Aus dem Institut für Bodenkunde und Standortslehre
Universität Hohenheim
Fachgebiet Bodenbiologie   Prof. Dr. Ellen Kandeler

Function and Composition of the Soil Microbial Community in Calcareous Grassland Exposed to Elevated Atmospheric Carbon Dioxide

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Diana Ebersberger
Diplom-Geographin
aus Neustadt an der Weinstraße

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Prodekan: Prof. Dr. Karl Stahr

Berichterstatterin, 1. Prüferin: Prof. Dr. Ellen Kandeler

Mitberichterstatter, 2. Prüfer: Prof. Dr. Andreas Fangmeier

3. Prüfer: Prof. Dr. Ulrich Haas

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A Introduction, summaries and conclusion

1. General Introduction

Human activities including fossil fuel burning and land-use change have caused the concentration of atmospheric carbon dioxide (CO_2) to increase in the last 200 years from about 280 parts per million (ppm) in the early days of industrialization to 370 ppm at the beginning of the 21st century. Projections of future atmospheric CO_2 concentrations range between 540 and 970 ppm by the end of the 21st century, depending on future anthropogenic emission scenarios (Prentice *et al.*, 2001). CO_2 is one of the so-called greenhouse gases and its increasing concentration may contribute to global warming. Since we live in a "carbon world", however, plants and ecosystems might also be affected more directly by "CO_2-fertilization", an issue that has received much less public attention (Körner, 2000). Any response of natural and agricultural plant communities to rising CO_2 concentrations might then mediate effects on soil biological communities.

In regard to photosynthesis, plant species are grouped into C_3, C_4 and CAM plants. Most plant species, especially in temperate regions, belong to the C_3 group. C_3 plants are less efficient in photosynthesis than C_4 plants: 20 - 50% of the fixed carbon is immediately lost by photorespiration. Hence, photosynthesis of C3 plants is stimulated by high levels of CO_2, but the responsiveness differs between species and genotypes. Under ideal conditions, increased photosynthesis rates translate into increased plant growth. Often, a higher proportion of the extra fixed carbon is allocated into roots, and the root-shoot-ratio increases (Rogers *et al.*, 1994). Even if no growth response is observed, CO_2 enrichment alters live plant-tissue composition. Commonly, the tissue concentration of nonstructural carbohydrates like starch and sugars increases. If nutrient supply is limited, stimulated photosynthesis under elevated CO_2 results in lower nutrient concentration in plant tissues than in ambient CO_2. In particular, lower N concentrations or wider C:N ratios are often observed (Körner, 2000). Over longer periods of CO_2 exposure, initial direct responses of plants become smaller because adaptations of photosynthesis to higher CO_2 occur. Besides C allocation of plants, elevated CO_2 also affects transpiration; it generally reduces stomatal conductance because aperture and stomata frequencies decline (Morison, 1998). As a consequence, plants use water more efficiently. In systems where water supply is a limiting factor, this indirect CO_2-effect of better water use efficiency might play a more important role in biomass increases than the direct photosynthesis stimulation effect (Volk *et al.*, 2000).

Direct CO_2-effects such as increased plant biomass are usually much smaller in natural ecosystems than in greenhouse studies or in agricultural systems, because other resources (e.g. nutrients) usually limit growth and because plants are competitive and interacting. Since plants species' and genotypes' responses to elevated CO_2 differ considerably, competitive shifts in natural complex systems are likely to occur under changing atmospheric conditions. This indirect CO_2-effect is probably more persistent than the direct ones (Körner, 2000).

In soils, CO_2 concentration exceeds atmospheric levels by a factor of 10, hence direct effects of CO_2 enrichment on soil organisms are unlikely. The effects of enrichment, however, will be mediated to soil microorganisms by plants. Plant responses will alter the carbon supply to heterotrophic microorganisms, whereby belowground carbon input will most likely increase. Less is known about the effects on the quality of C-inputs. So far, only little experimental evidence is available on the quality of rhizodeposits, solely deriving from pot studies, and nothing is known about the rhizodeposition of complex communities under elevated CO_2. Whether microorganisms can utilize extra C-input also depends on the availability of other nutrients, especially N. Enhanced plant and root growth, accompanied by increased plant nutrient uptake, may affect the dynamic equilibrium between plants and microbes in nutrient acquisition. Altered soil moisture conditions under elevated CO_2 directly influence the living conditions of soil microorganisms. Water limitations of microbial activities might be reduced under elevated CO_2 (Hu *et al.*, 1999). The importance of microbial community responses to CO_2 enrichment was highlighted by Norby (1997): "The soil system is incredibly complex, with uncounted bacterial, fungal and microfaunal species living and interacting amidst a matrix of plant roots and organic and inorganic particles, and awash in a nutrient and organic bath. Even if increased CO_2 does not lead directly to carbon accumulation, a faster cycling rate could induce myriad changes in species diversity and functions. These fundamental shifts in ecosystem physiology could in the long run be the most important controllers of carbon pools."

As pointed out above, CO_2 responses are strongly ecosystem-specific, varying with plant community studied, nutrient availability, soil properties and climatic conditions. In the following chapter, the long-term CO_2 enrichment in calcareous grassland is described in greater detail and its main results are presented.

2. The long-term CO_2 enrichment in calcareous grassland

Calcareous grasslands are man-made ecosystems that often originated many centuries ago by extensive use for grazing. They are among the most species-rich habitats in Central Europe, and many rare species, including orchids, are often found. A calcareous grassland in the Swiss Jura mountains was selected by the Institute for Botany in Basel as a research site to study the structure, dynamics and functions of biodiversity under changing atmospheric conditions. A long-term CO_2 enrichment experiment was set up; it contributed to the Global Change and Terrestrial Ecosystem Project (GCTE) of the International Geosphere-Biosphere Programme (IGBP) (Körner, 1995).

The grassland is located on a southwest-facing slope in the north-western part of Switzerland (47°27'30''N, 7°34'E, 520 m a.s.l.). Mean annual temperature is around 8.5 °C and annual precipitation is around 900 mm. On calcareous debris, a Rendzina developed. The thickness of the Ah horizon varies between 10 and 20 cm, and its texture is silty clay loam (Ogermann *et al.*, 1994). For at least several decades the site has been used as cattle pasture, leading to the characteristic vegetation of the *Mesobromion.* The dominant species is *Bromus erectus* Huds., which contributes more than 40% to the overall aboveground biomass. Overall, gramoinides contribute nearly 70% to aboveground biomass, non-legume dicots 15%, legumes 6%, and mosses 10%. With few exceptions, species in this community all are long-living perennials (Huovinen-Hufschmid and Körner, 1998).

For CO_2 exposure a novel system was developed. The Screen-Aided CO_2 Control (SACC) system uses much less CO_2 per experiment and replicate than Free Air Carbon enrichment (FACE) and has a smaller impact on the microclimate than Open Top Chambers (OTCs) (Leadley *et al.*, 1997). Each screen unit consists of clear plastic and a pipe at the base of the screen, through which CO_2-enriched or ambient air is directed into the unit. The units are hexagonal, 50 cm in height, enclose a ground area of 1.27 m², and leave a gap of 7 cm to the ground. The gap allows free convection of air and unrestricted movement of small animals. The screen breaks the wind, which along with the air blown into the screen creates turbulent mixing within the unit and thereby relatively uniform CO_2 concentrations. A fully automated system monitors CO_2 concentrations and regulates the CO_2 injection rate for each unit every ten minutes to maintain the preset CO_2 concentration of 600 ppm. Impacts on microclimate were smaller than usually observed in the case of OTCs: Temperatures in SACC units were app. 1.0 K higher over 24 hours compared to control plots, and on hot days peak temperature differences were 2.5 K at maximum. Precipitation in the centre area of SACC units exceeded 90% of that outside

the chamber, resulting in slightly lower soil water contents in plots with SACC units than in control plots (Leadley *et al.*, 1997).

The plots of the field experiment, including unscreened ambient CO_2 plots (control, 365 µl CO_2 l^{-1}), screened ambient CO_2 plots (365 µl CO_2 l^{-1}) and screened elevated CO_2 plots (600 µl CO_2 $^{l-1}$), were organized in four blocks à 6 plots (8 replicates per treatment, 24 plots in total). The blocks were oriented perpendicular to the slope. The CO_2 enrichment of undisturbed vegetation plots started in March 1994 and operated 24 hours a day, except from December to February, when it was shut down. The experiment was terminated in June 1999.

In the first two growing seasons of treatment, ecosystem-level day-time CO_2 uptake was increased at elevated CO_2 (25-60%), while night-time CO_2 release did not change significantly (Stocker *et al.*, 1997). In response to CO_2 exposure, plant biomass increased significantly between 20% to 29% in the 2nd to 4th growing season. In the 2nd and 3rd year of the experiment, significant differences in responsiveness between functional groups (legumes, non-leguminous, forbs and graminoids) were detected, but the order of responsiveness differed between years. By the 3rd year of CO_2 enrichment, large species-specific differences in CO_2 response had developed. *Carex flacca* and *Lotus corniculatus* increased their relative contribution to community biomass by 271 and 249%. Their strong positive response can be related to increased soil moisture under CO_2 exposure, which may allow *C. flacca* and *L. corniculatus* to out-compete less mesophytic species (Leadley *et al.*, 1999). Increased soil moisture at elevated CO_2 can be explained by reduced leaf conductance. The observed effects on leaf conductance were strongly buffered by leaf boundary layer and canopy conductance, so only small, non-significant decreases of evapotranspiration were found. However, these minute responses of reduced water loss accumulated over-time and resulted in significantly higher soil moisture in plots exposed to elevated CO_2. The largest differences of soil moisture between CO_2 treatments were usually found at intermediate soil moisture levels. After heavy precipitation, at high soil moisture levels, differences were small (Niklaus *et al.*, 1998b). A study on the root system over the first two years of the experiment revealed no differences in root mass (Leadley *et al.*, 1999). Minirhizotron observations also revealed no effects of CO_2 enrichment on root production or mortality, but a greater proportion of roots was found in the top layer (0-6 cm) (Arnone *et al.*, 2000). Plant aboveground C:N ratios were increased at elevated CO_2, and total amounts of N removed in biomass harvest were not affected by CO_2 exposure. This indicated that the observed plant biomass increases were solely attained by dilution of N. The C/N ratio of legumes was not affected by CO_2 enrichment, therefore legume growth was not limited by available soil N. In a greenhouse study with monoliths from the field site, addition of P lead to increased

plant N pools by stimulating the legumes. This indicates that the overall productivity of the system is N-limited, whereas effects of elevated CO_2 on legume growth and N fixation are limited by P (Niklaus *et al.*, 1998a).

In the 3rd year of the field experiment, microbial biomass and carbon were investigated. Microbial biomass carbon did not change under elevated CO_2, but microbial N did increase significantly, resulting in a narrower microbial C:N ratio (Niklaus, 1998). After 6 years of enrichment, the C pools in plant and surface litter had increased, but microbial C and soil organic C were not affected (Niklaus *et al.*, 2001b). In a greenhouse ^{13}C pulse labelling study, intact turves taken from the grassland were labelled for two photoperiods with $^{13}CO_2$. The distribution of the ^{13}C label was then tracked in plants, newly produced fine roots, earthworms, soil microorganisms and density fractions for the rest of the growing season. Plant ^{13}C pools increased significantly belowground, but the CO_2 enrichment had no effect on ^{13}C in soil microorganisms, fine roots, earthworms, or density fractions (Niklaus *et al.*, 2001a). The greenhouse and the *in situ* study indicate that C sequestration under elevated CO_2 occurred only in rapidly turning over pools, such as plant biomass or detritus, and that potential extra C inputs were rapidly re-mineralised (Niklaus *et al.*, 2001b).

3. Outline of the thesis

Previous results of the CO_2 enrichment in calcareous grassland have shown that the plant community responded by a shift in species composition and increased plant pools of carbon. A prominent feature was altered soil moisture conditions. The pool size microbial biomass, however, did not respond to changes in C supply and soil moisture. This thesis aims to analyse the responses of the soil microbial community to the long-term CO_2 enrichment in greater detail, both in terms of its function and its composition. Classical soil biological analysis as well as modern molecular methods were applied; in addition to the field experiment, a laboratory incubation experiment was also set up. The thesis is composed of 4 parts:

I. The impact of altered litter quality at elevated CO_2 was studied at the microhabitat level. In the laboratory incubation experiment, we focused on the response of xylanase and invertase to changes in litter quality at elevated CO_2 at the soil-litter interface (**Chapter B**).

On the field scale, the response of the microbial community to plant-mediated effects of CO_2-enrichment was examined by

II. investigating the functional diversity by means of activities of enzymes involved in C-, N-, P-, and S-cycling and N-mineralisation (**Chapter C**) and
III. analysing microbial community structure using phospholipid fatty acid (PLFA) profiles and analysing the bacterial community structure using Polymerase Chain Reaction (PCR) followed by Denaturing Gradient Gel Electrophoresis (DGGE) (**Chapter D**).
IV. In a synthesis with data of other researchers, data on soil microorganisms, soil fauna, soil structure and N cycle were compiled (**Chapter E**).

4. Effects of litter produced under elevated CO_2 on invertase and xylanase activity at the soil-litter interface

Elevated CO_2 often alters the live-tissue composition of plants, e.g. the C/N ratio widens and the content of nonstructural carbohydrates increases. Litter quality can also be affected by altered allocation patterns and changes in plant community structure. Though enzymes play a key role in mineralising organic substances, little is known about the effects of litter quality at elevated CO_2 on enzyme activities.

Laboratory incubation experiments were conducted to explore the relationship between litter quality under elevated CO_2 and enzymes involved in carbon cycling. Mixed, naturally senescent litter and soil material from ambient CO_2 and elevated CO_2 plots of the long-term CO_2 enrichment in calcareous grassland were incubated for 10, 30 and 60 days. Using a microtome cutting device, we took soil samples in predefined distances (0.250 mm – 14 mm) from the litter layer. Litter and soil samples were analysed for invertase and xylanase activity.

The lower litter quality produced under elevated CO_2 yielded lower invertase and xylanase activities of the litter. The reduced enzyme activities can slow down decomposition, at least during the initial stages. Litter addition stimulated the activities of both enzymes in adjacent soil but, in contrast to our expectations, in most cases no strong gradient of microbial activity developed within the soil. The relatively high contents of clay and organic carbon may have prevented the formation of enzymatic gradients. Litter quality did not affect invertase activity in adjacent soil, whereas soil xylanase activity was higher in soil compartments close to litter from elevated CO_2 plots. Possibly, more polymeric substances were released from elevated CO_2 litter as a result of its lower xylanase activity. The laboratory effects of litter quality on soil invertase and xylanase did not mirror the field study results. We conclude that CO_2-induced belowground C-inputs (e.g. increased root mass) and altered soil moisture conditions control enzyme activities more than altered litter quality.

5. Long-term CO_2 enrichment stimulates N-mineralisation and enzyme activities in calcareous grassland

Though microbial decomposition and mineralisation are mediated by soil enzymes, relatively few studies on the effects of CO_2 enrichment on soil microorganisms and their activity have measured enzyme activities. We investigated N-mineralisation and activities of the enzymes invertase, xylanase, urease, protease, arylsulfatase and alkaline phosphates in calcareous grassland, in spring and summer of the 6th growing season of CO_2 exposure.

In spring, N-mineralisation increased significantly by 30% at elevated CO_2, while there was no significant difference between treatments in summer (+3%). The response of soil enzymes was also more pronounced in spring, when alkaline phosphatase and urease increased most strongly by 32 and 21%, respectively. In summer, differences of activities between CO_2 treatments were greatest in the case of urease and protease (+21% and +17% at elevated CO_2).

N-mineralisation and enzyme activities were stimulated at elevated CO_2, though microbial biomass did not respond. Contrary to our expectations, we could only partly relate this stimulation to increased soil moisture under elevated CO_2. At both samplings, soil water content was close to field capacity and the water-saving effect under elevated CO_2 due to reduced evapotranspiration was not yet reflected in different soil water contents. Possibly, more pronounced effects of altered soil moisture conditions could be detected at drier conditions. The higher soil enzyme activities and N-mineralisation might also be related to extra C entering the soil and stimulating soil microbial activity, because the responses of soil enzymes were in the same order of magnitude as the increases in root biomass measured in March and June 1999.

In this study, enzyme activities appear to be more sensitive indicators for changes in belowground C- and N-turnover than the size of the microbial biomass pool. However, more direct measurements of turnover in other studies – e.g. ^{13}C labelling experiments, soil respiration measurements, fine root turnover, and cumulated N in aboveground plant mass removed in course of the field experiment – showed no change. Perhaps the increased C- and N-mineralisation capacity under elevated CO_2, observed as stimulated enzyme activities and N-mineralisation, translates into faster decomposition during periods with otherwise optimal conditions, for example during warm spells with high soil moisture and substrate availability.

