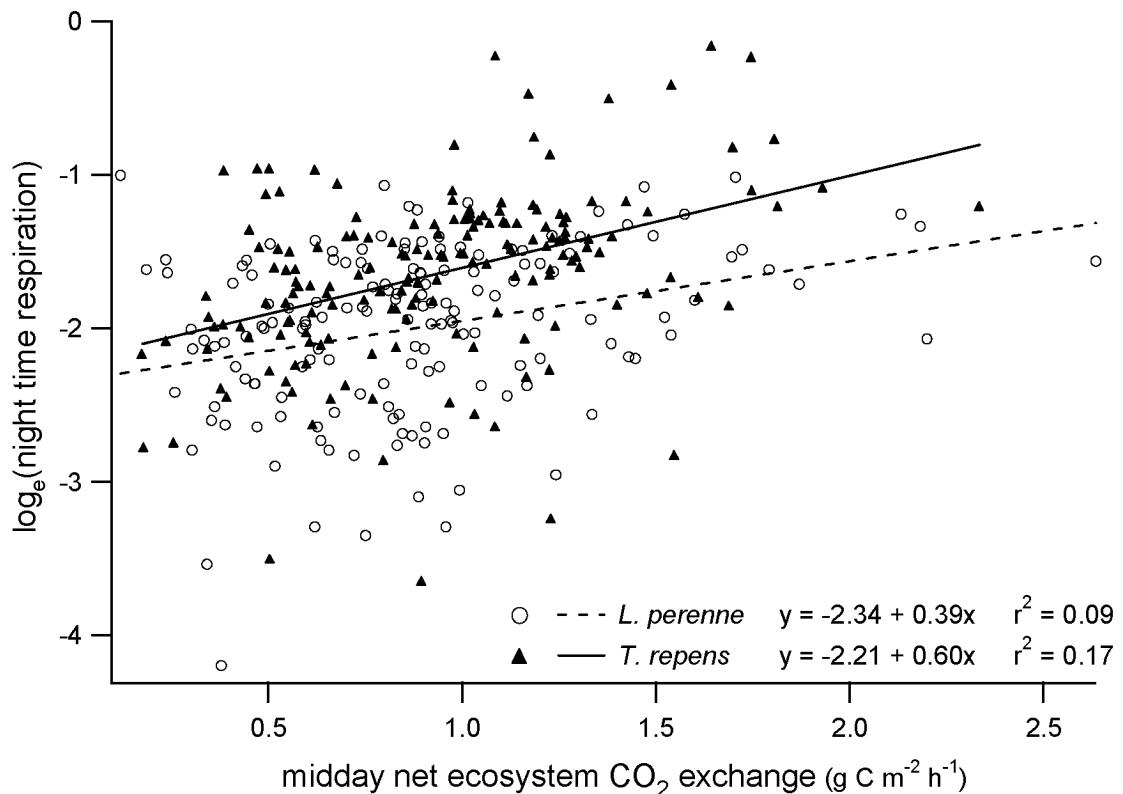


Figure 5.1 Linear regression between midday net ecosystem CO₂ exchange (mNEE) and log_e-transformed night-time ecosystem respiration (NER)

5.5.1 Elevated pCO₂ strongly increased NER

NER of the two species *L. perenne* and *T. repens* was increased at elevated pCO₂; this stimulation was higher at low N supply than at high N supply (Table 5.1).

Elevated pCO₂ has direct and indirect effects on plant respiration but indirect effects are most probably of greater importance (Amthor 1997). Direct effects of elevated pCO₂ are leading to decreased specific plant respiration, which has been shown to be correlated with inhibition of the respiratory enzymes cytochrome-c-oxidase and succinate dehydrogenase (Drake *et al.* 1999). Nevertheless, a direct causal link between reduced specific respiration and enzyme inhibition is doubtful and mechanisms that could explain this result remain unclear. Since we observed a large increase of NER at elevated pCO₂, direct effects that would reduce respiration are probably of minor importance in our system.

Elevated pCO₂ increased night-time degradation of starch in *T. repens* leaves and subsequent translocation (Fischer 1998) and the export of carbohydrates in starch-storing species accounted for a large fraction of nocturnal respiration (Bouma *et al.* 1995). Consequently, enhanced content and higher export rates of non-structural carbohydrates of leaves at elevated pCO₂ may have increased respiration, especially in *T. repens*.

Maintenance respiration per unit phytomass tends to be reduced at elevated pCO₂ due to lower tissue protein concentration and a higher C-to-N ratio (Amthor 1997). However, at the whole-plant level or on a ground-area basis, a decrease in specific shoot respiration at elevated pCO₂ (Schapendonk *et al.* 1997) was compensated by a higher biomass, resulting in similar or increased total respiration (Casella & Soussana 1997). These results indicate that plant growth and biomass are the most important factors affecting ecosystem respiration. *T. repens* responded to elevated pCO₂ at both N levels with a large increase of harvestable biomass (Hebeisen *et al.* 1997b; Richter 2003). In *L. perenne* harvestable biomass responded positively to elevated pCO₂ at high N supply but remained unaffected at low N supply (Daupp *et al.* 2000). This response pattern of harvestable biomass is not consistent with that of NER, indicating that NER in *L. perenne* may be more dominated by processes related with residual biomass (roots and stubble) than by production of harvestable biomass.

Contrary to harvestable biomass, total biomass of *L. perenne* increased at elevated pCO₂ independent of levels of N supply (Daupp *et al.* 2001). In *L. perenne* as well as in *T. repens* higher root growth at elevated pCO₂ (Jongen *et al.* 1995) contributed to the increase in total biomass. Schneider (2003) showed that in *L. perenne* residual above- and below-ground biomass increased largely at elevated pCO₂ and more pronounced at low N supply. Since the response of residual plant biomass to elevated pCO₂ is similar to that of NER, respiration of these plant parts is suggested to account for an important fraction of NER. This is in accordance with Ham *et al.* (1995), where in a tallgrass prairie a large fraction of ecosystem respiration originated from roots and soil.

In spring wheat, 75% of below-ground respiration originated from roots (Kuzyakov & Cheng 2001) and in grassland about 40% (Craine *et al.* 1999);

showing that below-ground respiration can largely be determined by roots. However, microbial biomass, which is also a component of below-ground respiration, increased at elevated pCO₂ in the Swiss-FACE experiment (Sowerby *et al.* 2000) and must have contributed to the higher respiration. Similarly, in a tallgrass prairie, Williams *et al.* (2000) found increased microbial activity at elevated pCO₂. Schortemeyer *et al.* (1996) and Montealegre *et al.* (2002) reported pCO₂ related changes in the soil microbial population structure below *T. repens*, which may also affect respiration.

The CO₂ effect did not vary significantly between the six periods of measurement (Table 5.1). Extreme conditions such as drought (Naumburg *et al.* 2003) and low temperatures (Long 1994) can reduce the CO₂ response of photosynthesis and growth. Due to the link between growth and respiration (Fig. 5.1) these conditions most probably also reduce the CO₂ response of respiration.

Luo *et al.* (1996) showed that CO₂ effects on below-ground respiration in a California grassland were smallest under very dry conditions. Since we found no effect of the period of measurement on the CO₂ response of NER, the variability of the climatic conditions at the experimental site was apparently too small to evoke such effects.

5.5.2 NER was markedly affected by N supply

Respiration of the two species differed in their response to N-fertilization levels. *L. perenne* swards showed a markedly increased respiration at high N supply whereas the N-response of *T. repens* was weak or even absent (Table 5.1). This species specific response to N supply is probably due to the symbiotic nitrogen fixation of *T. repens*, whose growth and hence growth-related respiration, are largely independent of N fertilization. In contrast, high N supply increased total biomass and N content of *L. perenne* (Daapp *et al.* 2001), which promoted both total and specific respiration.

Leaf turnover is faster at high N as growth is less or not at all N limited (Craine & Reich 2001), and one can also assume that root turnover is faster at high N supply.

The faster turnover of plant biomass would consequently favour increased rates of respiration when N supply is high. Consistent with this assumption, Van der Werf *et al.* (1993) found higher rates of specific root and shoot respiration in four grass species grown at high N supply. In our experiment, microbial activity and biomass may have been greater at high N supply shortly after application of fertilizer, leading temporarily to higher heterotrophic respiration.

The effect of N on NER was influenced by the period of measurement and was strikingly small in spring. Possibly, a high decomposition of roots during reproductive growth (Troughton 1957) led to a larger contribution of below-ground respiration during this period. This could have reduced the effect of N supply on NER, because root biomass and its senescence are little affected by N supply. Additionally, in early spring, low soil temperatures may have limited the microbial response to high N supply.

5.5.3 NER was species specific

Across all treatments mean NER was 2 to 56 mg C m⁻² h⁻¹ higher in *T. repens* than in *L. perenne* (Table 5.1). There seem to be different factors contributing to this difference. First, tissue nitrogen and protein concentration is higher in *T. repens*. According to Wullschleger *et al.* (1992) this may increase specific maintenance respiration. Second, symbiotic nitrogen fixation has higher carbon costs than nitrate reduction and consequently may increase below-ground respiration in *T. repens*, despite of lower root biomass compared to *L. perenne*. Additionally, the nocturnal degradation of starch and subsequent export from *T. repens* leaves (Fischer 1998) is an costly process in terms of energy.

The mean NER across all treatments (0.136 to 0.245 g C m⁻² h⁻¹) obtained in our experiment were comparable to those of Franzluebbers *et al.* (2002) in a tallgrass prairie (0.235 g C m⁻² h⁻¹). Maximum rates of night-time ecosystem respiration of deciduous forests (0.264 g C m⁻² h⁻¹) and C₃ crops (0.151 g C m⁻² h⁻¹) were in the same magnitude, too (Buchmann & Schulze 1999). These results demonstrate that respiration of various temperate ecosystems may differ by not more than a factor

of two. However, our results show that in a grassland ecosystem NER can be considerably affected by plant species composition.

5.5.4 Midday net ecosystem CO₂ exchange (mNEE) was affecting NER

Since NER was increased at elevated pCO₂, the higher mNEE at elevated pCO₂ observed in the Swiss FACE experiment (Chapter 3) will at least in part be compensated, consequently resulting in a reduction or dissipation of additional carbon sequestration.

Our measurements showed that midday net ecosystem CO₂ exchange (mNEE) of the preceding day was positively associated with NER (Fig. 5.1). This suggests that due to fast transport of photosynthates to the roots, respiration is linked to the availability of recently assimilated carbon. This hypothesis is supported by Kuzyakov & Cheng (2001) who found total below-ground CO₂ efflux in spring wheat to be closely coupled with photosynthesis. Respiration utilizes 40 to 60% of the carbon imported to roots (Van der Werf 1996) and as a consequence, Craine *et al.* (1999) found the soil CO₂ flux in a Minnesota grassland to be largely controlled by the availability of photosynthates to the roots. However, McCutchan & Monson (2001) showed that leaf respiration of perennial herbaceous species is not necessarily controlled by the availability of carbohydrates.

5.5.5 Seasonality of effects of canopy and soil temperature on NER

NER was positively correlated with soil temperature throughout the growing season for *L. perenne* and in summer for *T. repens* (Table 5.2). This was attributed to enhanced maintenance respiration (Gifford 1995) and stimulated microbial activity at higher soil temperatures. In accordance to this, increasing soil temperature stimulated soil CO₂ flux in a tallgrass prairie (Mielnick & Dugas 2000).

NER was negatively correlated to relative PAR absorption of the canopy during summer in *L. perenne*. In contrast, in *T. repens* NER increased with increasing relative PAR absorption during spring and autumn, but remained unaffected during summer (Table 5.2).

Table 5.2 Results of the multiple linear regression on night-time ecosystem respiration including soil temperature (Soil-T) and relative PAR absorption (Rel. abs.). Values are representing partial regression coefficients and correlation coefficients (R^2). Levels of significance: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Species	N (kg m ⁻² a ⁻¹)	Spring				Summer				Autumn			
		Soil-T	Rel. abs.	R^2	Soil-T	Rel. abs.	R^2	Soil-T	Rel. abs.	Soil-T	Rel. abs.	R^2	Soil-T
<i>L. perenne</i>	14	1.07*	n.s.	0.11	2.88***	-1.09***	0.58	1.29*	n.s.	n.s.	n.s.	0.07	
	56	n.s.	0.48*	0.13	2.26***	-0.43*	0.46	0.71*	n.s.	n.s.	n.s.	0.08	
<i>T. repens</i>	14	n.s.	0.68***	0.30	4.07**	n.s.	0.20	n.s.	1.04***	0.17			
	56	n.s.	0.65***	0.40	1.73**	n.s.	0.18	n.s.	0.72***	0.21			

In summer, NER of *L. perenne* was highest when soil coverage was low, indicating that an important proportion of total ecosystem respiration could be attributed to below-ground respiration. Shortly after cutting a large fraction of roots are decomposing (U. Zimmermann, personal communication), leading to high below-ground respiration. Furthermore, the N fertilizer application probably has temporarily increased microbial activity that may be greatest in summer due to highest soil temperatures.

The species differed in their response to soil temperature and relative PAR absorption, suggesting that their night-time respiration was influenced differently by a variety of factors. One explanation could be their difference in biomass allocation. Below-ground biomass allocation and root-to-shoot ratio are much higher in the grass species *L. perenne*; and the relative importance of root respiration is likely to be greater in this species. Therefore, factors affecting root respiration will be more important in *L. perenne*, whereas factors affecting above-ground respiration will be more important in *T. repens*. Other explanations may be different seasons of reproductive growth, alternative ways of nitrogen acquisition and contrasting storage carbohydrates. The export of fructans in *L. perenne* may be less energy-demanding and function at a different time scale than the degradation of starch and subsequent export in *T. repens*.

5.6 Conclusion

Nitrogen supply stimulated night-time ecosystem respiration markedly, showing that a faster turnover rate may increase respiratory losses in highly fertile grassland systems. Effects of soil temperature and canopy closure on NER varied between seasons and were species specific. Elevated pCO₂ induced a large increase of NER, demonstrating that at the ecosystem level indirect effects of CO₂ enrichment such as higher biomass and microbial activity will dominate over possibly existing direct effects on enzyme metabolism. Across the periods of measurement the effect of pCO₂ was rather stable; this may have important implications in a changing climate.

Since the nocturnal CO₂ fluxes leaving the ecosystem were enhanced at elevated pCO₂, the higher net CO₂ uptake during day-time will at least in part be compensated for, resulting in a reduction or dissipation of additional carbon sequestration in grassland ecosystems.

6 Approach of a grassland ecosystem carbon balance

Terrestrial ecosystems are assumed to act as carbon sinks that may mitigate the increase in atmospheric pCO₂ (Amthor 1995). Because grasslands cover large land areas and their soils have a high carbon storage capacity, they may considerably contribute to this carbon sink.