6. Effects of long-term CO_2 enrichment on microbial community structure in calcareous grassland

We analysed the microbial community structure in a species-rich calcareous grassland which had been exposed to elevated CO_2 for 6 growing seasons. Previous studies have shown that microbial biomass and basal respiration did not respond to CO_2 enrichment, whereas N-mineralisation and enzyme activities were stimulated. As these results indicate that changes in the functionality of the soil microorganisms were not offset by changes in the bulk parameter microbial biomass, we aimed to analyse the microbial community in greater detail. We used two different approaches to study microbial community structure: (1) Phospholipid fatty acid (PLFA) profiles, because this technique provides quantitative information on community structure and allows a discrimination between fungi and bacteria and (2) DNA fingerprints, obtained by Polymerase Chain Reaction (PCR) of 16S rDNA fragments followed by Denaturing Gradient Gel Electrophoresis (DGGE), which allow a detailed analysis of the bacterial community structure. Bacterial diversity was assessed based on Shannon diversity indices.

In the case of PLFA, only the reduced portion of i17:0 at elevated CO_2 in spring was significant. The overall profiles, analysed by partial redundancy analysis (RDA), were not affected, and the ratio between bacterial and fungal PLFA did not change. DNA fingerprints were highly complex. For the summer sampling, RDA revealed significant variation in DNA fingerprints in response to CO_2 enrichment. This variation must be attributed to low intensity bands because dominant bands did not differ between treatments. Species richness and diversity, as assessed by the number of detected bands and Shannon Diversity index, were not affected by elevated CO_2.

A possible cause for the minute, but significant changes of bacterial community structure is altered rhizodeposition. Experimental information on the chemistry of rhizodeposits of complex plant communities in undisturbed soils at elevated CO_2 is currently lacking. As rhizodepostion differs from species to species, and plant response to CO_2 exposure is species-specific, altered overall rhizodeposition could either result from altered rhizodeposition of single species or from changes in plant community structure.

7. Six years of *in situ* CO_2 enrichment evoke changes in soil structure and soil biota of nutrient-poor grassland

This synthesis presents data on soil microorganisms, soil fauna (protozoans, nematodes, acarians, collembolans), soil structure and nitrogen cycle of calcareous grassland after CO_2 exposure for six growing seasons.

Microbial biomass, soil basal respiration, and the metabolic quotient were not altered significantly. PLFA analysis revealed no significant shift in the ratio of fungi to bacteria. Microbial grazer populations (protozoans, bacterivorous and fungivorous nematodes, acarians and collembolans) and root-feeding nematodes were not affected by elevated CO_2. However, total nematode numbers averaged slightly lower under elevated CO_2 (-16%) and nematode mass was significantly reduced by 43%, caused by a reduction in large-diameter nematodes classified as omnivorous and predacious. CO_2 exposure resulted in a shift towards smaller aggregate sizes at both micro- and macro-aggregate scales; this was caused by higher soil moisture under elevated CO_2. Reduced aggregate sizes reduce pore neck diameters. This can confine the locomotion of large-diameter nematodes and therefore possibly accounts for their decrease.

The CO_2 enrichment also affected the nitrogen cycle. The N stocks in living plants and surface litter increased, but N in soil organic matter and microorganisms remained unaltered. N mineralisation increased considerably, but microbial N did not differ between treatments, indicating that net N immobilization rates were unaltered.

8. Concluding remarks

This thesis studied the response of the soil microbial community to CO_2 enrichment in calcareous grassland. In a laboratory incubation experiment, lower litter quality at elevated CO_2 affected xylanase but not invertase activity in adjacent soil. In the field, enzyme activities and N-mineralisation were stimulated by plant responses to elevated CO_2, especially in spring. Results of the laboratory experiment did not go in line with results of the field experiment. In conclusion, altered litter quality at elevated CO_2 seemed to be of minor importance in this system. The stimulation of microbial activity in the field study can be related to increased C-inputs by plants, e.g. increased root mass, and to higher soil moisture at elevated CO_2. We also detected minute, but significant, variation in DGGE fingerprints of 16S rDNA fragments in response to CO_2 enrichment. A possible cause for these effects is altered rhizodeposit quality, either due to altered rhizodeposition of single plants or to shifts in the plant community structure. Experimental evidence of rhizodeposition in complex, natural communities is currently lacking.

Both the function and structure of the soil microbial community were affected by plant community response to CO_2 enrichment. The response of microbial activity, however, was more pronounced in spring, whereas variation in bacterial community structure was more distinct in summer. This study did not directly address the link between function and composition. Broad-range approaches were applied to assess microbial activity and to analyse microbial community composition: The studied enzymes are produced by a wide range of microorganisms, many microbial groups contribute to single PLFA, and a eubacterial primer set was used in PCR. Linking the function and composition of microbial communities requires focusing on single functional genes which encode proteins. This, however, only yields information on small sub-communities. A novel approach in environmental microbiology to overcome these limitations are microarrays, which can compile many functional genes. Wu *et al.* (2001), for example, developed microarrays containing about 100 functional genes which code for key enzymes in processes of denitrification, nitrification and methane oxidation.

9. References

Arnone III, J. A., Zaller, J. G., Spehn, E. M., Niklaus, P. A., Wells, C. E., and Körner, C. (2000). Dynamics of root systems in native grasslands: effects of elevated atmospheric CO_2. *New Phytologist* **147**, 73-85.

Hu, S., Firestone, M. K., and Chapin III, F. S. (1999). Soil microbial feedbacks to atmospheric CO_2 enrichment. *Trends in Ecology & Evolution* **14**, 433-437.

Huovinen-Hufschmid, C., and Körner, C. (1998). Microscale distribution of species and biomass in grassland underlines the importance of biotic determinants for biodiversity. *Botanica Helvetica* **106**, 69-83.

Körner, C. (1995). Biodiversity and CO_2: Global change is under way. *GAIA* **4**, 234-243.

Körner, C. (2000). Biosphere responses to CO_2 enrichment. *Ecological Applications* **10**, 1590-1619.

Leadley, P. W., Niklaus, P., Stocker, R., and Körner, C. (1997). Screen-aided CO_2 control (SACC): a middle-ground between FACE and open-top chamber. *Acta Oecologia* **18**, 207-219.

Leadley, P. W., Niklaus, P. A., Stocker, R., and Körner, C. (1999). A field study of the effects of elevated CO_2 on plant biomass and community structure in a calcareous grassland. *Oecologia* **118**, 39-49.

Morison, J. I. L. (1998). Stomatal response to increased CO_2 concentration. *Journal of Experimental Botany* **49**, 443-452.

Niklaus, P. A. (1998). Effects of elevated atmospheric CO_2 on soil microbiota in calcareous grassland. *Global Change Biology* **4**, 451-458.

Niklaus, P. A., Göckler, E., Siegwolf, R., and Körner, C. (2001a). Carbon allocation in calcareous grassland under elevated CO_2: A combined ^{13}C pulse labelling/soil physical fractionation study. *Functional Ecology* **15**, 43-50.

Niklaus, P. A., Leadley, P. W., Stöcklin, J., and Körner, C. (1998a). Nutrient relations in calcareous grassland under elevated CO_2. *Oecologia* **116**, 67-75.

Niklaus, P. A., Spinnler, D., and Körner, C. (1998b). Soil moisture dynamics of calcareous grassland under elevated CO_2. *Oecologia* **117**, 201-208.

Niklaus, P. A., Wohlfender, M., Siegwolf, R., and Körner, C. (2001b). Effects of six years atmospheric CO_2 enrichment on plant, soil, and soil microbial C of a calcareous grassland. *Plant and Soil* **233**, 189-202.

Norby, R. (1997). Inside the black box. *Nature* **388**, 522-523.

Ogermann, P., Spycher, B., Schaub, D., and Sollberger, R. (1994). Die Landschaftsstruktur im Raum Nenzlingen - geoökologisch gesehen. *Regio Basiliensis* **35**, 91-100.

Prentice, I. C., Farquhar, G. D., Fasham, M. J. R., Goulden, M. L., Heimann, M., Jaramillo, V. J., Kheshgi, H. S., Le Quere, C., Scholes, R. J., and Wallace, D. W. R. (2001). The carbon cycle and atmospheric carbon dioxide. *In* "Climate Change 2001: The Scientific Basis. Contribution of Working Group I to the Third Assessment Report of the Intergovernmental Panel on Climate Change" (J. T. Houghton, Y. Ding, D. J. Griggs, M. Noguer, P. J. van der Linden, X. Dai, K. Maskell and C. A. Johnson, eds.), pp. 881. Cambridge University Press, Cambridge, New York.

Rogers, H. H., Runion, G. B., and Krupa, S. V. (1994). Plant responses to atmospheric CO_2 enrichment with emphasis on roots and the rhizosphere. *Environmental Pollution* **83**, 155-189.

Stocker, R., Leadley, P. W., and Körner, C. (1997). Carbon and water fluxes in a calcareous grassland under elevated CO_2. *Functional Ecology* **11**, 222-230.

Volk, M., Niklaus, P., and Körner, C. (2000). Soil moisture effects determine CO_2 responses of grassland species. *Oecologia* **125**, 380-388.

Wu, L., Thompson, D. K., Li, G., Hurt, R. A., Tiedje, J. M., and Zhou, J. (2001). Development and evaluation of functional gene arrays for detection of selected genes in the environment. *Applied and Environmental Microbiology* **67**, 5780-5790.

B Effects of litter produced under elevated CO_2 on invertase and xylanase activity at the soil-litter interface

Diana Ebersberger and Ellen Kandeler

Abstract

Laboratory incubation experiments were conducted to explore the relationship between litter quality under elevated carbon dioxide (CO_2) and enzymes involved in carbon cycling. Mixed litter and soil material from a long-term CO_2 enrichment experiment were incubated and samples taken after 10, 30 and 60 days. Soil samples were taken close to the litter layer using a microtome cutting device. Litter and soil samples were analysed for invertase and xylanase activity. The lower litter quality produced under elevated CO_2 led to lower litter invertase and xylanase activities. Litter addition stimulated these activities in the adjacent soil. Invertase activities of adjacent soil were not affected by litter quality, while soil xylanase activity was higher in soil compartments adjacent to litter from elevated CO_2 plots. The reduced enzyme activities of litter produced under elevated CO_2 can slow decomposition, at least during the initial stages. Since the effects of litter quality on enzyme activities in adjacent soil were small, we conclude that CO_2-induced belowground C-inputs (e.g. increased root mass) and altered soil moisture conditions are more important controls of enzyme activities than altered litter quality.

Keywords: decomposition, litter quality, invertase, xylanase

Introduction

The current increase in atmospheric CO_2 concentration affects carbon cycling in terrestrial ecosystems because photosynthesis, particularly in C3 plants, is generally stimulated by elevated CO_2 concentrations, at least in the short-term (Rogers *et al.*, 1994). If other resource constraints are small, CO_2 enrichment can boost net primary productivity. Even in the absence of plant growth responses, elevated CO_2 alters live-plant-tissue composition. It also increases leaf concentrations of total non-structural carbohydrates and reduces tissue protein, thereby increasing C/N ratios of the green parts of plants (Rogers *et al.*, 1994; Cotrufo *et al.*, 1998; Körner, 2000). Although leaf chemistry can change considerably under elevated CO_2, differences in litter quality between ambient and elevated CO_2 are often small or even absent, and green leaf chemistry might not be a reliable indicator of litter chemistry (Hirschel *et al.*, 1997). Arp *et al.* (1997) hypothesized that nitrogen resorption during leaf senescence is less efficient under elevated CO_2. In a meta-analysis of field studies, Norby *et al.* (2001) found that, under ideal conditions, the N resorption efficiency did not change under elevated CO_2; they concluded that other environmental influences on resorption, e.g. herbivory and early frosts, increased the variability in litter N concentrations. Apart from leaf chemistry changes, changes in litter quality could also derive from shifts in plant community composition. Plant response to CO_2 enrichment is species-specific, and in natural ecosystems this may permit certain species to compete more efficiently for other limited resources like water and nutrients (Körner, 1995). Dukes and Field (2000) concluded from a decomposition study in a Californian grassland that changes in allocation patterns and species composition are the dominant mechanisms for the altered decomposition rates.

Although numerous studies have been conducted on the influence of litter quality on microbial processes during decomposition under elevated carbon dioxide (e.g. Henning *et al.*, 1996; Randlett *et al.*, 1996; Ball and Drake, 1997; Torbert *et al.*, 1998; Sowerby *et al.*, 2000; Torbert *et al.*, 2000; van Ginkel *et al.*, 2000), the effects on enzyme processes are unknown. Enzymes catalyse the mineralisation of organic material and are crucial in decomposition processes. Sinsabaugh and Moorhead (1997) argue that enzymes are sensitive indicators of litter quality. Microorganisms and their enzymes are not uniformly distributed in soil, but mainly colonize the rhizosphere, in larger micropores or on deposits of organic matter (Foster, 1988). Kandeler *et al.* (1999) reported, that in an agricultural soil gradients of enzyme activity developed in adjacent soil during the decomposition of maize straw. Gaillard *et al.* (1999) also demonstrated microbial gradients in soil during decomposition of wheat straw. These studies indicate that it is

promising to examine effects of litter quality on microscale enzyme activities at the soil-litter interface. We used soil and litter material from the long-term CO_2 enrichment in calcareous grassland. In this species-rich grassland, *Carex flacca* and *Lotus corniculatus* increased their relative contribution to community biomass under elevated CO_2 (Leadley *et al.*, 1999). The foliar chemistry of grasses changed significantly in response to CO_2 exposure: their nitrogen content fell considerably, while their starch content increased markly (Goverde *et al.*, 2002).

We hypothesise that the different litter quality at ambient and elevated CO_2 triggers differing enzyme activity gradients in the soil-litter interface. We expect litter quality at elevated CO_2 to be lower, and therefore enzyme activity of the litter and of the adjacent soil to be lower. To test this hypothesis we conducted a laboratory incubation experiment in which litter obtained from a long-term CO_2 enrichment experiment was put on soil cores. These cores were incubated for 10, 30 and 60 days. Soil samples were obtained directly adjacent to the litter using a microtome cutting device. Xylanase and invertase activities of the litter and soil samples were measured. Xylanase is involved in the degradation of the major polymeric constituents of plant litter (Rodriguez-Kabana, 1982; Sinsabaugh *et al.*, 1991; Schinner *et al.*, 1996), whereas invertase catalyses the hydrolysis of sucrose to glucose and fructose (Frankenberger and Johanson, 1983; Ross, 1983). By using mixed litter from a long-term field experiment we were able to examine the overall effect of litter grown at elevated CO_2 on enzyme activity, integrating changes in litter chemistry and species composition.

Materials and methods

Litter and soil material

We obtained soil and litter material from a long-term CO_2 enrichment study conducted in a calcareous grassland in Switzerland for six growing season. Treatments in that study included unscreened control plots (356 µl CO_2 l^{-1}), screened ambient CO_2 plots (356 µl CO_2 l^{-1}), and screened elevated CO_2 plots (600 µl CO_2 l^{-1}), using the SACC-system for CO_2 exposure (Screen-aided-CO_2-control, Leadley *et al.*, 1997). A detailed description can be found in Leadley *et al.* (1999). The species-rich grassland, comprising one hundred vesicular species, is dominated by *Bromus erectus* Huds (for details see Huovinen-Hufschmid and Körner, 1998). In late June 1999, after six growing seasons under CO_2 enrichment, naturally senescent litter was sampled from all plots. For the incubation experiment, litter from replicates of the screened elevated CO_2 treatment, as well as litter from replicates of the screened ambient CO_2 treatments was mixed. Litter

properties, C/N ratio and fibre analysis data, obtained according to Goering and Van Soest (1970), are shown in Table B1. Litter quality differed between treatments. Litter from elevated CO_2 plots had a wider C/N-ratio and higher contents of ash, hemicellulose, cellulose and lignin. The soil is classified as rendzina. The texture of the Ah-horizon was 7% sand, 52% silt and 41% clay, and its organic C and N contents are 3.9% and 0.33%, respectively (Niklaus *et al.*, 2003).

Table B1 Initial properties of mixed litter originating from calcareous grassland exposed to ambient and elevated CO_2 for six growing seasons. Contents are given in % of total dry weight.

	Ambient CO_2	Elevated CO_2
C/N-ratio	42	44
Ash	25.47 (0.29)	27.52 (0.28)
Cellulose + Lignin	53.08 (0.50)	53.93 (0.41)
Hemicellulose	9.03 (0.14)	10.71 (0.03)

Laboratory incubation experiment

Soil from the Ah-horizon (0-15 cm) of the control plots of the field experiment was air dried and sieved through a 2 mm screen. The soil was then adjusted to a gravimetric water content of 35% (corresponding to 50% of the maximum water holding capacity) and incubated at 15 °C for 4 weeks. During this incubation, easily available substrates such as fine roots and carbon supplies resulting from air-drying and sieving (e.g. microbial debris) were expected to be consumed by microbial biomass.

After pre-incubation, 115.3 g moist soil material (corresponding to 85 g dry soil) was packed into PCV tubes (3 cm height, 5.6 cm diameter, 74 cm^3 volume) and consolidated to reach a bulk density of 1.15 g cm^{-3}, which corresponds to the field soil bulk density (Ogermann *et al.* 1994). A total of 54 tubes was prepared and completely filled with soil. On top of 36 tubes, we placed 0.60 g mixed litter, cut to ca. 2 mm length, originating either from ambient or elevated CO_2 plots; 18 tubes of each. A nylon mesh was placed on top of the litter and attached to the tube to ensure a firm contact between litter and soil. Eighteen tubes were incubated without litter addition, as a control treatment.