Potential forces driving increased net ecosystem carbon input at elevated pCO₂ include higher photosynthesis, preferential allocation to residual plant biomass and reduced respiration as a result of changes in decomposition processes. In the Swiss FACE experiment, assimilation at leaf level (Ainsworth *et al.* 2003) and at ecosystem level (Chapter 4) were considerably greater after ten or nine growing seasons of CO₂ fumigation, indicating that photosynthetic acclimation to elevated pCO₂ was only small. Consequently, assimilation would have raised a potential for additional net C input. Daeppe *et al.* (2001), Suter *et al.* (2002) and Schneider (2003) showed that growth at elevated pCO₂ increased the allocation to below-ground and residual above-ground biomass. However, if the increase in assimilation was matched by a similar increase in respiration, the higher C influxes do not necessarily lead to a higher carbon sequestration into the soil. Furthermore, other factors such as nitrogen availability and species composition may also affect the net C input in grassland.

Changes in soil carbon pool sizes are often not detectable with direct measurements because of the relatively small changes in relation to the large amount of organic carbon in grassland soils. By contrast, changes in the net carbon input based on ecosystem CO₂ fluxes are more readily detected, because gas exchange measurements are not affected by variability of below-ground carbon pool sizes.

6.1 Calculation

The net ecosystem carbon balance is the net CO₂ uptake during the photoperiod minus the night-time respiration and the carbon exported by harvests. The ecosystem carbon balance of the Swiss FACE experiment was established using the net ecosystem CO₂ exchange data presented in the Chapters 4 & 5. For technical reasons an insufficient number of reliable 24-hour-measurements were obtained in my experiment. Therefore, the carbon balance was calculated from the midday net ecosystem CO₂ exchange (mNEE) and night-time ecosystem respiration (NER), representing the two major components of net ecosystem CO₂ exchange (Fig. 6.1). The ecosystem carbon balance was established for the growing season 2001, because data of relative PAR absorption of the plant canopy were available for every regrowth period of that year.

Net ecosystem CO₂ uptake during the photoperiod was estimated using the equations for mNEE in dependency on intercepted PAR (Fig. 4.1). For every day during the growing season mNEE was calculated from the sum of intercepted PAR during midday (11 a.m. to 3 p.m.). The available diurnal measurements showed that averaged over the growing season the net CO₂ uptake during the midday period represented 50% (39 to 64%) of the net CO₂ uptake during the photoperiod. Therefore, the net CO₂ uptake of the midday period was multiplied by two to obtain an estimate of the daily net CO₂ uptake during the photoperiod. The growing season (beginning of April to end of October) included 210 days.

Night-time respiration was estimated using the mean value for the 2001 growing season. Throughout the growing season, these data showed no strong correlations with any measured variables. Consequently, accounting for them in the calculation would not have significantly improved the estimate of NER. The total NER of the growing season was obtained by multiplying the NER per hour by the average length of the dark period during the growing season (9.97 h) and by the number of days.

Before choosing the method outlined above for estimating the C balance, several other methods of calculation and different bases of data were evaluated. Similar conclusions were obtained about the response of the ecosystem carbon balance to N and pCO₂ using all procedures. Thus, despite the inaccuracies associated with the estimates presented here, I believe they provide a useful picture of the overall trends in the C balance.

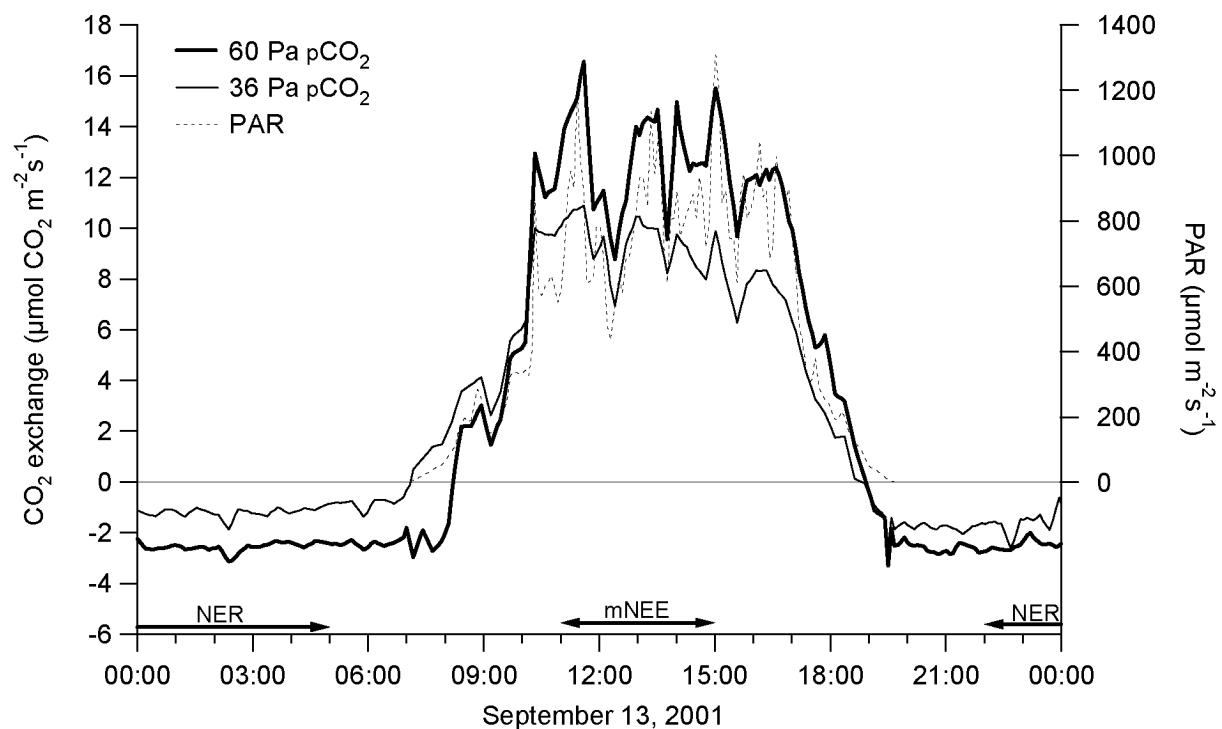


Figure 6.1 Example of a diurnal of ecosystem CO₂ exchange in a highly fertilized *Lolium perenne* sward exposed to ambient and elevated pCO₂. Arrows at the bottom indicate the periods when midday net ecosystem CO₂ exchange (mNEE) and night-time ecosystem respiration (NER) were determined.

6.2 Results

Like mNEE (Chapter 4), the day-time net CO₂ exchange per growing season also increased at elevated pCO₂. In *L. perenne* elevated pCO₂ stimulated day-time net CO₂ exchange by 253 and 291 g C m⁻² a⁻¹ at low and high N supply, respectively. In

T. repens the CO₂ response of day-time net CO₂ exchange was 264 and 205 g C m⁻² a⁻¹ at low and high N supply, respectively. Thus, compared to low N supply, high N supply led to slightly lower net CO₂ exchange during the photoperiod. This negative response to N supply decreased day-time net CO₂ exchange by 138 and 100 g C m⁻² a⁻¹ in *L. perenne* and 103 and 162 g C m⁻² a⁻¹ in *T. repens* at ambient and elevated pCO₂, respectively.

In general, the 2001 season responses of NER to pCO₂ and N were similar to those for mean NER (Table 5.1). Elevated pCO₂ increased NER by 197 and 157 g C m⁻² a⁻¹ in *L. perenne* and by 279 and 143 g C m⁻² a⁻¹ in *T. repens* at low and high N supply, respectively. High N supply increased NER by 101 and 61 g C m⁻² a⁻¹ in *L. perenne* and by 44 and -92 g C m⁻² a⁻¹ in *T. repens* at ambient and elevated pCO₂, respectively.

In *L. perenne* carbon exports by harvest were slightly increased at elevated pCO₂ (7 and 32 g C m⁻² a⁻¹ at low and high N, respectively). In *T. repens*, elevated pCO₂ had a negative effect on the amount of harvested carbon at low N supply (54 g C m⁻² a⁻¹) but a positive one (46 g C m⁻² a⁻¹) at high N supply.

The carbon imports and exports listed below affected net ecosystem carbon balance as follows. In the growing season 2001, elevated pCO₂ was estimated to slightly stimulate the net carbon input to the ecosystem (Table 6.1) but this effect was statistically not significant. In *L. perenne*, this increase was 49 (9%) and 102 g C m⁻² a⁻¹ (50%) at low and high N supply, respectively. In *T. repens*, the net C input at elevated pCO₂ was increased by 32 (8%) and 9 g C m⁻² a⁻¹ (3%) at low and high N supply, respectively.

The estimated net carbon input was found to be markedly higher at low N supply than at high N supply ($p < 0.0001$). High N supply reduced net ecosystem carbon input by 360 (64%) and 308 g C m⁻² a⁻¹ (50%) in *L. perenne* and by 100 (27%) and 123 g C m⁻² a⁻¹ (31%) in *T. repens* at ambient and elevated pCO₂, respectively. In *L. perenne* the net ecosystem C input was higher than in *T. repens* ($p < 0.01$).

Table 6.1 Net ecosystem carbon balance for the growing season 2001. Means \pm standard error

Species	N	pCO ₂	Day-time net C uptake (g C m ⁻² a ⁻¹)	Night-time respiration (g C m ⁻² a ⁻¹)	Harvested C (g C m ⁻² a ⁻¹)	Net ecosystem C input
						(g C m ⁻² a ⁻¹)
<i>L. perenne</i>	14	36	1062	341	155	566 \pm 29
	14	60	1315	538	162	615 \pm 31
	56	36	924	442	277	205 \pm 83
	56	60	1215	599	309	307 \pm 57
<i>T. repens</i>	14	36	1072	479	220	372 \pm 48
	14	60	1336	758	174	404 \pm 30
	56	36	969	523	174	272 \pm 15
	56	60	1174	666	228	281 \pm 45

6.3 Discussion

6.3.1 Effects of elevated pCO₂ on net C input were marginal

The net ecosystem carbon input tended to be higher at elevated pCO₂, especially in *L. perenne* at high N supply, but the differences were not statistically significant.

Van Kessel *et al.* (2000) showed that the fraction of newly fixed C in soil of the Swiss FACE experiment only increased during the first three years of the experiment. Then, the labile C pool, which accounted for 20 to 30% of soil organic C was completely turned over. Total soil organic C (0 to 10 cm) that was approximately 3 kg C m⁻² was not significantly affected by elevated pCO₂ in 1998 after six years of CO₂ enrichment (Van Kessel *et al.* 2000). However, in *T. repens*, there was an increase in soil organic carbon of 390 and 150 g C m⁻² at elevated pCO₂ at low and high N supply, respectively; but due to large variation between replicates this difference was not statistically significant. Mean soil organic carbon stocks (1 m depth) for grasslands in Switzerland were estimated to range between 6 and 12 kg C m⁻² depending on management and soil type (Leifeld *et al.* 2003). Consequently, despite of being not significant on an annual basis, an additional increase of 50 and 102 g C m⁻² a⁻¹ in *L. perenne* (at low and high N, respectively), as I

estimated for 2001, would have considerably enhanced the soil carbon content after ten years of CO₂ enrichment. Nevertheless, after six and eight years no change in soil organic C was found.

In an experiment with *L. perenne* at elevated pCO₂, Casella & Soussana (1997) reported an increase of the net ecosystem carbon input of 140 and 270 g C m⁻² per growing season at low and high N supply, respectively. My findings show the same trend as their results: the net carbon input induced by elevated pCO₂ was greater at high N supply than at low N supply. However, contrary to my estimates, the effect of CO₂ was more pronounced and statistically significant. This difference may be related to the age of the swards and to differences in the growing conditions. The data of Casella & Soussana (1997) were obtained under controlled conditions in a newly established experiment, whereas our field experiment was already running for eight years when the carbon balance was calculated. Schneider (2003) and Richter (2003) showed that elevated pCO₂ caused feedback mechanisms in the soil in the long-term and nitrogen availability changed in the course of years. Just after a step increase of pCO₂ at the beginning of an experiment, the CO₂ response of net ecosystem carbon input may be higher than in an ecosystem that is adapted to elevated pCO₂. The soil carbon content is unlikely to increase linearly in the course of years, negative feedbacks and replenishment of the pool can be expected, which bring the ecosystem to a new equilibrium.

The data of Van Kessel *et al.* (2000) as well as my estimated C balance suggest that there was no considerable additional C sequestration induced by elevated pCO₂. As reported in Chapter 5, increased ecosystem respiration dissipated more or less completely any additional net C input.

In some years, e.g. in 2001 for *L. perenne*, there may have been a tendency to a higher net C input at elevated pCO₂ but this is probably not a consistent trend in the long-term. Schimel *et al.* (2000) showed that the US carbon sink strongly varies from year to year as a result of climate variability. Thus, one has to be aware that data obtained from one or two growing seasons will not give a definitive answer to the question of long-term carbon sequestration. My results suggest that growing conditions in 2001 may have been more favorable for carbon sequestration under elevated pCO₂ than in an average year.

6.3.2 High N supply decreased net C input

Net ecosystem carbon input was considerably higher at low N supply than at high N supply (Table 6.1). Whereas after six years of pCO₂ enrichment Van Kessel *et al.* (2000) observed no differences in total soil C between the N supply levels, there was a trend evident, though not statistically significant, to more total soil C at low N supply in 2000 (Van Groenigen *et al.* 2002). Eight years after starting the experiment the total C content of the soil at low N supply was 300 and 700 g C m⁻² greater than at high N supply, at ambient and elevated pCO₂, respectively. This difference is consistent with the carbon balances estimates presented here which indicate that at least in the last years there was more C sequestration at low N supply. A more positive carbon balance at low N supply is also consistent with the results of Casella & Soussana (1997) who measured a net ecosystem C input per growing season at ambient pCO₂ of 440 and 280 g C m⁻² at low and at high N supply, respectively.

The higher net C input at low N supply was attributed to similar day-time net CO₂ uptake in combination with decreased night-time respiration and less harvested biomass (Table 6.1). Midday net ecosystem CO₂ exchange (mNEE) was lower at high N supply than at low N supply for low amounts of intercepted PAR (Fig 4.1). This relation was inverse when interception of PAR was high. Since throughout the growing season intercepted PAR was more often in the lower range, average mNEE of both N supply levels were quite similar.

The apparent effect of N on the net C input may be reinforced by the methodology of calculation. As already discussed in Chapter 4, there may be greater residual leaf area after cut at low N supply than at high N supply but this was not reflected in the measurements of intercepted PAR. Therefore, mNEE per intercepted PAR at low N supply would have been overestimated in the first days after cut, thus leading also to an overestimate of net carbon input at low N supply.