Tubes were incubated at 15 °C. Twice a week, tubes were weighed and water contents were adjusted to their initial value. After 10, 30 and 60 days, 18 cores (6 each from ambient CO_2 litter, elevated CO_2 litter and control treatments) were sampled. The

litter layer was carefully separated from the soil core, and soil core and litter were then stored at –20 °C.

Samples were obtained from the frozen soil cores in distances from the core surface of 0.250 mm, 0.500 mm, 0.750 mm, 1.00 mm, 1.25 mm, 1.50 mm, 1.75 mm, 2.0 mm, 2.5 mm, 3.0 mm, 3.5 mm, 4.0 mm, 6.0 mm , 8.0 mm, 10.0 mm, 12.0 mm and 14.0 mm using a cryostat microtome (HM 500 M, MICROM, Walldorf, Germany). During cutting, the temperature was kept at –21 °C. The samples were stored at –21 °C until enzymatic analysis.

Enzyme analysis

For each date and each treatment, litter and the corresponding microtome samples of 3 cores were analysed for invertase or xylanase activity. Invertase activity was measured by incubating 0.250 g moist soil or litter material with 5 ml 35 mM sucrose solution and 5 ml 2 M acetate buffer (pH 5.5) for 3 h at 50 °C. Released reducing sugars reduced potassium hexacyanoferrate (III) in an alkaline solution. Potassium hexacyanoferrite (II) was estimated using the Prussian blue colorimetric procedure (Schinner *et al.*, 1996). For the determination of xylanase activity, 0.250 g soil or litter was incubated in 5 ml of a substrate solution (1.2% w/v xylan from oat spelts suspended in 2 M acetate buffer) and 5 ml 2 M acetate buffer (pH 5.5) for 24 h at 50 °C. Reducing sugars were measured as described for invertase activity. All enzymatic analyses were carried out in duplicate.

Statistical analysis

All results were calculated on a dry weight basis. The mean enzyme activities of soil adjacent to the litter were calculated based on the six microtome samples up to 1.5 mm distance from the core surface for each core. This distance corresponded to the range of influence of litter organic matter (Kandeler *et al.*, 1999). The mean enzyme activities of soil further away from the litter were calculated based on the microtome samples more than 2 mm from the litter or core surface, respectively. Enzyme activities of litter and soil were analysed by analysis of variance (ANOVA) using the STATISTICA software package. All data were log transformed to obtain normalised data prior to analysis. In the case of litter, ANOVA factors were incubation time and CO_2 treatment of the litter. *A priori* linear contrasts were used to test for effects of litter origin on enzyme activity. In the analysis of soil enzyme activities, ANOVA factors were incubation time, litter quality/addition (from ambient CO_2/from elevated CO_2/without litter) and distance to

litter or core surface (adjacent/remote). Effects of litter quality and litter addition on enzyme activity of adjacent soil, and differences between adjacent and remote soil, were tested by *a priori* linear contrasts.

In order to study the formation of gradients of enzyme activities within the soil core, enzyme activities were further analysed by polynomial inverse regression (3rd order) relating distance to litter layer (x) and activity (y) using Sigma Plot 2000.

Results

Figures B1 and B2 show invertase and xylanase activities of mixed litter from a calcareous grassland exposed to ambient and elevated CO_2 after 10, 30 and 60 days of incubation with soil. The ANOVA factor CO_2 treatment of litter was significant for both studied enzyme activities. Invertase activity of litter from the elevated CO_2 treatment was significantly reduced by 27% after 10 days of incubation and by 36% after 30 days. The reduction by 12% after 60 days was insignificant. Xylanase activity was significantly lower for litter produced at elevated CO_2 at all dates. After 10 days of incubation, it was reduced by 38%, after 30 days by 22% and after 60 days by 32%.

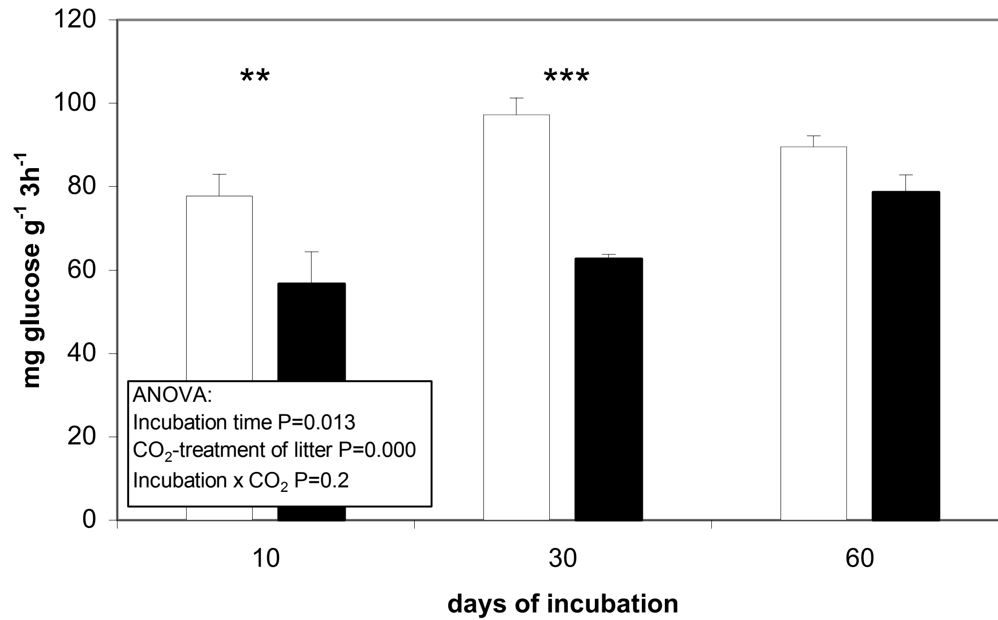

Figure B1 Invertase activity of mixed litter from calcareous grassland produced under ambient (white bars) and elevated (black bars) CO_2-concentrations, incubated on top of soil cores for 10, 30 and 60 days. Means and standard errors of 3 replicates are given. *$P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$.

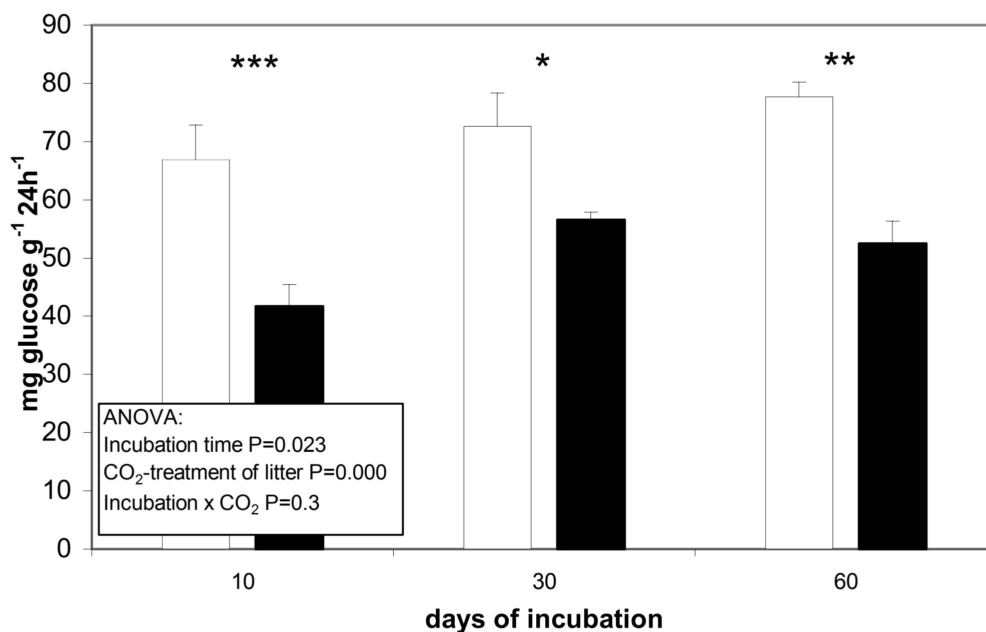

Figure B2 Xylanase activity of mixed litter from calcareous grassland produced under ambient (white bars) and elevated (black bars) CO_2-concentrations, incubated on top of soil cores for 10, 30 and 60 days. Means and standard errors of 3 replicates are given. *$P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$.

Table B2 presents the invertase activities of the soil. Incubation time, litter addition and distance to litter layer or core surface were significant ANOVA factors (Table B3). Invertase activities of soil adjacent to litter from ambient and elevated CO_2 plots did not differ significantly, but activities of control cores without litter addition were significantly lower ($P<10^{-4}$). In cores with litter addition, invertase activity was significantly higher in soil adjacent to litter compared with remote soil ($P<10^{-4}$), while no differences were found within control cores.

Table B2 Invertase activity of soil (mg glucose g^{-1} 3 h^{-1}) after 10, 30 and 60 days of incubation either with mixed litter produced under ambient CO_2 concentrations or elevated CO_2 or without litter. Adjacent refers to the soil compartment in 0.0-1.5 mm distance to the core surface/litter; remote refers to the soil compartment 2.0-14.0 mm away from the core surface/litter. Means and standard errors of 3 replicates are given.

Duration of incubation	Litter addition	Distance from litter/core surface	
		adjacent	remote
10 days	ambient-CO_2 litter	5.74 (0.25)	5.90 (0.67)
	elevated-CO_2 litter	6.25 (0.53)	5.81 (0.64)
	without litter addition	5.60 (0.45)	5.47 (0.46)
30 days	ambient-CO_2 litter	6.88 (0.24)	6.49 (0.09)
	elevated-CO_2 litter	7.02 (0.49)	4.16 (0.16)
	without litter addition	5.09 (0.22)	4.90 (0.35)
60 days	ambient-CO_2 litter	8.51 (0.15)	6.98 (0.25)
	elevated-CO_2 litter	6.62 (0.19)	6.66 (0.19)
	without litter addition	6.27 (0.15)	5.76 (0.02)

Table B3 Results of analysis of variance of invertase activities. ANOVA factors were duration of incubation, litter addition and distance from core surface.

Source of variation	df	MS	F	P
Duration	2	0.031	22.74	10^{-6}
Litter	2	0.031	22.41	10^{-6}
Distance	1	0.028	20.30	10^{-4}
Duration x Litter	4	0.006	4.45	0.01
Duration x Distance	2	0.006	4.66	0.02
Litter x Distance	2	0.005	3.41	0.05
Duration x Litter x Distance	4	0.009	6.81	10^{-3}

Table B4 presents xylanase activities of the soil cores after 10, 30, and 60 days. Incubation time, litter addition and distance to litter layer were also significant factors of the ANOVA (Table B5). Activity was significantly higher in soil adjacent to litter from elevated CO_2-plots (P<0.004). Litter addition significantly stimulated xylanase activity (P<0.001) in adjacent soil compared with the upper compartment of cores without litter addition. Furthermore, in cores with litter addition, activity in adjacent soil was higher than in remote soil (P<0.004). In cores without litter addition, no significant differences between remote and adjacent soil compartments were found.

Table B4 Xylanase activity of soil (mg glucose g^{-1} 24 h^{-1}) after 10, 30 and 60 days of incubation either with mixed litter produced under ambient CO_2 concentrations or elevated CO_2 or without litter. Adjacent refers to the soil compartment in 0.0-1.5 mm distance to the core surface/litter; remote refers to soil compartment 2.0-14.0 mm away from the core surface/litter. Means and standard errors of 3 replicates are given.

Duration of incubation	Litter addition	Distance from litter/core surface	
		adjacent	remote
10 days	ambient-CO_2 litter	4.20 (0.30)	3.85 (0.41)
	elevated-CO_2 litter	5.77 (0.07)	4.77 (0.09)
	without litter addition	4.77 (0.34)	4.92 (0.34)
30 days	ambient-CO_2 litter	6.20 (0.77)	4.92 (1.21)
	elevated-CO_2 litter	7.02 (1.56)	6.94 (1.00)
	without litter addition	5.45 (0.43)	4.29 (0.19)
60 days	ambient-CO_2 litter	5.95 (0.63)	4.78 (0.34)
	elevated-CO_2 litter	8.00 (1.09)	5.86 (0.14)
	without litter addition	3.90 (0.13)	4.02 (0.29)

Table B5 Results of analysis of variance of xylanase activities. ANOVA factors were duration of incubation, litter addition and distance from core surface.

Source of variation	df	MS	F	P
Duration	2	0.030	6.16	0.01
Litter	2	0.099	19.98	10^{-6}
Distance	1	0.044	8.89	0.01
Duration x Litter	4	0.022	4.39	0.01
Duration x Distance	2	0.002	0.31	0.8
Litter x Distance	2	0.003	0.70	0.5
Duration x Litter x Distance	4	0.008	1.53	0.2

In most cases, the regressions revealed no, or only a weak, relationship between distance to litter layer (x) and enzyme activity (y). In only two out of 12 cases with litter addition a considerable (R^2>50%) and highly significant regression ($P<0.0001$) could be calculated; these gradients are shown in figures B3 (invertase activity after 30 days of incubation with litter from elevated CO_2 treatment) and B4 (invertase activity, 60 days, ambient-CO_2 litter). No relationship was found between gradient formation, source of litter or incubation time. As only two gradients were detected, the question of whether different litter quality triggers different gradients in the soil-litter interface could not be evaluated.

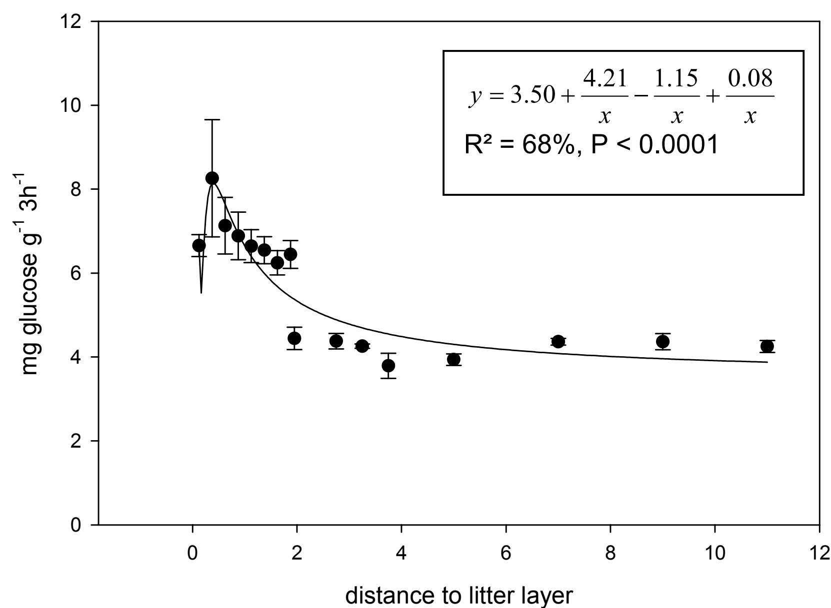

Figure B3 Invertase activity in soil core after 30 days of incubation with litter from elevated-CO_2 plots

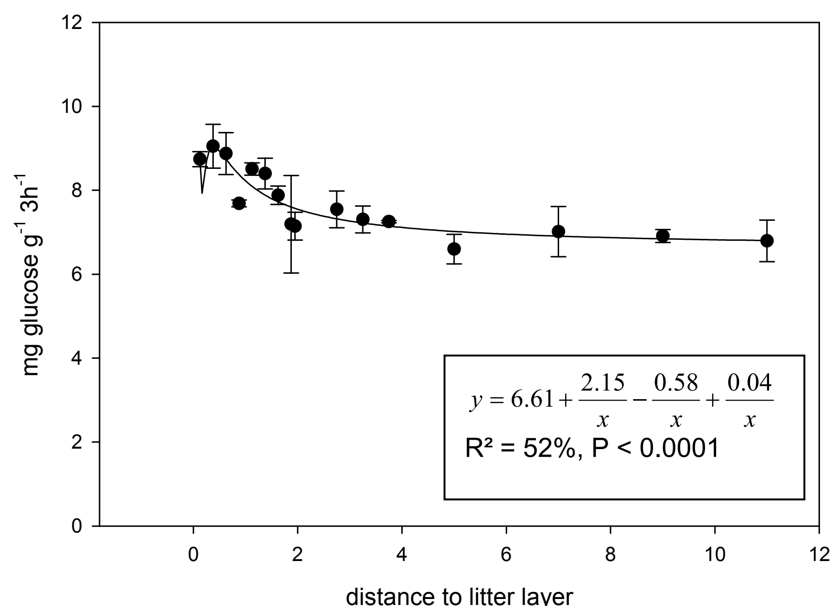

Figure B4 Invertase activity in soil core after 60 days of incubation with litter from ambient-CO_2 plots

Discussion

In the laboratory incubation experiment, xylanase and invertase activities of litter produced under elevated atmospheric carbon dioxide were significantly reduced. Slightly lower litter quality at elevated CO_2 (compare Table 1) was sufficient to reduce enzyme activities. Litter composition influences both the physiological regulation of enzyme secretion and the physicochemical processes of adsorption and stabilization (Sinsabaugh *et al.*, 2002). Luxhoi *et al.* (2002) analysed invertase and xylanase activities of incubated green and brown litter from different species and showed that litter quality affected enzyme activity during decomposition. Suppressed activity of enzymes involved in C-cycling can lower decomposition rates of elevated-CO_2 litter. Lower mass loss of litter produced under elevated CO_2 during initial stages of decomposition was observed in several litter bag experiments (Kemp *et al.*, 1994; Hättenschwiller *et al.*, 1999; Frederiksen *et al.*, 2001).