Compared to photosynthesis, growth was more nitrogen limited at low N supply. The resulting discrepancy between assimilation and growth favored accumulation of carbohydrates in leaves (Fischer *et al.* 1997; Isopp *et al.* 2000a). A large proportion of these nonstructural carbohydrates were exported to stubble and

roots. Decomposition of this residual plant biomass constitutes a carbon flux to the soil that can potentially lead to carbon sequestration. Above-ground necromass in *L. perenne* was higher at low N supply than at high N supply (Blum *et al.* 1997; Schneider 2003). In *L. perenne* low N supply increases the ratio of residual to harvestable biomass (Daupp *et al.* 2001), leading absolutely and relatively to smaller carbon exports by harvests. These factors positively affect the ecosystem C balance at low N supply.

However, these results do not imply that natural unfertilized vegetations always represent a greater carbon sink than nitrogen rich agricultural grasslands. A severe nitrogen limitation in natural grassland could inhibit any CO₂ response of the assimilation (Stitt & Krapp 1999) and consequently of the carbon balance. On the other hand, excessive N supply seems to increase disproportionately carbon exports by respiration and by harvest or grazing and therefore, to reduce net ecosystem C input compared to moderate N supply.

6.3.3 Net C input differed between species

The net ecosystem carbon input was slightly higher in *L. perenne* than in *T. repens*, particularly at low N supply (Table 6.1). This may be due to the higher root biomass of *L. perenne* that builds the potential for a carbon flux to the soil. Additionally, the night-time respiration of *L. perenne* was lower than in *T. repens* (Chapter 5), causing smaller carbon losses from the ecosystem. As a consequence of these species specific differences in the C balance, CO₂ and N-related changes in the species composition (Hebeisen *et al.* 1997a; Lüscher *et al.* 1998; Navas *et al.* 1999) would affect the net ecosystem carbon input.

6.4 Conclusion

Elevated pCO₂ clearly increased net ecosystem CO₂ exchange during day-time as well as night-time respiration. Because both processes were affected, there was no

significant increase of net ecosystem carbon sequestration. N supply was of minor importance for day-time net CO₂ exchange but strongly affected night-time respiration, resulting in more net carbon input at low N supply. Consequently, in respect to the ecosystem carbon balance, effects of N supply dominated over effects of pCO₂.

7 General discussion

7.1 Elevated pCO₂ increased ecosystem CO₂ fluxes, but N supply affected the net ecosystem C input

The Swiss FACE experiment is the longest running field experiment investigating effects of elevated pCO₂ on managed grassland on fertile soil. This experimental array offered a great advantage because the ecosystem was continuously exposed to elevated pCO₂ for nine growing seasons and the results obtained are therefore indicative of the long-term effects of elevated pCO₂ on ecosystem processes.

As shown in Chapter 4, elevated pCO₂ clearly increased (up to 24%) midday net ecosystem CO₂ exchange (mNEE) in the long-term in *Lolium perenne* and *Trifolium repens* at both levels of N supply. These results indicate that in field experiments photosynthetic acclimation to elevated pCO₂ is also in the long-term of minor importance.

Elevated pCO₂ strongly stimulated assimilation in leaves at the top of the canopy, whereas assimilation was not affected by pCO₂ in lower leaves. This leaf position effect on the assimilatory CO₂ response resulted in a smaller effect of elevated pCO₂ on mNEE than on photosynthesis of individual leaves measured in the same experimental site by Ainsworth *et al.* (2003).

The night-time ecosystem respiration (NER) in grassland was markedly increased (up to 39%) at elevated pCO₂ in both species and at both low and high N supply (Chapter 5). The response of NER to elevated pCO₂ did not vary significantly throughout the growing season, indicating that it did not depend on environmental conditions and stage of plant development. Higher total plant- and microbial biomass were most probably the main causes for higher NER at elevated pCO₂.

General discussion

Effects such as decreased tissue protein concentration and inhibition of enzyme activity, which may reduce specific respiration, were obviously of minor importance for respiration at the ecosystem level.

Elevated pCO₂ tended to increase net ecosystem C input but this small effect was not statistically significant. The increased net CO₂ uptake at elevated pCO₂ during day-time was mostly compensated for by a higher night-time respiration. N supply clearly affected the net ecosystem C input. Because night-time respiration, but not day-time assimilation, increased at high N supply, the net ecosystem C input was larger at low N supply than at high N supply.

These results demonstrate that elevated pCO₂ clearly increased the carbon fluxes (CO₂ uptake and release) in a managed grassland ecosystem but had little effect on the ecosystem carbon balance that was primarily affected by the amount of supplied N.

7.2 Ecosystem level effects on the assimilatory CO₂ response - consequences for harvestable biomass

The relatively large increase of mNEE at elevated pCO₂ indicates that even after nine years acclimation of photosynthesis to elevated pCO₂ was weak. This result is in agreement with photosynthesis measurements at individual leaves by Ainsworth *et al.* (2003) at the same site. In *L. perenne* at low N supply, the sink for C was reduced when N limitation became important towards the end of a regrowth period and photosynthesis was down-regulated (Isopp *et al.* 2000b). Biomass removal by cutting restored the sink strength and eliminated the photosynthetic acclimation (Rogers *et al.* 1998), demonstrating that management has an important effect on the photosynthetic CO₂ response.

The response of harvestable biomass to elevated pCO₂ was lower than expected from the increase of individual leaf photosynthesis, particularly at low N supply.

At the top of the canopy, where individual leaf photosynthesis usually was measured, assimilation increased greatly at elevated pCO₂, while low in the canopy the assimilatory response to elevated pCO₂ was absent (Chapter 4). These contrasting effects are most probably related to vertical heterogeneity of light conditions and leaf age. Leaves apparently gain no advantage of elevated pCO₂ when light is the limiting resource. Additionally, it is probable that photosynthesis of older leaves, which have a lower sink activity than expanding leaves, responds less to elevated pCO₂. As a consequence, extrapolation from photosynthesis data from individual leaves results in an overestimation of the CO₂ effect of assimilation at the canopy level.

Although N availability has little direct effect on photosynthetic rates, nitrogen is often the limiting resource for plant growth. This limitation is highest under elevated pCO₂ at low N supply when not all photosynthates can be transformed into structural biomass, leading to an accumulation of non-structural carbohydrates (Stitt & Schulze 1994). These compounds are exported to stubble and roots and are not necessarily used later on for additional production of harvestable biomass. In legumes or under high N supply harvestable biomass responded to elevated pCO₂, because N limitation of growth was here of minor importance.

At elevated pCO₂, there is preferential allocation to residual biomass due to changed source-sink relations (Daepp *et al.* 2001; Schneider 2003). The higher fraction of non-photosynthetic plant tissue resulted in increased carbon losses by plant respiration (Chapter 5). Consequently, this had a negative effect on the CO₂ response of harvestable biomass.

The leaf position effect on the assimilatory CO₂ response, N limitation of growth, and increased respiration due to more residual biomass were the main causes for the relatively small increase of harvestable biomass at elevated pCO₂.

7.3 Intra- and inter-ecosystem variability of respiration

Night-time respiration (NER) was strongly enhanced by high N supply, and in *T. repens* NER was higher than in *L. perenne* (Chapter 5). This nitrogen and species dependency caused NER to range between 136 and 245 mg C m⁻² h⁻¹. Across different ecosystems, respiration was lowest (117 mg C m⁻² h⁻¹) in broadleaf evergreen forests and highest (359 mg C m⁻² h⁻¹) in C₄ crops (Buchmann & Schulze 1999). The data presented here suggest that intra-ecosystem variation of respiration may be almost as large as variability between ecosystems. My results (Chapter 5) suggested that NER is higher in legumes than in grasses, presumably because of symbiotic nitrogen fixation and differences in storage carbohydrates. Symbiotic nitrogen fixation in legumes is more energy demanding than nitrate reduction in grasses and is consequently associated with higher respiration. Furthermore, degradation of starch in *T. repens* and subsequent translocation is a highly energy demanding, nocturnal process (Fischer 1998) while fructan translocation in grasses probably requires less energy and is not strongly associated with the night period. Soil organic C content and management are additional factors that affect respiration. The soil CO₂ flux of grassland and crops was found to be two to three times higher in peat soil than in mineral soil (Lohila *et al.* 2003), most likely caused by higher organic C content of the peat soil. Cutting or grazing reduced soil respiration in a tallgrass prairie compared to unmanaged conditions due to biomass reduction (Bremer *et al.* 1998).

These results show that the influence upon carbon losses of factors such as species composition, the nutrient status and the management of an ecosystem may be as significant as the ecosystem type itself. This has important implications for carbon losses from terrestrial ecosystem in the context of land-use change.

In my experiment, variability of NER throughout the growing season was considerable and may be attributed to several factors. Firstly, soil temperature has a large effect on heterotrophic respiration and consequently soil respiration (Mielnick & Dugas 2000). Secondly, carbohydrate availability, which is determined by the assimilation rate, affects both plant- and microbial respiration. Finally, throughout

the growing season, the cutting regime causes strong dynamics in growth rates and standing biomass and these in turn determine growth- and maintenance respiration.

One could argue that night-time respiration was probably not representative for respiration during the day. However, Grahammer *et al.* (1991) showed that day-time and night-time soil respiration in a grassland were not significantly different when the soil water content exceeded a minimal value. The same may not be true for above-ground plant respiration. In any case, it was not necessary to extrapolate from night-time to day-time respiration because the net ecosystem CO₂ exchange measurements accounted for respiration during the photo-period.

7.4 Net ecosystem carbon input as affected by land-use change and time – Is the FACE a carbon sink?

Lal (2003) suggested that appropriate land-use change may enhance carbon sequestration and consequently mitigate the increase of atmospheric pCO₂. It is estimated that after a change in land use from arable crops to grassland or forest the carbon sink is increased for a period of 50 to 70 years (Sauerbeck 2001). Before the Swiss FACE experiment started the site was grown with annual crops. Therefore, the considerable net C input in the Swiss FACE experiment calculated for the growing season 2001 (Table 6.1) may be related to the land-use change eight years earlier.

A comparison of the results of Van Kessel *et al.* (2000) and Van Groenigen *et al.* (2002) shows clearly that the total C content under a managed grassland tends to increase in the time course of years. Within two years, the C content of the soil (0 to 10 cm) apparently increased by up to 800 g C m⁻² (27%). However, one has to be aware that differences caused by methodology cannot completely be excluded. Casella & Soussana (1997) calculated a net input of 280 to 580 g C m⁻² per growing season, which is comparable to my estimates of 205 to 615 g C m⁻² a⁻¹ (Table 6.1). All these estimates exceed by far the calculation of Watson *et al.* (2000) and Leifeld

et al. (2003) who predicted that conversion of arable land to grassland would lead to an annual sequestration of 50 to 100 g C m⁻² a⁻¹. One has to be aware that the C sequestration will decrease in the time course, caused by C saturation of the soil (Sauerbeck 2001). Flanagan *et al.* (2002) showed that the carbon balance of ecosystems is strongly influenced by weather conditions and therefore, varies between years. In 2001, favorable growing conditions may have led to more C being sequestered than the long-term average.

The net C input calculated on a growing season basis may overestimate the annual net C input. Volk & Niklaus (2002) showed that respiration rates in a calcareous grassland were considerable (\sim 150 g C m⁻²) during winter time but they were not affected by elevated pCO₂. Fluxes of gases other than CO₂ may be affected by elevated pCO₂, e.g. CH₄ (Ineson *et al.* 1998) but the amounts involved are too small to make a significant contribution to the ecosystem carbon balance.

Modeling the effects of rising pCO₂ on carbon sequestration in the USA has led to the suggestion that effects of land-use change may be larger than effects of pCO₂ (Schimel *et al.* 2000). This hypothesis is supported by my results: the net ecosystem carbon input was mainly affected by N supply and plant species and only to a small extent by pCO₂.

7.5 Needs for further research

Based on the available results it would be of great interest to investigate the following questions:

- I. How large are the relative contributions of the different CO₂ sources (shoot, root, microbes) to total ecosystem respiration and how are these CO₂ fluxes affected by elevated pCO₂?

Total ecosystem respiration was found to be increased at elevated pCO₂, but it remains unknown if shoot, root and microbial respiration respond equally. Since biomass allocation was affected by elevated pCO₂, leading to a higher

root-to-shoot ratio, it is reasonable to assume that root respiration, as a proportion of total respiration, will be higher, too. The stable isotope technique using ^{13}C and ^{18}O in the combination with ecosystem CO_2 exchange measurements may elucidate these processes (Amundson *et al.* 1998). CO_2 originating from below ground (root and microbes) has a different $^{18}\text{O}:\text{O}^{16}$ isotopic ratio than CO_2 originating from shoot respiration. When growing plants on a soil previously grown with C_4 -plants or exposed to ^{13}C -depleted CO_2 , the $^{13}\text{C}:\text{O}^{12}$ isotopic ratio of CO_2 originating from microbial respiration is different from that associated with root respiration.

- II. Can grassland sequester additional carbon in the long-term when pCO_2 is increasing and how large would this sink be? This question could not definitely be answered with the data presented here. Incorporation of the available data into ecosystem models and further experiments are needed to answer this question.
- III. Why was the net C input higher when N supply was low? Possibly, N availability to plants is primarily determining microbial activity (Hodge *et al.* 2000) and thus, low N supply suppresses the decomposition of plant residues. The question concerning competition between microbes and plants for nutrients merits further attention.
- IV. Is the difference between the CO_2 fluxes of *L. perenne* and *T. repens* typical for grasses and legumes? Experiments with other species of these functional groups would give further information on this. It would also be possible to use non-fixing mutants of legumes in order to test the effect of N_2 -fixation on the C fluxes.