Invertase and xylanase activity were approximately 10 times higher in litter than in soil. Litter or particulate organic matter are colonized by microorganisms and these produce high amounts of enzymes that are then adsorbed onto the litter (Luxhoi *et al.*, 2002). In a study on the location of microorganisms using ^{14}C labelled substrate, Chotte *et al.* (1997) concluded that amended particulate residues are populated by microorganisms, and that microorganisms within the soil matrix (microaggregates 2-50 µm) are stimulated by soluble compounds originating from this residues. In our study, litter addition stimulated

enzyme activities in adjacent soil. Though litter addition and hence soluble compounds stimulated this activity, in only 2 out of 12 cases a significant relationship between enzyme activity and distance to litter layer was detected. This contrasts with the study of Gaillard *et al.* (1999), who found strong gradients of dehydrogenase activity within the first 4 mm in soil cores incubated with wheat straw. Kandeler *et al.* (1999) also observed the development of gradients of xylanase, invertase and protease activity after 30 days of incubation of maize straw. In both of these experiments, however, the soil clay (and organic carbon) contents were considerably lower than in the present study. In a decomposition experiment xylanase and invertase activity associated with the clay fraction responded only weakly to a maize straw amendment (Stemmer *et al.*, 1999). Little is known about the movement of enzymes and organic substances on the micro-scale. A high clay content possibly prevents gradient formation within the soil. The relatively high organic carbon content also yield higher background variability of enzyme activity. Additionally, the point source character of the added litter was possibly weaker in this grassland soil than in the experiments of Kandeler *et al.* (1999) and Gaillard *et al.* (1999).

Litter quality had no effect on invertase activities in adjacent soil. For xylanase, lower activity in litter from elevated CO_2 plots corresponded with increased activity in adjacent soil (versus soil amended with ambient-CO_2 litter). Perhaps more polymeric organic substances were released from elevated-CO_2 litter as a result of its lower xylanase activity. Soil invertase and xylanase activities in the laboratory incubation did not mirror activities measured for bulk soil samples taken at the same date when litter was sampled. In the grassland soil, invertase activity was higher and xylanase activity lower in elevated CO_2 plots (Ebersberger *et al.*, 2003).

We conclude that in species-rich grassland, changes of belowground C-inputs (e.g. increased root mass) and higher soil moisture under elevated CO_2 are more important controls of soil enzyme activities than lower litter quality. However, the reduced enzyme activities of litter produced under elevated CO_2 could slow decomposition, at least during initial stages of decomposition.

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References

Arp, W. J., Kuikman, P. J., and Gorissen, A. (1997). Climate Change: The potential to affect ecosystem functions through changes in the amount and quality of litter. In "Driven by nature: plant litter quality and decomposition" (G. Cadisch and K. E. Giller, eds.), pp. 187-200. CAB International, Wallingford.

Ball, A. S., and Drake, B. G. (1997). Short-term decomposition of litter produced by plants grown in ambient and elevated atmospheric concentrations. *Global Change Biology* **3**, 29-35.

Chotte, J. L., Ladd, J. N., and Amato, M. (1997). Sites of microbial assimilation, and turnover of soluble and particulate ^{14}C-labelled substrates decomposing in a clay soil. *Soil Biology & Biochemistry* **30**, 205-218.

Cotrufo, M. F., Ineson, P., and Scott, A. (1998). Elevated CO_2 reduces the nitrogen concentration of plant tissues. *Global Change Biology* **4**, 43-54.

Dukes, J. S., and Field, C. B. (2000). Diverse mechanisms for CO_2 effects on grassland litter decomposition. *Global Change Biology* **6**, 145-154.

Ebersberger, D., Niklaus, P. A., and Kandeler, E. (2003). Long-term CO_2 enrichment stimulates N-mineralisation and enzyme activities in calcareous grassland. *Soil Biology and Biochemistry* **35**, 965-972.

Foster, R. C. (1988). Microenvironments of soil microorganisms. *Biology and Fertility of Soils* **6**, 189-203.

Frankenberger, W. T., and Johanson, J. B. (1983). Factors affecting invertase activity in soils. *Plant and Soil* **74**, 313-323.

Frederiksen, H. B., Rønn, R., and Christensen, S. (2001). Effect of elevated atmospheric CO_2 and vegetation type on microbiota associated with decomposing straw. *Global Change Biology* **7**, 313-321.

Gaillard, V., Chenu, C., Recous, B., and Richard, G. (1999). Carbon, nitrogen and microbial gradients induced by plant residues decomposing in soil. *European Journal of Soil Science* **50**, 567-578.

Goering, H. K., and Van Soest, P. J. (1970). Forage Fiber analyses (Apparatus, Reagents, Procedures, and Some Applications). *In* "Agriculture Handbook No. 379", pp. 387-598. Agriculture Research Service, USDA.

Goverde, M., Erhardt, A., and Niklaus, P. A. (2002). *In situ* development of a satyrid butterfly on calcareous grassland exposed to elevated carbon dioxide. *Ecology* **83**, 1399-1411.

Hättenschwiller, S., Bühler, S., and Körner, C. (1999). Quality, decomposition and isopod consumption of tree litter produced under elevated CO_2. *OIKOS* **85**, 271-281.

Henning, F. P., Wood, C. W., Rogers, H. H., Runion, G. B., and Prior, S. A. (1996). Composition and Decomposition of soybean and sorghum tissues grown under elevated atmospheric carbon dioxide. *Journal of Environmental Quality* **25**, 822-827.

Hirschel, G., Körner, C., and Arnone III, J. A. (1997). Will rising atmospheric CO_2 affect leaf litter quality and *in situ* decomposition rates in native plant communities? *Oecologia* **110**, 387-392.

Huovinen-Hufschmid, C., and Körner, C. (1998). Microscale distribution of species and biomass in grassland underlines the importance of biotic determinants for biodiversity. *Botanica Helvetica* **106**, 69-83.

Kandeler, E., Luxhøi, J., Tscherko, D., and Magid, J. (1999). Xylanase, invertase and protease at the soil-litter interface of a loamy sand. *Soil Biology and Biochemistry* **31**, 1171-1179.

Kemp, P. R., Waldecker, D. G., Qwensby, C. E., Reynolds, J. F., and Virginia, R. A. (1994). Effects of elevated CO_2 and nitrogen fertilization pretreatments on decomposition on tallgrass prairie litter. *Plant and Soil* **165**, 115-127.

Körner, C. (1995). Biodiversity and CO_2: Global change is under way. *GAIA* **4**, 234-243.

Körner, C. (2000). Biosphere responses to CO_2 enrichment. *Ecological Applications* **10**, 1590-1619.

Leadley, P. W., Niklaus, P., Stocker, R., and Körner, C. (1997). Screen-aided CO_2 control (SACC): a middle-ground between FACE and open-top chamber. *Acta Oecologia* **18**, 207-219.

Leadley, P. W., Niklaus, P. A., Stocker, R., and Körner, C. (1999). A field study of the effects of elevated CO_2 on plant biomass and community structure in a calcareous grassland. *Oecologia* **118**, 39-49.

Luxhøi, J., Magid, J., Tscherko, D., and Kandeler, E. (2002). Dynamics of invertase, xylanase and coupled indices of decomposing green and brown plant residues. *Soil Biology and Biochemistry* **34**, 501-508.

Niklaus, P. A., Alphei, J., Ebersberger, D., Kampichler, C., Kandeler, E., and Tscherko, D. (2003). Six years of in situ CO_2 enrichment evoke changes in soil structure and soil biota of nutrient-poor grassland. *Global Change Biology* **9**, 965-972.

Norby, R. J., Cotrufo, M. F., Ineson, P., O´Neill, E. G., and Canadell, J. G. (2001). Elevated CO_2, litter chemistry, and decomposition: a synthesis. *Oecologia* **127**, 153-165.

Ogermann, P., Spycher, B., Schaub, D., and Sollberger, R. (1994). Die Landschaftsstruktur im Raum Nenzlingen - geoökologisch gesehen. *Regio Basiliensis* **35**, 91-100.

Randlett, D. L., Zak, D. R., Pregitzer, K. S., and Curtis, P. S. (1996). Elevated atmospheric carbon dioxide and leaf litter chemistry: Influences on microbial respiration and net nitrogen mineralization. *Soil Science Society of America Journal* **60**, 1571-1577.

Rodriguez-Kábana, R. (1982). The effects of crop rotation and fertilization on soil xylanase activity in a soil of the southeastern United States. *Plant and Soil* **64**, 237-247.

Rogers, H. H., Runion, G. B., and Krupa, S. V. (1994). Plant responses to atmospheric CO_2 enrichment with emphasis on roots and the rhizosphere. *Environmental Pollution* **83**, 155-189.

Ross, D. J. (1983). Invertase and amylase and respiratory activities as influenced by clay minerals, soil-clay fractions and topsoils under grassland. *Soil Biology & Biochemistry* **15**, 287-293.

Schinner, F., Öhlinger, R., Kandeler, E., and Margesin, R. (1996). "Methods in Soil Biology" Springer, Berlin Heidelberg New York.

Sinsabaugh, R. L., Antibus, R. K., and Linkins, A. E. (1991). An enzymatic approach to the analysis of microbial activity during plant litter decomposition. *Agriculture, Ecosystems and Environment* **34**, 43-54.

Sinsabaugh, R. L., Carreiro, M. M., and Alvarez, S. (2002). Enzyme and microbial dynamics of litter decomposition. *In* "Enzymes in the Environment Activity, Ecology, and Applications" (R. G. Burns and R. P. Dick, eds.), pp. 249-266. Marcel Dekker, Inc., New York, Basel.

Sinsabaugh, R. L., and Moorhead, D. L. (1997). Synthesis of litter quality and enzymatic approaches to decomposition modelling. *In* "Driven by nature: Plant litter quality and decomposition" (G. Cadisch and K. E. Giller, eds.), pp. 363-375. CAB International, Wallingford.

Sowerby, A., Blum, H., Gray, T. R. G., and Ball, A. S. (2000). The decomposition of *Lolium perenne* in soils exposed to elevated CO_2: comparisons of mass loss of litter with soil respiration and soil microbial biomass. *Soil Biology & Biochemistry* **32**, 1359-1366.

Stemmer, M., Gerzabek, M. H., and Kandeler, E. (1999). Invertase and xylanase activity of bulk soil and particle-size fractions during maize straw decomposition. *Soil Biology & Biochemistry* **31**, 9-18.

Torbert, H. A., Prior, S. A., Rogers, H. H., and Runion, G. B. (1998). Crop residue decomposition as affected by growth under elevated atmospheric CO_2. *Soil Science* **163**, 412-419.

Torbert, H. A., Prior, S. A., Rogers, H. H., and Wood, C. W. (2000). Review of elevated atmospheric CO_2 effects on agro-ecosystems: residue decomposition processes and soil C storage. *Plant and Soil* **224**, 59-73.

van Ginkel, J. H., Gorissen, A., and Polci, D. (2000). Elevated atmospheric carbon dioxide concentration: effects of increased carbon input in a *Lolium perenne* soil on microorganisms and decomposition. *Soil Biology & Biochemistry* **32**, 449-456.

C Long-term CO_2 enrichment stimulates N-mineralisation and enzyme activities in calcareous grassland

Diana Ebersberger, Pascal A. Niklaus, and Ellen Kandeler

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Abstract

Elevated concentration of atmospheric carbon dioxide will affect carbon cycling in terrestrial ecosystems. Possible effects include increased carbon input into the soil through the rhizosphere, altered nutrient concentrations of plant litter and altered soil moisture. Consequently, the ongoing rise in atmospheric carbon dioxide might indirectly influence soil biota, decomposition and nutrient transformations.

N-mineralisation and activities of the enzymes invertase, xylanase, urease, protease, arylsulfatase, and alkaline phosphatase were investigated in spring and summer in calcareous grassland, which had been exposed to ambient and elevated CO_2 concentrations (365 and 600 µl l^{-1}) for six growing seasons.

In spring, N-mineralisation increased significantly by 30% at elevated CO_2, while there was no significant difference between treatments in summer (+3%). The response of soil enzymes to CO_2 enrichment was also more pronounced in spring, when alkaline phosphatase and urease activities were increased most strongly by 32% and 21%. In summer, differences of activities between CO_2 treatments were greatest in the case of urease and protease (+21% and +17% at elevated CO_2).

The stimulation of N-mineralisation and enzyme activities at elevated CO_2 was probably caused by higher soil moisture and/or increased root biomass. We conclude that elevated CO_2 will enhance belowground C- and N-cycling in grasslands.

Keywords: elevated CO_2, enzymes, grassland, N-mineralisation

Introduction

Since the beginning of industrialisation the global concentration of atmospheric carbon dioxide (CO_2) has risen from about 280 ppm to 365 ppm, mainly due to fossil fuel burning and land-use change. Currently, the concentration is rising 1.5 ppm per year on average (IPCC, 2001). Direct effects of elevated CO_2 on soil organisms are unlikely because CO_2 concentrations in soils are already 10-50 times higher than in the atmosphere (Lamborg *et al.*, 1983). There are, however, three plant-mediated mechanisms by which increased atmospheric CO_2 concentration might influence soil microbial communities:

1) Elevated CO_2 stimulates photosynthesis of plants, and consequently net primary productivity increases. At least part of the extra C fixed is allocated belowground. This can result in increased root biomass, root-shoot-ratio, fine root biomass and fine root turnover (Rogers *et al.*, 1994).

2) Chemistry of green leaves is altered; generally, the carbon to nitrogen ratio increases in green leaf tissue, in part due to starch accumulation. As soil microorganisms are often constrained by available C (Paul and Clark, 1996), it is likely that they respond to these changes by increasing biomass and/or activity. However, naturally senesced litter often does not show these changes in C/N (Hirschel *et al.*, 1997; Norby *et al.*, 2001). Also, the concentration of phenolic compounds such as lignin and tannins sometimes increases, reducing decomposability of the plant material.

3) Elevated CO_2 reduces stomatal conductance of plants which results in higher water use efficiency. At a plant community level, this often results in decreased stand transpiration and higher soil water content (Körner, 2000). Soil matrix potential is an important control of soil microbial activity, directly through osmosis and indirectly by altering the supply of nutrients. Up to a certain threshold, increasing soil moisture is beneficial to soil microbes and their activity (Killham, 1994).

Responses of plants to elevated CO_2 are well studied, and they generally vary with the species studied and nutritional conditions. Microbial responses to elevated CO_2 in complex natural ecosystems are less well understood (Kampichler *et al.*, 1998). In their review, Zak *et al.* (2000) showed that the response of soil microorganisms to elevated CO_2 is highly variable, no matter whether activity, biomass or effects on the N-cycle were studied. This variability cannot be explained by plant life forms. Studies that reported changes of soil microbial parameters at elevated CO_2 often dealt with soil-plant systems characterised by high belowground carbon-input by plants in combination with low carbon content of the soil (Zak *et al.*, 2000). In contrast, undisturbed natural systems showed little or no responses, even after several years of CO_2 treatments (Körner *et al.*, 1997). Most

information on soil microbial response to elevated CO_2 originates from short-term experiments and/or experiments with disturbed soil. Extrapolation of these results to mature ecosystems and to longer time scales is limited (Hu *et al.*, 1999).

Soil microorganisms hold a key position in terrestrial ecosystems as they mineralise organic matter. Therefore, any effect of elevated CO_2 on soil microorganisms may in turn feed back on the response of plant communities to rising CO_2 and thus the sequestration of extra carbon. Though microbial decomposition and mineralisation are mediated by soil enzymes, relatively few studies have included measurements of enzyme activities (Kandeler *et al.*, 1998; Körner and Arnone, 1992; Kang *et al.*, 2001).

The objective of the present study was to assess the effect of elevated CO_2 on N-mineralisation and soil enzyme activities in a long-term *in situ* CO_2 enrichment experiment in calcareous grassland. Previous measurements had shown that microbial biomass did not change (Niklaus, 1998) and that increased soil moisture under elevated CO_2 was prominent throughout the growing period (Niklaus *et al.*, 1998). Higher soil moisture caused changes in soil structure (Niklaus *et al.*, 2003). In the sixth year of this experiment, soil was sampled in spring and summer and N-mineralisation and activities of enzymes related to the C-, N-, P- and S-cycling were measured. We hypothesised that

(1) N-mineralisation and enzyme activities were higher at elevated CO_2, though total microbial biomass remained unaffected, and,
(2) that the higher soil moisture under elevated CO_2 is the driving factor for changes in soil microbial activity, since organic carbon content of this grassland soil is rather high.