8 References

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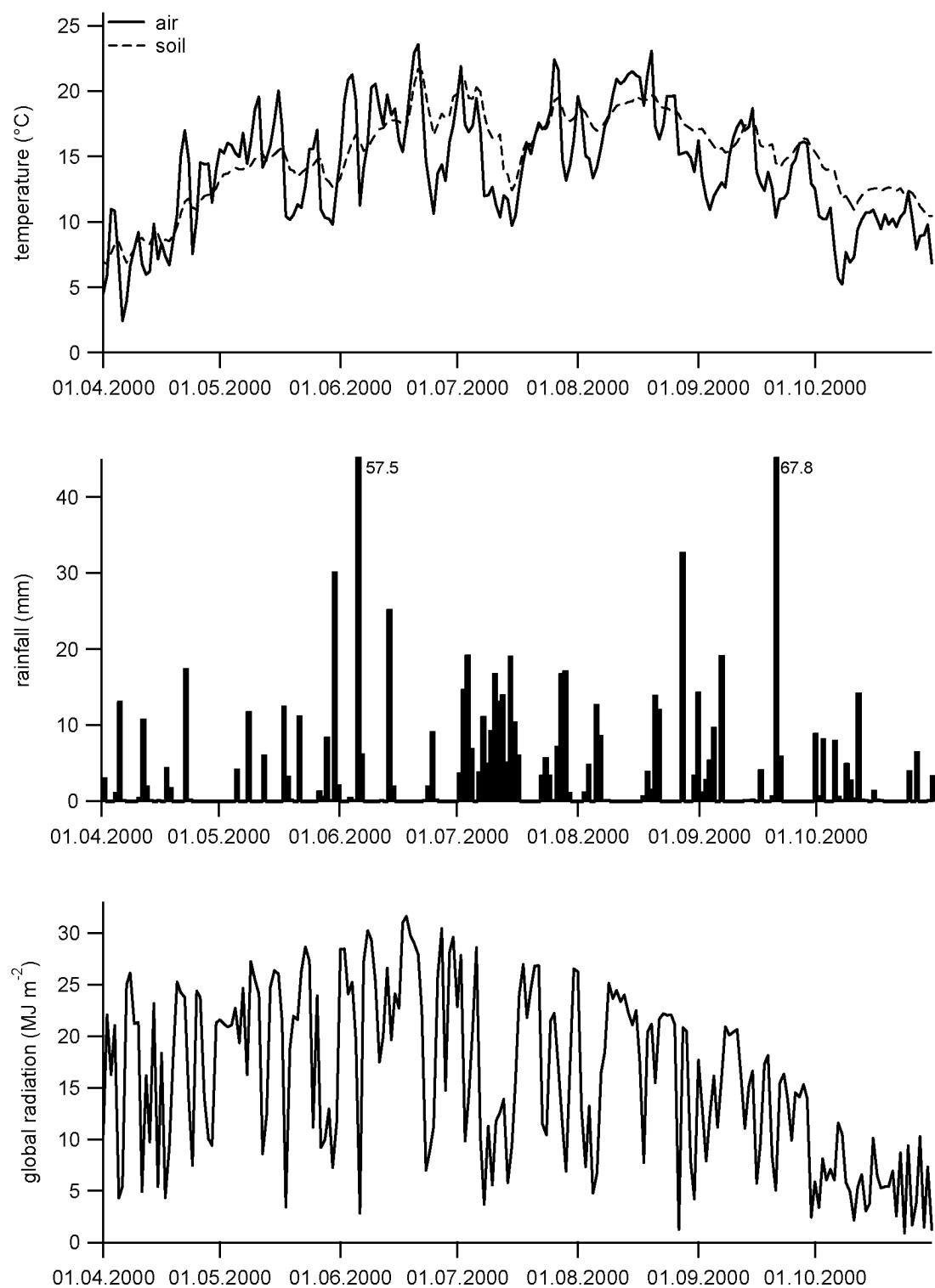
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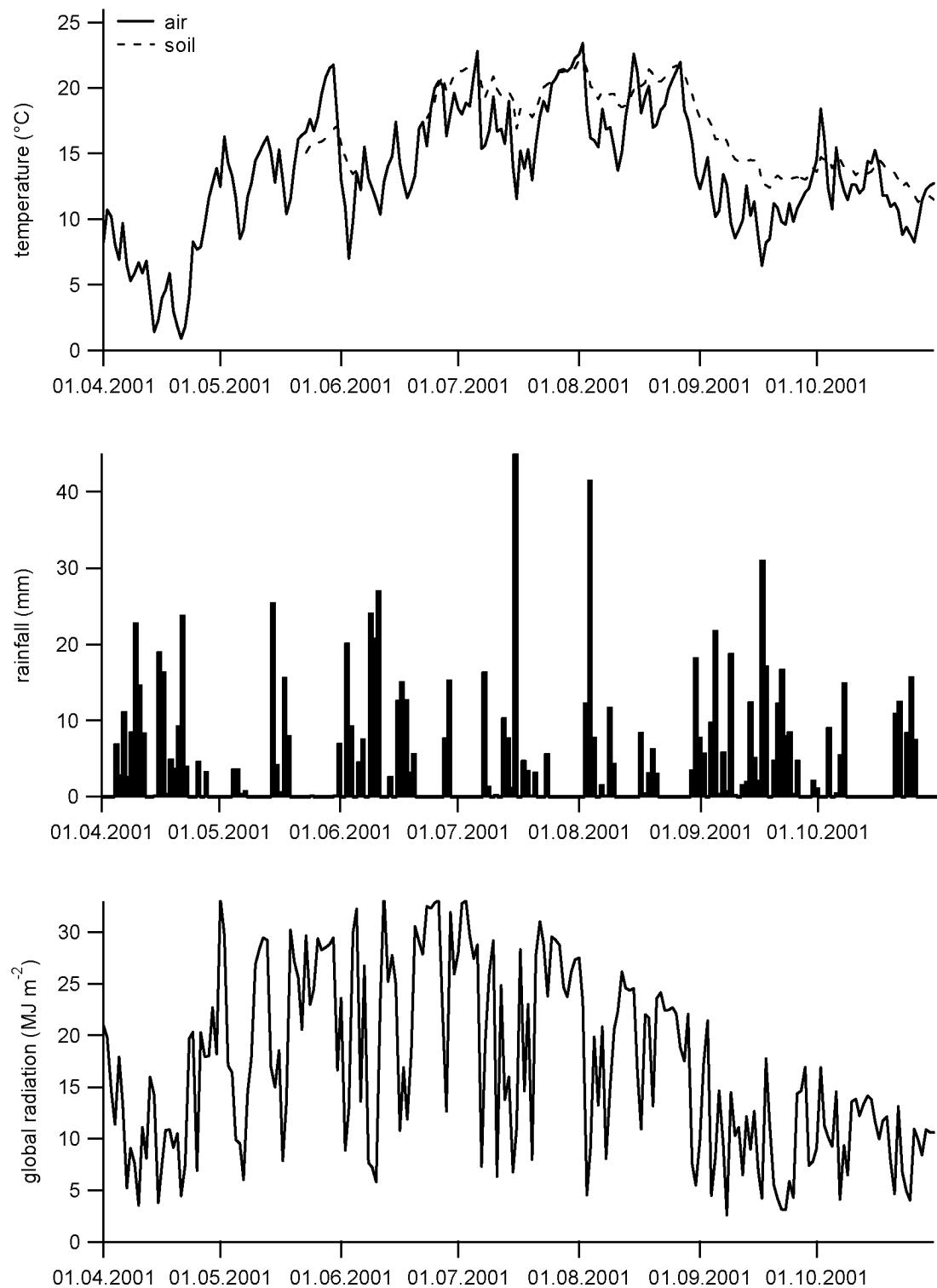
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Appendix - meteorological data