Materials and methods

Study site and experimental design

The study site is located in the foothills of the Swiss Jura Mountains in NE-Switzerland on a south-west facing slope (20°) at an altitude of 520 m (47°33‘ N, 7°34‘ E, Leadley *et al.*, 1999). Soil is classified as transition Rendzina derived from calcareous debris. Texture and chemical properties are given in Table C1. The species-rich grassland contains over 100 vascular plant species and is dominated by *Bromus erectus* Huds. Average annual precipitation is about 900 mm, average annual air temperature between 8.5-9.0 °C (Ogermann *et al.*, 1994). Before the field experiment was set up, the grassland was extensively used as cattle pasture.

Table C1 Texture and chemical properties of the soil, a Swiss calcareous grassland

Soil property	
Clay g kg^{-1}	305
Silt g kg^{-1}	560
Sand g kg^{-1}	135
pH	6.5
N_t%	0.38
P mg 100 g^{-1}	< 1
K mg 100 g^{-1}	6.9
Mg mg 100g^{-1}	5.0
C_{org}%	3.9
N_t%	0.33

The CO_2 enrichment experiment was started at the end of March 1994. Twenty-four plots (1.27 m² area each) were selected and 3 treatments (8 replicates each) assigned randomly in a complete block design. Treatments were unscreened control plots (365 µl CO_2 l^{-1}), screened ambient plots (365 µl CO_2 l^{-1}), and screened elevated CO_2 plots (600 µl CO_2 l^{-1}), using the SACC-system for CO_2 exposure (Screen-aided-CO_2-control, Leadley *et al.*, 1997). The system operated 24 hours a day and was only shut down between December and end of February each year.

Soil sampling and storage

Soil was sampled at the end of April ('spring') and the end of June 1999 ('summer'), when the field experiment was terminated. From each plot, 3 subsamples each containing approximately 30 g dry soil were taken from the top 10 cm of the A_h horizon and pooled afterwards. Soil samples were stored at -20°C. Before sieving (5 mm mesh size), samples were allow to thaw for three days at 4°C. During soil microbial analysis, which was finished within two weeks, samples were kept at 4°C.

Soil microbial activity

Net N-mineralisation was determined by incubating 5.0 g soil under waterlogged conditions in an enclosed tube at 40°C for 7 days (Keeney, 1982). Released ammonium was extracted with 2M KCl and measured colorimetrically (Kandeler and Gerber, 1988).

Urease activity was measured by incubating 0.5 g moist soil with 1.5 ml of a 79.9 mM urea solution for 2 h at 37°C. Produced NH_4^+ was extracted with 12 ml 1M KCl/10mM

HCl and determined colorimetrically by an indophenol reaction as described by Kandeler and Gerber (1988).

For the determination of alkaline phosphatase, 0.3 g soil was incubated in 2 ml 200 mM borate buffer (pH 10.0) and 1 ml buffered disodium phenylphosphate solution at 37 °C for 3 h. Released phenol was measured by colour reaction (Hoffmann, 1968).

The modified method of Ladd and Butler (1972) was used to estimate protease activity by incubating 0.3 g moist soil in 5.0 ml 5mM TRIS buffer (pH 8.1) and buffered 5.0 ml casein-solution (2% w/v) for 2 h at 50 °C. The aromatic amino acids produced were extracted with trichloracetic acid (0.92 M) and measured colorimetically after adding Folin-Ciocalteau-reagent and expressed as tyrosine equivalents.

To measure xylanase activity, 0.3 g fresh soil was incubated with 0.9 ml of a substrate solution (1.2% w/v xylan from oat spelts suspended in 2 M acetate buffer, pH 5.5) and 0.9 ml 2 M acetate buffer (pH 5.5) for 24 h at 50 °C. Released reducing sugars reduced potassium hexacyanoferrat (III) in an alkaline solution. Potassium hexacyano-ferrat (II) produced was estimated by a colorimetrical procedure according to the Prussian blue reaction (Schinner *et al.*, 1996).

Invertase activity was determined by incubating 0.3 g soil with 0.9 ml 35 mM sucrose solution and 0.9 ml 2 M acetate buffer for 3 h at 50 °C. Reducing sugars were determined as described for xylanase activity (Schinner *et al.*, 1996). Results of invertase and xylanase activity were expressed as glucose equivalents.

Arylsulfatase activity was assayed by incubating 0.3 g moist soil in 1.2 ml 0.5 M acetate buffer (pH 5.8) and 0.3 ml 0.02 M p-nitrophenyl-sulfate solution for 1 h at 37 °C. After adding 0.5 M NaOH, released nitrophenol was estimated colorimetically (Tabatabai and Bremner, 1970).

Analysis of each replicate sample (total n of 48) was carried out in duplicate, except for alkaline phosphatase and xylanase activities which were determined in triplicate. All results were calculated on a oven dry soil weight basis.

Soil water content

Soil water content was determined by drying 10 g moist soil to constant mass at 105 °C. Measurements were carried out in duplicate.

Statistical analysis

Before statistical analysis, soil microbial activity and water content data were standardised by dividing each value by the mean value of the respective parameter in the respective season (spring or summer). Standardised activities were then log-transformed to obtain normally distributed data. In analysis of variance (ANOVA), all factors were fitted sequentially (type I sum of squares) using SPSS 8.0 (SPSS Inc., Chicago, IL). Two different models were used for analysis of screen and CO_2-effects: (1) Effects on the activity of individual enzymes, N-mineralisation and soil water content were tested in a model comprising data from both sampling dates and, according to the hierarchical design, ANOVA factors were (in that order) Block, Screen/CO_2, Plot(CO_2), Season, Season $\times$ CO_2. (2) Two ANOVAs, one for the April and one for the June measurements, were used to test for overall responses of enzyme activities to the Screen/CO_2 treatment. These ANOVAs included activities of all enzymes, containing the factors Block, Screen/CO_2, Plot(CO_2), Enzyme, and CO_2 $\times$ Enzyme. Effects of the Screen/CO_2 treatment were tested against Plot, the CO_2 $\times$ Enzyme interaction against the residual. For both models, *a priori* linear contrasts were used to test for effects of screening and CO_2 enrichment alone.

For both samplings, Pearson's correlation was calculated as measure of correlation between soil water content and individual enzyme activities and N-mineralisation respectively.

Ecological experiments using natural, unhomogeneized soils in combination with relatively low replication result in lower statistical power to detect effects than typical laboratory studies. The probability of type I errors has therefore to be balanced against the increasing probability of type-II errors (accepting the null hypothesis when it is false, i.e., failing to declare a real difference as statistically significant; c.f. Scheiner, 2001). We therefore consider effects with $P \leq 0.05$ as significant, and effects with $P \leq 0.1$ as marginally significant. Error estimates given in the text and error bars in figures are standard errors of the means. In figures, (*) indicates $P \leq 0.1$, * $P \leq 0.05$ and ** $P \leq 0.01$, *** $P \leq 0.001$.

Results

Figures C1 and C2 show the response of N-mineralisation and soil enzyme activities of a calcareous grassland to 6 growing seasons of elevated atmospheric CO_2. Data were in the range previously reported for grassland (Tscherko and Kandeler, 2000).

In spring, N-mineralisation increased by 30% in elevated compared to screened ambient CO_2 plots (P= 0.02, *a priori* linear contrast) (Figure C1). In summer, N-mineralisation was not increased under CO_2 enrichment (3.0%, P=0.6). At both sampling dates, all measured enzyme activities were higher under elevated CO_2 compared to the screened ambient treatment (Figure C2), with the only exception of xylanase activity in summer. In spring, alkaline phosphatase responded most strongly to the CO_2 enhancement with a significant increase in activity of 32% (P=0.02), followed by urease activity, which was 21% higher at elevated CO_2 (p=0.13). In summer, urease activity raised by same magnitude (+21%, P=0.2). Protease and invertase activity were marginally significantly higher by 17% (P=0.09) and 14% (P=0.07) respectively under elevated CO_2 in summer.

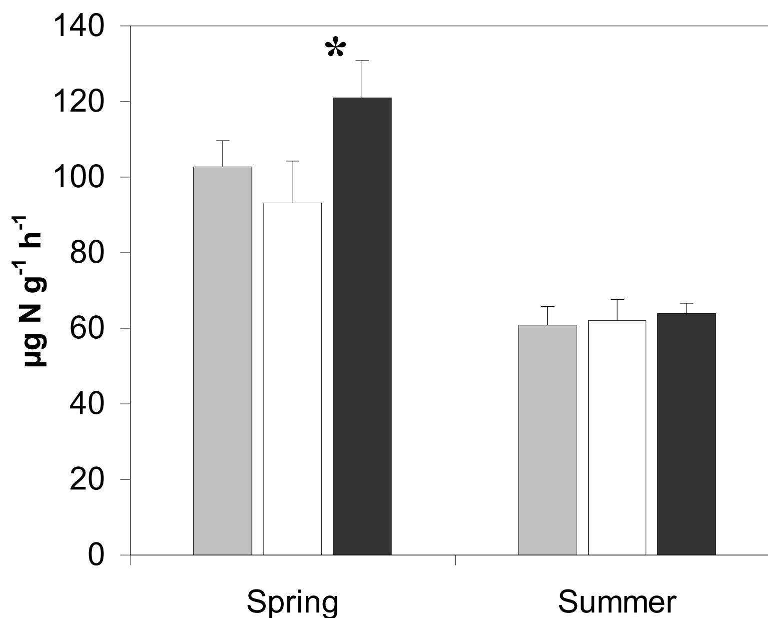

Figure C1 N-Mineralisation in unscreened control (grey bars), screened ambient CO_2 (white bars) and screened elevated CO_2 (black bars) plots in a calcareous grassland in spring and summer 1999 after 6 years of CO_2 enrichment. Means of 8 field replicates and standard errors are shown. * $P \leq 0.05$

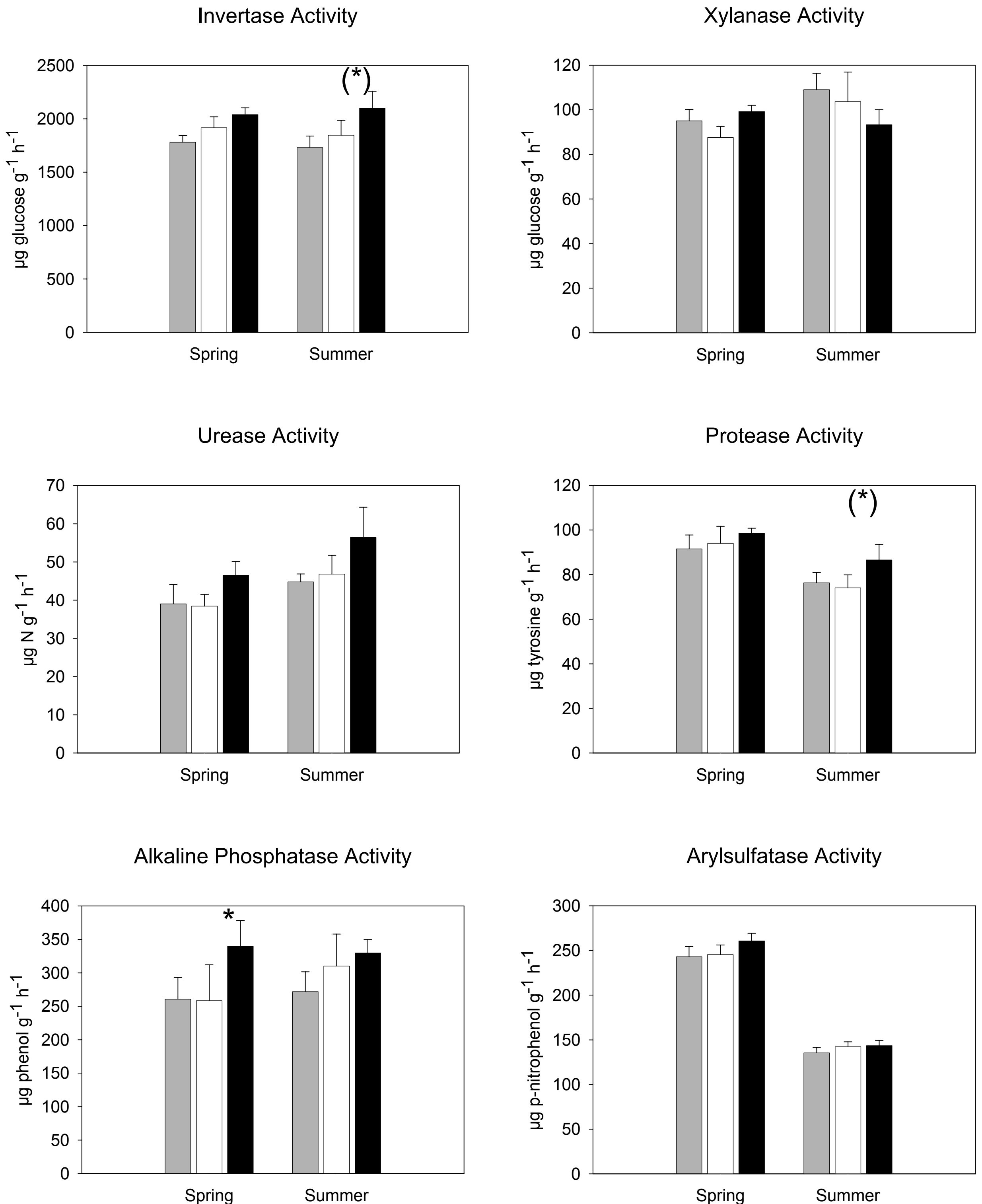

Figure C2 Enzyme activities in unscreened control (grey bars), screened ambient CO_2 (white bars) and screened elevated CO_2 (black bars) plots in a calcareous grassland in spring and summer 1999 after 6 years of CO_2 enrichment. Means of 8 field replicates and standard errors are shown. (*) $P \leq 0.1$, * $P \leq 0.05$

The ANOVAs testing the overall response of enzyme activities in spring and summer (model 2) showed that the interaction between CO_2 and enzyme activities was insignificant (Table C2) at both samplings. This indicates a uniform response pattern for all individual enzyme activities. The overall response of enzyme activities to CO_2 exposure was significant in spring (P=0.04, *a priori* linear contrast). At the summer sampling, the trend towards increased activities was not statistically significant (P=0.2). At both sampling dates, no effects of the treatment screens on the overall enzyme activity were found (spring P=0.9, summer P=0.8).

Table C2 Results of ANOVAs for spring and summer data including all enzyme activities with factors Block, Screening/CO_2, Plot(Screening/CO_2) and CO_2/Screening x Enzyme

		Spring			Summer		
Source of variation	df	MS	F	P	MS	F	P
Block	7	0.025			0.025		
Screening/CO_2	2	0.065	3.32	0.07	0.026	2.19	0.2
Screening[1]	1	0.001	0.03	0.9	0.001	0.05	0.8
CO_2[1]	1	0.106	5.38	0.04	0.033	1.67	0.2
Plot(Screening/CO_2)	14	0.020			0.012		
Enzyme	5	0.006	0.59	0.7	0.001	0.11	1.0
Screening/CO_2 x Enzyme	10	0.008	0.74	0.7	0.008	0.79	0.6
Residual	105	0.010			0.010		

[1)] *a priori* linear contrasts, tested against plot

At both samplings, soil water contents were higher in elevated CO_2 compared to screened ambient CO_2 plots (6.4% relative increase in spring, 3.7% in summer; Figure C3). However, differences between treatments were not significant. This is because soils had recently been saturated and the CO_2-effect onto evapotranspiration had not yet built up to a significant difference in soil moisture. In spring, alkaline phosphatase activity (r=0.64, P=0.001), N-mineralisation (r=0.59, P=0.004) and protease activity (r=0.43, P=0.045) were positively correlated with soil water content. In summer, no significant correlation between soil moisture and enzyme activities was found.

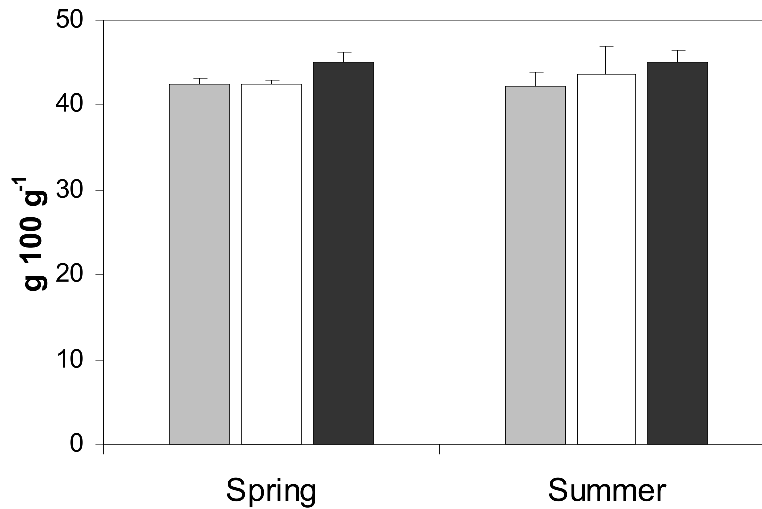

Figure C3 Soil water content in unscreened control (grey bars), screened ambient CO_2 (white bars) and screened elevated CO_2 (black bars) plots in a calcareous grassland in spring and summer 1999 after 6 years of CO_2 enrichment. Means of 8 field replicates and standard errors are shown. Differences between treatments are not significant.

Discussion

At the spring sampling and to a lower extent at the summer sampling, microbial activity, measured as enzyme activities and N-mineralisation, were higher in soils that had been exposed to elevated CO_2 for six years. Confirming our first hypothesis, microbial activities were stimulated by CO_2 enrichment, though microbial biomass did not respond to elevated CO_2. This was demonstrated by several measurement of soil microbial biomass in 1999, which are compiled in table C3. Total PLFA contents were determined on the same soil samples which were analysed for enzyme activities. Microbial biomass data by Niklaus *et al.* (2001b) for June 1999 were determined for samples taken at the same date.

Table C3 Plant and microbial biomass in March, April, and June 1999 in a Swiss calcareous grassland exposed to elevated CO_2 for six growing seasons

Organisms	Date	Units	Layer	Experimental treatment			CO_2-effect	Reference
				unscreened control	screened ambient CO_2	screened elevated CO_2		
Plants	March 1999	g C m^{-2}	Shoots	57.7 ± 11.2	61.4 ± 6.6	80.9 ± 12.8	+32%, n.s.	P. A. Niklaus, unpublished
		g C m^{-2}	Roots, 0-	890.9 ± 105.8	869.6 ± 171.4	929.5 ± 29.1	+7%, n.s.	
	June 1999	g C m^{-2}	Shoots	n.a.	142 ± 5	165 ± 9	+17%, P = 0.006	Niklaus *et al.* (2001b)
		g C m^{-2}	Roots, 0-	n.a.	571 ± 49	711 ± 31	+24%, P = 0.02	
Soil microbes	March 1999	μg C (g soil)$^{-1}$	0-10 cm	1267 ± 50	1376 ± 64	1478 ± 45	+7%, n.s.	P. A. Niklaus, unpublished
	April 1999	nmol PLFA (g soil)$^{-1}$	0-10 cm	123.8 ± 15.7	121.2 ± 9.5	98.04 ± 11.0	-21%, n.s.	D. Ebersberger, unpublished
	June 1999	μg C (g soil)$^{-1}$	0-10 cm	1171 ± 65	1324 ± 60	1351 ± 48	+2%, n.s.	Niklaus *et al.* (2001b)
		nmol PLFA (g soil)$^{-1}$	0-10 cm	71.7 ± 7.1	88.7 ± 7.7	86.9 ± 4.7	± 0%, n.s.	D. Ebersberger, unpublished

Regarding our second hypothesis that higher soil moisture is the driving factor for the effects of elevated CO_2, we could only partly relate this stimulation to altered soil moisture. At our study site, soil water contents were generally higher under elevated CO_2 and the amplitude of diurnal top-soil moisture cycles was reduced (Niklaus *et al.*, 1998). At the samplings undertaken for this study, soil water contents were also slightly, but not significantly, higher at elevated CO_2. Though at both samplings all measured process rates were higher at elevated CO_2 (with the exception of xylanase in summer), a correlation with soil water was only found in the case of N-mineralisation, alkaline phosphatase and protease, and only for the spring sampling. However, indirect evidence supporting this hypothesis could be derived from the fact that differences between treatments in microbial activities were greater in spring than in summer, as found for soil water contents. At both samplings, soil water content was close to field capacity. Such high soil moisture is only reached shortly after rainfall, and differences between CO_2 treatments are typically absent or small because the water saving effect under elevated CO_2 (reduced evapotranspiration) needs some time to translate into significant soil moisture differences (Niklaus *et al.*, 1998). Therefore, it is possible that more pronounced effects would have been detected under drier conditions, also because soil microorganisms would be more limited by water. Hungate *et al.* (1997a) explained higher gross N-mineralisation in the first growing season of CO_2 enrichment (ambient CO_2 concentration + 350 $\mu l\ l^{-1}$) in serpentine and sandstone annual grassland by increased soil moisture. Rice *et al.* (1994) related higher microbial activity, measured as soil respiration, to better soil water conditions under double-ambient CO_2 concentration in a tall grass prairie.

Increased soil moisture is not the only possible explanation for the observed effects. The increase in soil enzyme activities and N-mineralisation might also be related to extra C entering the soil and stimulating soil microbial activity. Additional C input could be due to greater root biomass, greater fine root turnover and higher rhizodeposition under elevated CO_2. In a ^{13}C labelling study using monoliths taken from this calcareous grassland, Niklaus *et al.* (2001a) found that root biomass increased under elevated CO_2, while rhizodeposition and root turnover were not affected. Minirhizotron observations in the field also suggested that root turnover was not altered, or even decreased, under elevated CO_2 (Arnone *et al.*, 2000). The responses of soil enzymes were in the same order as the increases in root biomass measured in March and June 1999 (see Table C3). If rhizodeposition per unit root weight had increased markedly, we would have expected enzyme activity to increase even more because the sampled top soil was densely explored by roots.

Since microbial biomass did not increase under elevated CO_2, higher enzyme activities could also be due to more plant-derived enzymes entering the soil as a consequence of the overall increase of root mass. However, root mass measured in June and enzyme activity was only weakly correlated in the case of alkaline phosphatase activity in spring (r=0.47, P=0.02) and this enzyme is not exuded by plant roots (Juma and Tabatabai, 1988). In a study of the root system in the first two years of this CO_2 enrichment study no differences in root mass were detected (Leadley *et al.*, 1999). A greater proportion of roots was, however, found in the top layer (0-6 cm) at elevated CO_2. The higher surface root density may induced higher activity of heterotrophic soil microorganisms (Arnone *et al.*, 2000).

Enhanced enzyme activities under elevated CO_2 were also reported by Ross *et al.* (1995) in a short-term chamber experiment with grassland turves exposed to elevated CO_2 for a total of 220 days (700 µl l^{-1} CO_2). Invertase activity increased significantly in the elevated CO_2 treatment at two of three sample dates. This was explained by a greater input of plant-derived invertase and greater production of invertase in response to increased C input. In a similar experiment which lasted 422 days, Ross *et al.* (1996) found only minor and insignificant differences of invertase activity at different CO_2 concentrations (350, 525, and 700 µl l^{-1} CO_2). Dhillion *et al.* (1996) studied a Mediterranean model ecosystem. After exposure to CO_2 concentrations of 700 µl l^{-1} for several months xylanase and dehydrogenase activities were significantly increased (61% and 13%). Cellulase and acid phosphatase activities were also considerably but insignificantly higher. Increased xylanase activity was related to greater fine root biomass and higher turnover of fine roots at elevated CO_2. Higher phosphatase activity reflected an increased need for P. Enzyme data from long-term field studies with CO_2 enrichment is scarce. Moorhead and Linkins (1997) studied enzyme activities associated with roots, ectomycorrhizae and organic and mineral soil in tussock tundra after 3 years of CO_2 enrichment. In contrast to rhizosphere compartments, in mineral soil no differences of acid phosphatase, exocellulase and endocellulase activity between ambient and double-ambient CO_2 treatments were found. Körner *et al.* (1997) exposed alpine grassland to elevated CO_2, with and without addition of complete mineral fertilizer (NPK and micronutrients). After three years of treatment, Mayr *et al.* (1999) used sensitive enzymes as indicators of C-cycling (CELase, beta-D-cellobiohydrolase; GLUase, beta-D-Glucosidase) and N-cycling (NAGase, N-acetyl-ß-D-glucosaminidase; APEase, L-Leucin-7-aminopeptidase). In the no-fertiliser treatment, CELase and NAGase showed significantly positive responses to elevated CO_2 (680 µl l^{-1}) in the 0-5 cm horizon only, while the underlying layers (5-15 cm) did not reveal any significant effects.

Temporal variability also must be considered. In spring, the stimulation of enzyme activities and N-mineralisation was more distinct than in summer. Hungate *et al.* (2000) found a similar temporal pattern for the response of soil microbiota in grasslands and concluded that an increased flux of carbon into the soil under elevated CO_2 was more pronounced in the early part of the growing season. However, Dhillion *et al.* (1996) reported that soil enzymes did not respond to elevated CO_2 at an early sampling date, but a significant effect was found at a later sampling. Thus, effects of elevated CO_2 on microbial activity may only be detectable at specific times and are closely linked to plant processes.

At both samplings, processes involved in N-cycling, urease and protease activity and N-mineralisation, were faster in elevated CO_2 soils. This indicates higher N-availability to plants and microbes. At the same time, net N immobilisation rates did not change since microbial N did not differ between treatments (Niklaus *et al.*, 2003). Increased N cycling in this study is remarkable since net N-mineralisation in grasslands is highly variable and generally does not respond significantly to elevated CO_2 (see review of Zak *et al.*, 2000).

Two main mechanisms were hypothesized concerning N-availability under elevated CO_2, both of which critically feed back on plant productivity (Diaz *et al.*, 1993; Zak *et al.*, 1993). Diaz *et al.* (1993) concluded that increased C availability under elevated CO_2 would boost microbial biomass, in turn leading to nutrient immobilisation and thus constrained plant responses. In contrast, Zak *et al.*, (1993) reported increased N mineralisation rates under elevated CO_2, supposedly due to a priming effect of rhizodeposition on the mineralisation of native soil organic matter, resulting in higher N-availability to plants. Our results support Zak's hypothesis. N-mineralisation and enzyme activities were stimulated at elevated CO_2, even with microbial biomass remaining the same. In June 1999 plant biomass was increased significantly at elevated CO_2 (Niklaus *et al.*, 2001b; Table C3). Niklaus (1998) found that soil microbial growth in this ecosystem was co-limited by available N, at least in the short term (1 week). Probably, plants benefited more than microbes from increased N mineralisation, most likely because C still limited microbial growth. Similar findings were reported for Californian annual grassland (Hu *et al.*, 2001).

Elevated CO_2 stimulated N-mineralisation and soil enzyme activities in this calcareous grassland. The increased mineralisation capacity is in line with results by Ross *et al.*, (1996) and Hungate *et al.* (1997b) who reported enhanced C- and N-cycling in grassland under elevated CO_2, at least during certain times of the year. In our study, enzyme activities appear to be more sensitive indicators for changes in belowground C- and N-turnover than the size of the microbial biomass pool. However, more direct

measurements of turnover such as ^{13}C labelling experiments, soil respiration measurements, fine root turnover as observed with a minirhizotron camera, and accumulated N in aboveground plant mass removed in the course of this study did not change. This raises the question whether substrate availability and environmental conditions were limiting decomposition instead of enzyme activities (at least regarding the long-term average), or whether the more direct measurements did not provide sufficient statistical power to detect differences. However, increased C- and N-mineralisation capacity under elevated CO_2 may translate into faster decomposition during periods with otherwise optimal conditions, for example during warm spells with high soil moisture and substrate availability. This is likely to occur in spring, and may be responsible for the faster regrowth of plants under elevated CO_2 (c.f. LAI development in Niklaus *et al.* 1998; a similar response was observed for net CO_2 exchange). The likely driving mechanisms for the increased soil enzyme activities are higher soil moisture and root biomass.

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References

Arnone III, J. A., Zaller, J. G., Spehn, E. M., Niklaus, P. A., Wells, C. E., and Körner, C. (2000). Dynamics of root systems in native grasslands: effects of elevated atmospheric CO_2. *New Phytologist* **147**, 73-85.

Dhillion, S. S., Roy, J., and Abrams, M. (1996). Assessing the impact of elevated CO_2 on soil microbial activity in a Mediterranean model ecosystem. *Plant and Soil* **187**, 333-342.

Diaz, S., Grime, J. P., Harris, J., and McPherson, E. (1993). Evidence of a feedback mechanism limiting plant response to elevated carbon dioxide. *Nature* **364**, 616-617.

Hirschel, G., Körner, C., and Arnone III, J. A. (1997). Will rising atmospheric CO_2 affect leaf litter quality and *in situ* decomposition rates in native plant communities? *Oecologia* **110**, 387-392.

Hoffmann, G. (1968). Eine photometrische Methode zur Bestimmung der Phosphatase-Aktivität in Böden. *Zeitschrift für Pflanzenernährung*, *Düngung und Bodenkunde* **118**, 161-172.

Hu, S., Chapin III, F. S., Firestone, M. K., Field, C. B., and Chiariello, N. R. (2001). Nitrogen limitation of microbial decomposition in a grassland under elevated CO_2. *Nature* **409**, 188-191.

Hu, S., Firestone, M. K., and Chapin III, F. S. (1999). Soil microbial feedbacks to atmospheric CO_2 enrichment. *Trends in Ecology & Evolution* **14**, 433-437.

Hungate, B. A., Chapin III, F. S., Zhong, H., Holland, E., and Field, C. B. (1997a). Stimulation of grassland nitrogen cycling under carbon dioxide enrichment. *Oecologia* **109**, 149-153.

Hungate, B. A., Holland, E. A., Jackson, R. B., Chapin III, F. S., Mooney, H. A., and Field, C. B. (1997b). The fate of carbon under carbon dioxide enrichment. *Nature* **388**, 576-579.

Hungate, B. A., Jager III, C. H., Gamara, G., Chapin III, F. S., and Field, C. B. (2000). Soil microbiota in two annual grasslands: responses to elevated CO_2. *Oecologia* **124**, 589-598.

IPCC (2001). “IPCC Third Assessment Report - Climate Change 2001 The Scientific Basis Technical Summary”, Geneva.

Juma, N. G., and Tabatabai, M. A. (1988). Comparison of genetic and thermodynamic parameters of soils and corn and soybean roots. *Soil Biology and Biochemistry* **20**, 533-539.

Kampichler, C., Kandeler, E., Bardgett, R. D., Jones, T. H., and Thompson, L. J. (1998). Impact of elevated atmospheric CO_2 concentration on soil microbial biomass and activity in a complex, weedy field model ecosystem. *Global Change Biology* **4**, 335-346.

Kandeler, E., and Gerber, H. (1988). Short-term assay of soil urease activity using colorimetric determination of ammonium. *Biology and Fertility of Soils* **6**, 68-72.

Kandeler, E., Tscherko, D., Bardgett, R. D., Hobbs, P. J., Kampichler, C., and Jones, T. H. (1998). The response of soil microorganisms and roots to elevated CO_2 and temperature in a terrestrial model ecosystem. *Plant and Soil* **202**, 251-262.

Kang, H., Freeman, C., and Ashenden, T. W. (2001). Effects of elevated CO_2 on fen peat biogeochemistry. *The Science of the Total Environment* **279**, 45-50.

Keeney, D. R. (1982). Nitrogen-availability indices. *In* “Methods of Soil Analysis, part 2” (A. L. Page, R. H. Miller and D. R. Keeney, eds.), pp. 711-733. American Society of Agronomy, Madison, Wis.

Killham, K. (1994). “Soil Ecology” Cambridge University Press, Cambridge.

Körner, C. (2000). Biosphere responses to CO_2 enrichment. *Ecological Applications* **10**, 1590-1619.

Körner, C., and Arnone III, J. A. (1992). Responses to elevated carbon dioxide in artificial tropical ecosystems. *Science* **257**, 1672-1675.

Körner, C., Diemer, M., Schäppi, B., Niklaus, P., and Arnone III, J. A. (1997). The responses of alpine grassland to four seasons of CO_2 enrichment: a synthesis. *Acta Oecologica* **18**, 165-175.

Ladd, J. N., and Butler, J. H. A. (1972). Short-term assay of soil proteolytic enzyme activities using proteins and dipeptide derivatives as substrates. *Soil Biology and Biochemistry* **4**, 19-30.

Lamborg, M. R., Hardy, R. W. F., and Paul, E. A. (1983). Microbial effects. *In* “CO_2 and Plants, The Response of Plants to Rising Levels of Atmospheric Carbon Dioxide” (E. R. Lemon, ed.), pp. 131-176. Westview Press, Boulder.

Leadley, P. W., Niklaus, P., Stocker, R., and Körner, C. (1997). Screen-aided CO_2 control (SACC): a middle-ground between FACE and open-top chamber. *Acta Oecologia* **18**, 207-219.

Leadley, P. W., Niklaus, P. A., Stocker, R., and Körner, C. (1999). A field study of the effects of elevated CO_2 on plant biomass and community structure in a calcareous grassland. *Oecologia* **118**, 39-49.

Mayr, C., Miller, M., and Insam, H. (1999). Elevated CO_2 alters commmunity-level physiological profiles and enzymes activities in alpine grassland. *Journal of Microbiological Methods* **36**, 35-43.

Moorhead, D. L., and Linkins, A. E. (1997). Elevated CO_2 alters belowground exoenzyme activities in tussock tundra. *Plant and Soil* **189**, 321-329.

Niklaus, P. A. (1998). Effects of elevated atmospheric CO_2 on soil microbiota in calcareous grassland. *Global Change Biology* **4**, 451-458.

Niklaus, P. A., Göckler, E., Siegwolf, R., and Körner, C. (2001a). Carbon allocation in calcareous grassland under elevated CO_2: A combined ^{13}C pulse labelling/soil physical fractionation study. *Functional Ecology* **15**, 43-50.

Niklaus, P. A., Spinnler, D., and Körner, C. (1998). Soil moisture dynamics of calcareous grassland under elevated CO_2. *Oecologia* **117**, 201-208.

Niklaus, P. A., Wohlfender, M., Siegwolf, R., and Körner, C. (2001b). Effects of six years atmospheric CO_2 enrichment on plant, soil, and soil microbial C of a calcareous grassland. *Plant and Soil* **233**, 189-202.