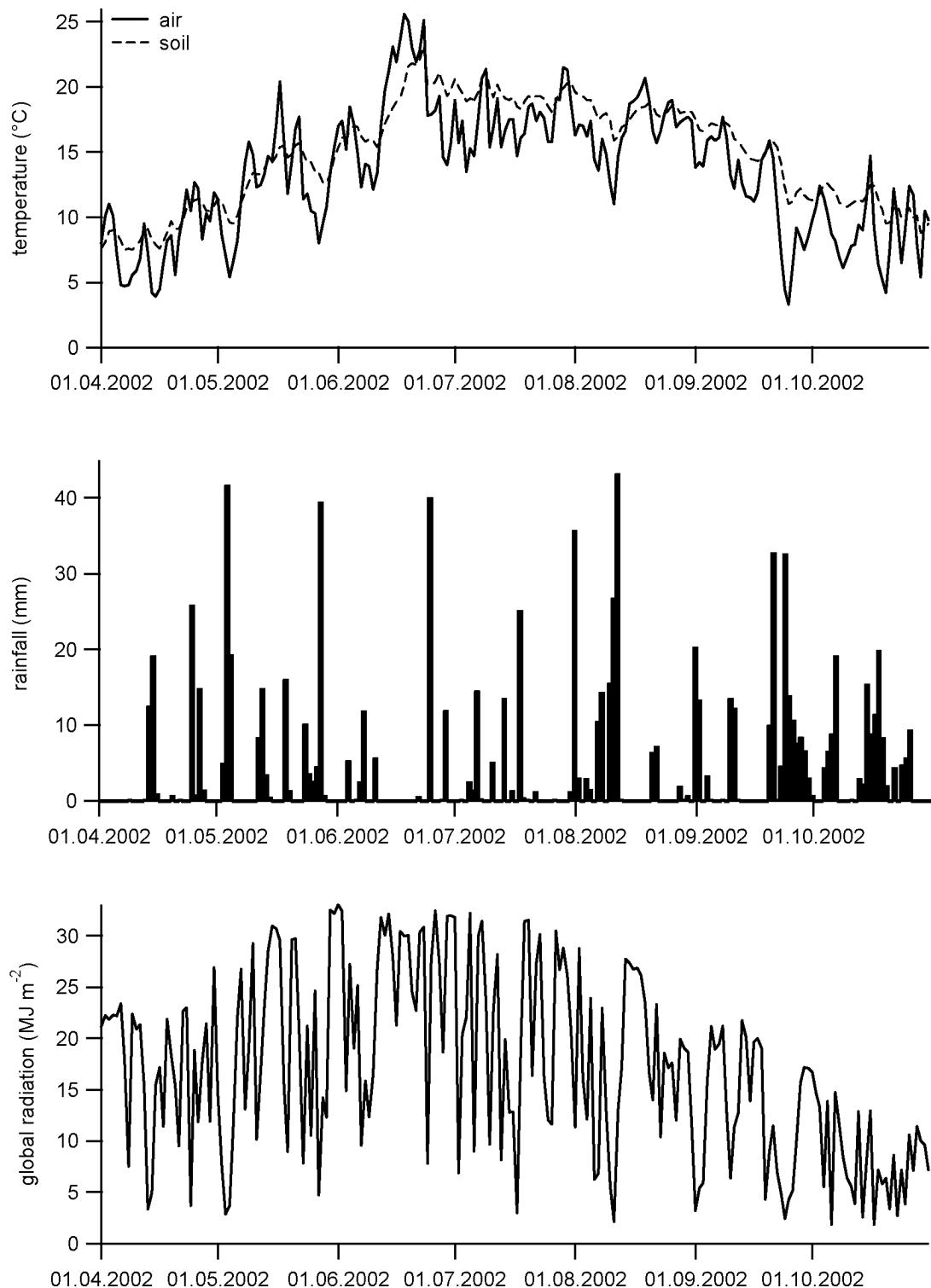


Meteorological conditions in Eschikon for the growing season 2000. Upper graph: daily means of air temperature at 2 m above-ground and of soil temperature at 10 cm below-ground. Middle graph: daily sum of rainfall. Lower graph: daily sum of global radiation.

Appendix



Meteorological conditions in Eschikon for the growing season 2001. Upper graph: daily means of air temperature at 2 m above-ground and of soil temperature at 10 cm below-ground. Middle graph: daily sum of rainfall. Lower graph: daily sum of global radiation.



Meteorological conditions in Eschikon for the growing season 2002. Upper graph: daily means of air temperature at 2 m above-ground and of soil temperature at 10 cm below-ground. Middle graph: daily sum of rainfall. Lower graph: daily sum of global radiation.

Herzlichen Dank

Professor Josef Nösberger danke ich herzlich, dass er mir dieses interessante Dissertationsthema anvertraut hat und mir somit ermöglichte ein Forschungsgebiet vertieft kennen zu lernen. Sein fachliches Wissen und seine Erfahrung aber auch sein psychologisches Geschick haben massgeblich zum Gelingen dieser Arbeit beigetragen.

Professor Peter Edwards danke ich herzlich, dass er das Referat für meine Dissertation übernommen hat. Seine Kommentare zu den Paper-Entwürfen waren sehr hilfreich.

Ein grosser Dank gebührt Herbert Blum, sein technisches Know-How und sein unermüdlicher Einsatz machten die Messanlage erst funktionsfähig.

Ein riesiges Dankeschön geht an meine Kollegen vom MUM-Team, ohne sie wäre diese Arbeit undenkbar gewesen. Ich danke Manuel Schneider und Michael Richter für die gute Zusammen- bzw. Team-Arbeit auf dem Feld und im Büro. Manuel danke ich für seine Freundschaft; seine grosse Motivation beeinflusste auch meine positiv.

Regula Muheim danke ich für die tolle Zusammenarbeit im ersten FACE Sommer. Dies hat sehr geholfen die Startschwierigkeiten zu meistern.

Andi Lüscher machte mich mit dem FACE-Experiment bekannt und sein statistisches und fachliches Wissen ermöglichte interessante Diskussionen.

Ein spezieller Dank geht an Werner Wild für seine tatkräftige Unterstützung im FACE und dafür, dass er beim Sortieren stets für eine heitere Stimmung sorgte. Thomas Flura danke ich für seine Mithilfe in Eschikon und seine offene Kritik. Je remercie Valentine Hebeisen pour son travail qu'elle a toujours fait avec joie et un grand engagement.

Dani Suter danke ich für interessante Diskussionen jeglicher Art und seinen Feldeinsatz im FACE-Sommer 2001. Markus Daepf erleichterte mir den Einstieg in das FACE-Experiment erheblich. Pavel Jager danke ich für seine wertvolle

Mitarbeit während den Ernten. Victor Escalante danke ich für seine tatkräftige Mitarbeit in Eschikon, speziell beim Zügeln der Messanlage.

I thank Lisa Ainsworth for her valuable contribution to the draft of the first paper. The discussions with her were very helpful and it was always a pleasure when she joined our group in Eschikon.

Robert Widmer gebührt ein Dank für sein Mitwirken beim Bau und bei Reparaturen der Gaswechsel-Messanlage sowie für den Unterhalt der FACE-Begasung. Anni Dürsteler danke ich für die Labor-Analysen und die Versüßung der Kaffeepausen.

Klaus Girgenrath vom Labor für organische Chemie danke ich für die Durchführung der ^{14}C -Analysen. Hans-Rudolf Roth vom Seminar für Statistik danke ich für die kompetente Statistik-Beratung.

Sabina Keller, Lydia Frey und Neville Millar danke ich, dass sie zusammen mit dem MUM-Team das soziale Leben der Gruppe aufrecht erhielten und wir auch ausserhalb der Arbeitszeit fröhliche Stunden zusammen verbringen durften.

Nicht zu vergessen sind an dieser Stelle die zahlreichen Hilfsassistentinnen und Hilfsassistenten, ohne die wir das Jäten und die Ernten nicht hätten bewältigen können.

„rbeat“ gebührt ein spezieller Dank für seine Verdienste als Systemadministrator. Allen Mitgliedern der Gruppe Graslandwissenschaften danke ich für das stets angenehme Arbeitsklima.

Ein herzliches Dankeschön geht auch an alle, die mich in den letzten Jahren durch ihre Freundschaft unterstützt haben. Meinen Eltern danke ich ganz herzlich für die umfassende Unterstützung während der ganzen Studienzeit und des Doktorats.

I don't have any solution, but I certainly admire the problem

Ashleigh Brilliant

Curriculum vitae

September 28, 1973	born in Lucerne
1980 – 1986	<i>Primarschule</i> in Lucerne
1986 – 1993	<i>Kantonsschule Alpenquai</i> in Lucerne <i>Matura</i> type C (mathematics/natural sciences)
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