Niklaus, P. A., Alphei, J., Ebersberger, D., Kampichler, C., Kandeler, E., and Tscherko, D. (2003). Six years of *in situ* CO_2 enrichment evoke changes in soil structure and soil biota of nutrient-poor grassland. *Global Change Biology* **9**, 585-600.

Norby, R. J., Cotrufo, M. F., Ineson, P., O´Neill, E. G., and Canadell, J. G. (2001). Elevated CO_2, litter chemistry, and decomposition: a synthesis. *Oecologia* **127**, 153-165.

Ogermann, P., Spycher, B., Schaub, D., and Sollberger, R. (1994). Die Landschaftsstruktur im Raum Nenzlingen - geoökologisch gesehen. *Regio Basiliensis* **35**, 91-100.

Paul, E. A., and Clark, F. E. (1996). “Soil Microbiology and Biochemistry” Academic Press, San Diego, CA.

Rice, C. W., Garcia, F. O., Hampton, C. O., and Owensby, C. E. (1994). Soil microbial response in tallgrass prairie to elevated CO_2. *Plant and Soil* **165**, 67-74.

Rogers, H. H., Runion, G. B., and Krupa, S. V. (1994). Plant responses to atmospheric CO_2 enrichment with emphasis on roots and the rhizosphere. *Environmental Pollution* **83**, 155-189.

Ross, D. J., Saggar, S., Tate, K. R., Feltham, C. W., and Newton, P. C. D. (1996). Elevated CO_2 effects on carbon and nitrogen cycling in grass/clover turves of a Psammaquent soil. *Plant and Soil* **182**, 185-198.

Ross, D. J., Tate, K. R., and Newton, P. C. D. (1995). Elevated CO_2 and temperature effects on soil carbon and nitrogen cycling in ryegrass/white clover turves of an Endoaquept soil. *Plant and Soil* **176**, 37-49.

Scheiner, S. M. (2001). “Design and analysis of ecological experiments” 2nd Ed. Oxford Press, Oxford.

Schinner, F., Öhlinger, R., Kandeler, E., and Margesin, R. (1996). "Methods in Soil Biology," Springer, Berlin Heidelberg New York.

Tabatabai, M. A., and Bremner, J. M. (1970). Arylsulfatase activity of soils. *Soil Science Society of America Proceedings* **34**, 225-229.

Tscherko, D., and Kandeler, E. (2000). Classification and monitoring of soil microbial biomass, N-mineralization and enzyme activities to indicate environmental changes. *Die Bodenkultur* **50**, 215-226.

Zak, D. R., Pregitzer, K. S., Curtis, P. S., Teeri, J. A., Fogel, R., and Randlett , D. L. (1993). Elevated atmospheric CO_2 and feedback between carbon and nitrogen cycles. *Plant and Soil* **151**, 105-117.

Zak, D. R., Pregitzer, K. S., King, J. S., and Holmes, W. E. (2000). Elevated atmospheric CO_2, fine roots and the response of soil microorganisms: a review and hypothesis. *New Phytologist* **147**, 201-222.

D Effects of long-term CO_2 enrichment on microbial community structure in calcareous grassland

Diana Ebersberger, Nicola Wermbter, Pascal A. Niklaus, and Ellen Kandeler

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Abstract

Elevated CO_2 generally increases plant productivity, and has been found to alter plant community composition in many ecosystems. Because soil microbes depend on plant-derived C and are often associated with specific plant species, elevated CO_2 has the potential to alter structure and functioning of soil microbial communities. We investigated soil microbial community structure of a species-rich semi-natural calcareous grassland that had been exposed to elevated CO_2 (600 µL L^{-1}) for 6 growing seasons. We analysed microbial community structure using phospholipid fatty acid (PLFA) profiles and DNA fingerprints obtained by Denaturing Gradient Gel Electrophoresis (DGGE) of 16S rDNA fragments amplified by the Polymerase Chain Reaction (PCR).

PLFA profiles were not affected by CO_2 enrichment and the ratio of fungal and bacterial PLFA did not change. Ordination analysis of DNA fingerprints revealed a significant relation between CO_2 enrichment and variation in DNA fingerprints in summer ($P=0.01$), but not in spring. This variation was due to changes in low-intensity bands, while dominant bands did not differ between CO_2 treatments. Diversity of the bacterial community, as assessed by number of bands in DNA fingerprints and calculation of Shannon diversity indices, was not affected by elevated CO_2.

Overall, only minor effects on microbial community structure were detected, corroborating earlier findings that soil carbon inputs did probably change much less than suggested by plant photosynthetic responses.

Keywords: 16S rDNA, DGGE, elevated CO_2, grassland, PLFA, soil microbial community

Introduction

Terrestrial ecosystems generally respond to the rising atmospheric CO_2 concentration with increased net primary productivity and increased water use efficiency (Berntson and Bazzaz, 1996; Morgan *et al.*, 2004). As a result, the amount and quality of organic substances entering the soil and fuelling microbial metabolism might change. Soil microorganisms and their activity might also be affected by increased soil moisture at elevated CO_2 (Hu *et al.*, 1999). To date, most research on responses of soil microbial communities has focused on microbial biomass, with inconsistent results, reporting no response, increases as well as decreases (Zak *et al.*, 1993; Diaz *et al.*, 1993; Allen *et al.*, 2000; Williams *et al.*, 2000; Zak *et al.*, 2000b). So far, only a few studies have opened the black box "microbial biomass" and addressed structural changes within the microbial communities. In studies doing so, often specific microbial populations have been examined, e.g., *Rhizobium* species (Schortemeyer *et al.*, 1996; Montealegre *et al.*, 2000) or mycorrhizal fungi *(Klironomos et al.*, 1996*)*. In studies using whole community approaches, PLFA profiling of microbial communities associated with trees dominate (Wiemken *et al.*, 2001; Zak *et al.*, 2000a; Zak *et al.*, 1996; Ringelberg *et al.*, 1997). With the exception of the study by Ringelberg *et al.*, only minor effects of CO_2 enrichment on PLFA profiles were found. Montealegre *et al.* (2002) detected changes in PLFA profiles in bulk soil but not in rhizosphere soil of *Trifolium repens* exposed to elevated CO_2 for three years. Various DNA-based approaches were also used to address the question of structural changes in the microbial community. In short-term experiments, Griffiths *et al.* (1998), using %G+C-profiling, found no effects of elevated CO_2 in rhizosphere communities of ryegrass and wheat. Bruce *et al.* (2000) also could not detect changes in DGGE profiles of bacterial communities from model terrestrial ecosystems exposed to elevated CO_2. In a FACE study with *Lolium perenne/Trifolium repens* swards, the dominance of *Pseudomonas spp.* was increased at elevated CO_2 in monocultures of *Lolium perenne*, whereas it was decreased in the case of *Trifolium repens* (Marilley *et al.*, 1999). These studies were all either conducted in artificial or agronomic systems. There is a lack of experimental evidence on the effects of long-term CO_2 enrichment on microbial community structure in complex undisturbed systems. Microbial responses to elevated CO_2 may differ in such systems, as, e.g., soil is fully explored by roots and responses of individual plant species are integrated on the plant-community level.

We analysed the microbial community structure in a species-rich calcareous grassland exposed to elevated CO_2 for 6 growing seasons. Plant productivity increased under elevated CO_2, with effects ranging from 5 to 31% for aboveground biomass and an average increase in root biomass of 16%. Individual species biomass did not increase

statistically significantly under elevated CO_2 except for two subdominant *Carex* species, which showed large increases. However, species were significantly more evenly distributed in elevated CO_2-communities, due to small increases in the biomass of low-abundant species (which were not statistically significant when tested at the species level) (Niklaus and Körner, 2004). Evapotranspiration was reduced under elevated $CO_{2,}$ which led to significantly higher soil moisture. The largest differences in soil moisture between ambient and elevated CO_2 treatments were usually found at intermediate soil moisture levels (Niklaus *et al.*, 1998). Previous studies have shown that microbial biomass and basal respiration did not respond to CO_2 enrichment (Niklaus, 1998; Niklaus *et al.*, 2001a), whereas N-mineralisation and enzyme activities were stimulated by elevated CO_2 (Ebersberger *et al.*, 2003) (Table D1).

We used two different methods: (1) PLFA profiles, which give quantitative information on community structure and allow discrimination between fungi and bacteria, and (2) DNA fingerprints, obtained by PCR-DGGE of 16S rDNA, which allow a detailed analysis of the bacterial community structure.

Table D1 Soil water content, microbial biomass and N-Mineralisation in spring and summer 1999 in a Swiss calcareous grassland exposed to elevated CO_2 for six growing seasons

	Date	Units	Experimental treatment			CO_2-effect	Reference
			unscreened control	screened ambient CO_2	screened elevated CO_2		
Soil water content	April 1999	g (100g soil)$^{-1}$	42.50 ± 0.77	42.45 ± 0.59	45.15 ± 1.01	+6%, n.s.	Ebersberger *et al.* (2003)
	June 1999	g (100g soil)$^{-1}$	42.31± 1.47	43.55 ± 3.48	45.14 ± 1.23	+4%, n.s.	
Microbial biomass	March 1999	μg C (g soil)$^{-1}$	1267 ± 50	1376 ± 64	1478 ± 45	+7%, n.s.	P. A. Niklaus, unpublished
	June 1999	μg C (g soil)$^{-1}$	1171 ± 65	1324 ± 60	1351 ± 48	+2%, n.s.	Niklaus *et al.* (2001b)
N-Mineralisation	April 1999	μg N (g soil 7d)$^{-1}$	102.7 ± 7.1	93.2 ± 11.0	121.0 ± 9.9	+30%, P=0.02	Ebersberger *et al.* (2003)
	June 1999	μg N (g soil 7d)$^{-1}$	60.9 ± 4.8	62.0 ± 5.6	63.8 ± 2.7	+3%, n.s.	

Materials and methods

Study site and experimental design

The field research site is located at the foothills of the Swiss Jura Mountains in NE-Switzerland on a south-west facing slope (20°) at an altitude of 520 m (47°33‘ N, 7°34‘ E, 520 m a.s.l., Leadley *et al.*, 1999). Average annual precipitation is about 900 mm, average annual air temperature between 8.5-9.0 °C. Soil is classified as transition rendzina derived from calcareous debris, with a loamy texture (Ogermann *et al.*, 1994). The species-rich grassland with over 100 vascular plant species is dominated by *Bromus erectus* Huds. Until the setting up of the field experiment, the nutrient-poor grassland had been extensively used as cattle pasture.

Twenty-four plots (1.27 m² area each) were selected and 3 treatments assigned randomly in a complete block design. Treatments included unscreened control plots (365 µl CO_2 l^{-1}), screened ambient plots (365 µl CO_2 l^{-1}), and screened elevated CO_2 plots (600 µl CO_2 l^{-1}), using the Screen Aided CO_2 Control system for CO_2 exposure (SACC, Leadley *et al.*, 1997). CO_2 enrichment started at the end of March 1994. The system operated 24 hours a day and was only shut down between December and end of February each year.

Soil sampling and storage

Soil sampling took place at the end of April (‘spring’) and end of June 1999 (‘summer’) when the field experiment was terminated. In the central area of each plot, 3 bulk soil subsamples (approx. 30 g each) were taken from the top layer (0-10 cm) and pooled. Soil samples were sieved through a 5 mm screen and stored at –20°C.

PLFA analysis

Phospholipid fatty acids (PLFA) were analysed following the procedure used by Bardgett *et al.* (1996). Lipids were extracted with a single-phase chloroform-methanol-citrate buffer mixture (Bligh and Dyer 1959; White *et al.*, 1979), and then fractionated using SI-columns (Varian, Harbor City, CA, USA). To the polar lipid fraction, including phospholipids, methyl-nonadecanoate was added, and phospholipids were then converted to fatty-acid methyl esters by mild alkaline methanolysis. Fatty acid methyl esters were analysed by capillary gas chromatography (Perkin-Elmer Autosystem XL, Norwalk, CT, USA, fitted with a 50 m capillary column [HP-5, Agilent, Palo Alto, CA,USA] and a flame ionisation detector). Methyl esters were assigned to phospholipid fatty acids using SUPELCO qualitative standards (Sigma-Aldrich, Taufkirchen, Germany). Mass

spectrometry was used to confirm the chemical structures of the methyl esters standards separated by gas chromatography. All samples were analysed using a set of 20 fatty acids, which were quantified in almost all plots.

The fatty acid nomenclature used was as follows: total number of carbon atoms: number of double bonds, followed by the position of the double bond from the methyl end of the molecule. The prefixes a and i indicate anteiso- and iso-branching, and cy refers to cyclopropyl fatty acids. Following Frostegård and Bååth (1996), bacterial biomass was estimated from the summed concentration of 9 bacterial PLFA (i15:0, a15:0, 15:0, i16:0, i17:0, 17:0, cy17:0, 18:1ω7, and cy19:0) and fungal biomass was estimated from the concentration of the marker 18:2ω6. The prefixes a and i indicate anteiso- and iso-branching, and cy refers to cyclopropyl fatty acids.

DNA fingerprints

Soil microbial community DNA was extracted using the FastDNA^{TM} SPIN Kit for Soil (Qbiogene, Carlsbad CA, USA), following the manufacturer's instructions. The extract was then stored at -20°C.

The DNA extracted from the soil was amplified using the primer set F984-968GC and R1378-1401 (Heuer *et al.*, 1997). These anneal to conserved regions of the 16S rDNA of eubacteria and contain a GC clamp. PCR was undertaken as described by Marschner *et al.* (2003). PCR-products were then purified using the QIAquick PCR Purification Kit (Qiagen, Valencia, CA, USA) according to the manufacture’s protocol.

DGGE was performed with 6% acrylamide gels containing a linear chemical gradient ranging from 35 to 55 %, the 100 % denaturing solution contained 7 M urea and 40% (v/v) formamide. Electrophoresis was carried out with the DCode-System (Bio-Rad Laboratories, Hercules, CA, USA) in a 1 x TAE buffer at a constant voltage of 90 Volt for 16h. Standard samples were always run in triplicate on each gel to control the gradient and facilitate comparison of the different gels. After electrophoresis, gels were stained with the silver stain kit (Bio-Rad Laboratories) following the manufacturer’s protocol. Developed gels were scanned and then analysed using image analysis software (Geldoc Quantity One 4.0.1, Bio-Rad Laboratories). The position and intensity of each band was included in a data matrix for each sampling date.

Shannon Diversity Index

For each sample, we calculated Shannon diversity indices H’ based on DNA-fingerprints as

$$H' = -\sum_i \frac{n_i}{N} \ln \frac{n_i}{N} \quad ,$$

where n_i the intensity of the ith band respectively, and N is the total sum of the intensity of all bands respectively.

Statistical analysis

Data of individual PLFA, sums of PLFA, Shannon diversity indices, and number of bands in DNA fingerprints were analysed by analysis of variance (ANOVA). All data was log-transformed prior to analysis. All ANOVA factors were fitted sequentially (type I sum of squares) using SPSS 10.0 (SPSS Inc., Chicago, IL). According to the hierarchical design, ANOVA factors were Block, SACC/CO_2, Plot(SACC/CO_2), Season, Season*CO_2. A priori linear contrasts were used to test for effects of screening and CO_2 enrichment alone.

PLFA profiles and DGGE fingerprints were analysed with CANOCO 4 for Windows software (Microcomputer Power, Ithaca, NY, USA). Partial redundancy analysis (RDA) was used to analyse the data. RDA is an ordination technique based on principal component analysis (PCA). In PCA, ordination axes describe maximum variance of multivariate data, while in RDA ordination axes are constrained to be linear combinations of environmental variables. Hence, RDA allows direct analysis of treatments effects in experiments. The significance of the ordination axis, thus the significance of the relationship between environmental data and species composition, can then be tested by the Monte Carlo permutation test. Detailed descriptions of these methods are given in ter Braak and Smilauer (1998) and ter Braak and Prentice (1988).

PLFA and DNA data and the two sampling dates were analysed separately. Proportions of individual PLFA and bands of DNA fingerprints were considered individual species in RDA. Species data were log-transformed. Blocks of the field experiment were included as covariables to account for block effects of field experiment and analysis.

For RDA, treatments were included as environmental variables and three data sets for each sampling date were used to test for treatment effects: (1) a data set comprising data from all plots, (2) a data set comprising data from screened ambient plots and unscreened control plots to test for effects of screening (A vs. C treatment) and (3) a data set comprising data from screened ambient CO_2 plots and screened elevated CO_2 plots to test for effects of CO_2 enrichment (E vs. A treatment). The Monte Carlo test was used to test the null hypothesis that screening and/or CO_2 enrichment respectively did not significantly affect PLFA profiles and DNA fingerprints. The Monte Carlo test was based on 9999 unrestricted permutations of the data within blocks.

Effects with $P \leq 0.05$ were considered significant, while effects with $P \leq 0.1$ were considered marginally significant. In the case of individual PLFAs, the significance levels were adjusted by Bonferroni-correction to account for multiple tests.

Results

PLFA

PLFA profiles were dominated by fatty acids 18:1ω7, 18:1ω9 and 16:0, which together accounted for more than 50% of the total PLFA (Table D2). Differences between ambient and elevated CO_2 plots were mostly insignificant. Only the reduced proportion of i17:0 at elevated CO_2 in comparison to ambient CO_2 in spring was significant ($F_{1,21}=11.59$, $P=0.003$). Branched fatty acids are widely present in Gram-positive bacteria (Basile *et al.*, 1995). In spring, total PLFA were reduced at elevated CO_2 compared to ambient CO_2, which could be related to the reduction of bacterial PLFA. Both effects were marginally significant ($P=0.09$). Fungal PLFA and the fraction of 18:2ω6 did not respond to CO_2 enrichment. The ratio between bacterial and fungal PLFA also was not affected by CO_2 exposure (data not shown). No significant effects of screening were detected. Marginally significant responses to screening were observed for total PLFA in summer.

Table D2 Fraction (%) of individual PLFA, total determined PLFA (nmol g^{-1}) , and identified PLFA specific for bacterial and fungi (nmol g^{-1}) at ambient and elevated CO_2 and in unscreened control plots in calcareous grassland after 6 years of CO_2 enrichment. Means (standard error) of 8 replicates are given. Bold and italic numbers indicate significant differences at P≤0.05 and P≤0.01.

	Spring			Summer		
PLFA	Ambient CO_2	Elevated CO_2	Control	Ambient CO_2	Elevated CO_2	Control
		(% of total)			(% of total)	
14:0	0.29 (0.17)	0.19 (0.13)	0.06 (0.04)	0.44 (0.14)	0.22 (0.10)	0.25 (0.13)
i15:0	2.93 (0.44)	2.94 (0.21)	2.95 (0.34)	4.14 (0.72)	3.21 (0.61)	2.97 (0.80)
a15:0	4.05 (0.53)	4.32 (0.52)	4.37 (0.51)	5.56 (0.92)	4.38 (0.71)	3.74 (0.75)
15:0	0.75 (0.19)	0.66 (0.19)	0.55 (0.08)	0.65 (0.16)	0.48 (0.11)	0.40 (0.17)
i16:0	3.88 (0.17)	3.51 (0.29)	3.69 (0.17)	4.57 (0.30)	4.10 (0.25)	4.04 (0.37)
16:1ω7	6.70 (0.50)	7.01 (0.38)	7.10 (0.29)	6.57 (0.40)	6.53 (0.50)	5.66 (0.60)
16:0	15.58 (0.35)	15.53 (0.32)	16.06 (0.46)	15.58 (0.55)	15.86 (0.59)	15.31 (0.86)
i17:0	**2.87 (0.22)**	**2.46 (0.10)**	2.50 (0.09)	2.98 (0.07)	2.79 (0.07)	3.09 (0.10)
cy17:0	4.38 (0.25)	4.32 (0.12)	4.23 (0.17)	5.02 (0.23)	5.25 (0.20)	5.32 (0.28)
17:0	1.15 (0.13)	1.06 (0.05)	0.97 (0.04)	1.05 (0.05)	0.98 (0.04)	0.98 (0.08)
18:3ω3	2.16 (0.42)	2.39 (0.17)	2.13 (0.23)	0.87 (0.35)	0.96 (0.64)	0.62 (0.38)
18:2ω6	4.93 (0.11)	5.14 (0.31)	4.92 (0.20)	4.04 (0.30)	4.37 (0.20)	4.32 (0.23)
18:1ω9	15.73 (0.49)	15.47 (0.44)	15.54 (0.61)	15.60 (0.99)	15.80 (0.68)	16.00 (0.93)
18:1ω7	23.35 (0.86)	24.27 (1.09)	23.86 (0.71)	21.95 (1.31)	24.22 (1.33)	24.94 (1.62)
18:0	4.47 (0.41)	3.98 (0.24)	3.87 (0.21)	3.75 (0.43)	3.91 (0.34)	4.40 (0.51)
cy19:0	3.42 (0.25)	3.26 (0.20)	3.92 (0.57)	4.25 (0.60)	4.12 (0.68)	4.50 (0.85)
20:5ω3	1.37 (0.14)	1.31 (0.22)	1.19 (0.17)	0.83 (0.15)	0.76 (0.10)	1.23 (0.16)
20:6ω6	0.13 (o.05)	0.18 (0.05)	0.25 (0.03)	0.22 (0.06)	0.19 (0.05)	0.07 (0.05)
20:2	0.71 (0.05)	0.83 (0.07)	0.76 (0.05)	0.97 (0.13)	0.94 (0.13)	1.11 (0.12)
20:0	1.15 (0.06)	1.15 (0.10)	1.05 (0.07)	0.94 (0.88)	0.92 (0.06)	1.06 (0.13)
		(nmol g^{-1})			(nmol g^{-1})	
Total PLFA	*121.16 (9.52)*	*98.04 (11.03)*	123.79 (15.67)	*88.86 (7.72)*	86.88 (4.70)	*71.17 (7.09)*
Bacterial PLFA	*56.46 (4.25)*	*45.63 (4.86)*	57.84 (7.01)	44.78 (4.23)	43.03 (2.36)	35.53 (3.17)
Fungal PLFA	5.96 (0.48)	5.14 (0.72)	6.16 (0.83)	3.58 (0.34)	3.75 (0.16)	3.06 (0.34)

Multivariate analysis using RDA with Monte Carlo test did not reveal any significant relationship between treatments and variation in PLFA profiles (Fig. D1, Table D3).

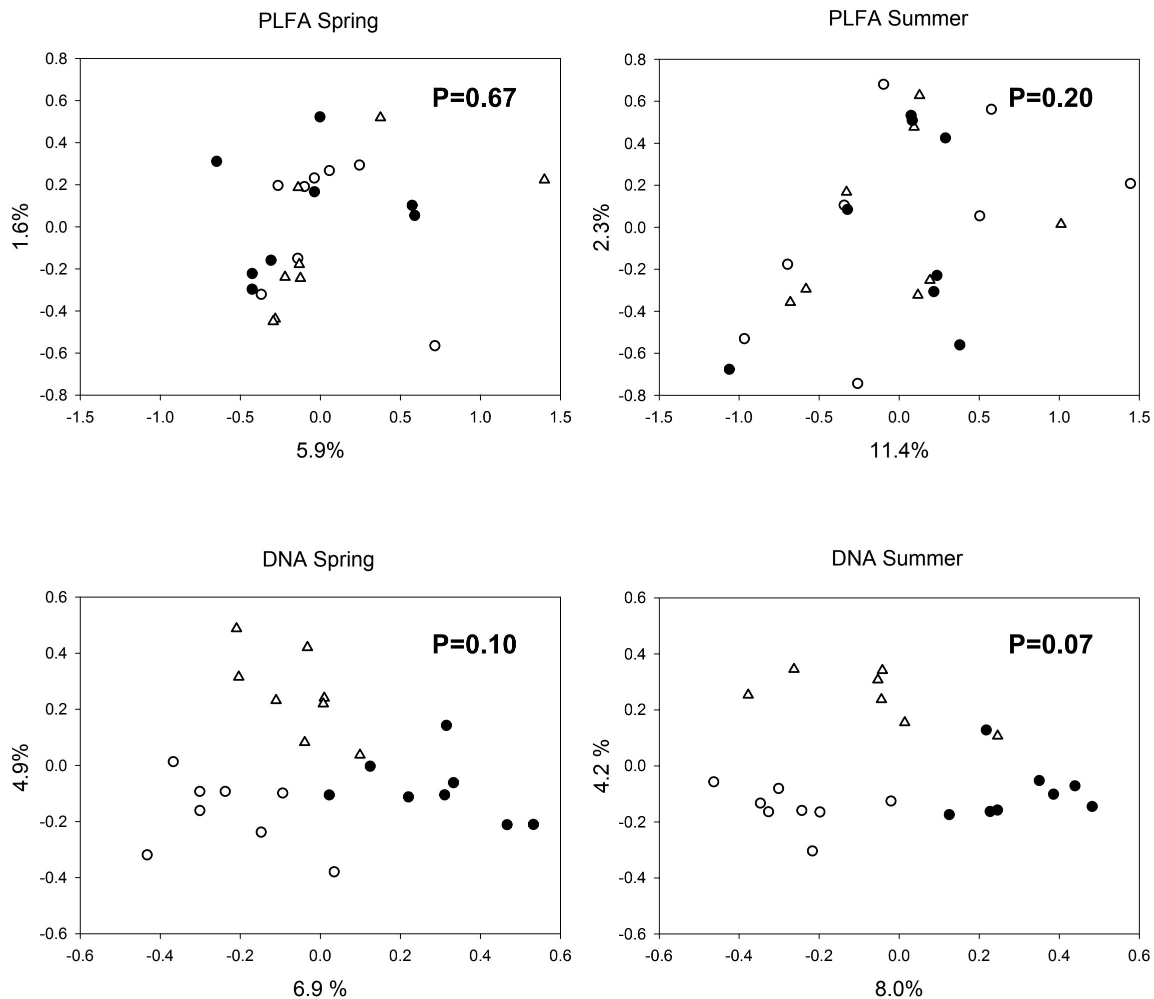

Figure D1 Sample scores of redundancy analysis of PLFA and DNA data in spring and summer 1999, after 6 growing seasons under elevated CO_2. △ unscreened ambient CO_2 plots, ○ screened ambient CO_2 plots, ● screened elevated CO_2 plots, explained variance and level of significance of ordination axes are given.
Only in the case of DNA fingerprints was a marginally significant relation between treatments and species variation detected.

Table D3 Explained variance (Expl. Var.) of species data and significance P (Monte Carlo permutation test) of first ordination axis in RDA. A vs. E: effect of CO_2 enrichment was analysed, A vs. C: effect of screening was analysed

		Spring		**Summer**	
		Expl. Var.	P	Expl. Var.	P
PLFA	A vs. E	3.9%	n.s.	5.5%	n.s.
	A vs. C	10.2%	n.s.	16.3%	n.s.
DNA fingerprints	A vs. E	11.6%	(*)	13.4%	**
	A vs. C	8.4%	n.s.	7.6%	n.s.

DNA fingerprints

DNA fingerprints were complex, with the number of bands detected in samples varying between 25 and 52. This variation could be attributed to differences in gels as the number of bands did not differ significantly between treatments (Table D4). An exemplary DGGE gel is shown in Fig. D2.

Table D4 Number of detected bands and Shannon-Index H‘ calculated for 16S rDNA fingerprints of soil bacterial communities for ambient CO_2, elevated CO_2 and unscreened control plots in calcareous grassland after 6 years of CO_2 enrichment, means (standard error) of 8 replicates are given. No significant differences between treatments were detected.

	Spring			Summer		
	Ambient CO_2	Elevated CO_2	Control	Ambient CO_2	Elevated CO_2	Control
Number of bands	37.5 (3.1)	39.6 (2.9)	41.0 (3.1)	35.3 (3.3)	34.4 (2.9)	36.4 (2.9)
Shannon-Index H‘	3.08 (0.30)	3.48 (0.32)	3.80 (0.36)	3.24 (0.07)	3.22 (0.07)	3.28 (0.07)

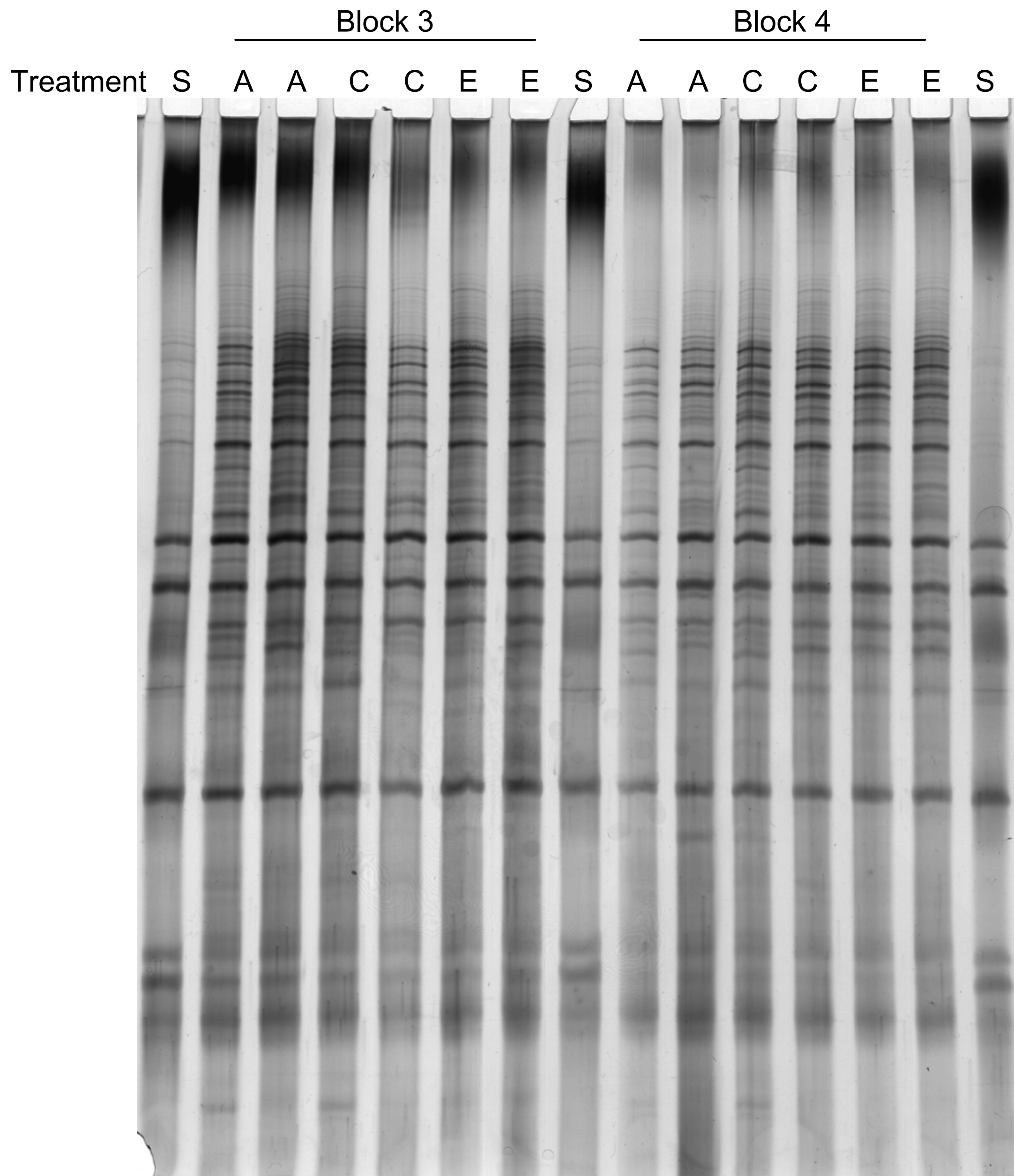

Figure D2 Exemplary DGGE gel showing bacterial DNA fingerprints of soil samples taken in summer 1999, after 6 growing seasons under elevated CO_2. S denotes standard bacterial mixture, A samples from screened ambient CO_2 plots, C unscreened ambient CO_2 control plots, and E screened elevated CO_2 plots.

RDA with a Monte Carlo permutation test revealed a significant relationship between variation in DNA fingerprints and CO_2 enrichment in summer (F=1.7, P=0.007); the first ordination axis explained 13.4 % of the overall variation in the data set. In spring, band variation was marginally significantly related to CO_2 enhancement (F=1.4, P=0.07).

At both samplings, screening did not affect the composition of the bacterial community (Table D3).

Though variation of DNA fingerprints associated with CO_2 treatments were found to be significant in summer and marginally significant in spring, the source of variation between samples from ambient CO_2 and elevated CO_2 plots could not be identified visually in the gel images. Dominant bands were similar in treatments and treatments probably differed in low intensity bands. Banding patterns were very complex, and varied between gels, due to subtle differences in the gel gradients and staining intensity. In multivariate analysis, these differences were taken into account by including the covariable block into the analysis, because samples were grouped on gels according to their block. The variation due to gel differences partly accounted for the relatively small explained variation by the significant ordination axes.

Shannon Diversity Indices

There were no differences in DNA diversity in spring (Table D4); in summer, DNA diversity averaged higher in elevated CO_2 plots than in the ambient CO_2 treatments, but differences were not statistically significant (Table D4).

Discussion

Six growing seasons of CO_2 enrichment in calcareous grassland caused minor significant changes in bacterial community structure, as shown by PCR-DGGE of 16S rDNA fragments analysed by ordination techniques. However, diversity and species richness, as estimated by Shannon diversity indices and the number of bands in DNA fingerprints, were not affected by CO_2 enrichment.

In case of PLFA, only the reduced fraction of i17:0 was significant while the overall profiles were not affected. This is in line with results of most CO_2-enrichment studies. Insam *et al.* (1999) studied microbial response to elevated CO_2 in a tropical ecosystem. Neither bacterial nor fungal PLFA fractions were altered by elevated CO_2, but 4 single PLFAs were reduced at elevated CO_2. In studies with *Populus tremuloides* and *Populus gradientata*, no changes in microbial community composition were found at elevated CO_2, as assessed by PLFA (Zak *et al.*, 2000a; Zak *et al.*, 1996). In a model beech-spruce ecosystem PLFA patterns at ambient and elevated CO_2 were also similar (Wiemken *et al.*, 2001). CO_2-exposure of *Pisum sativum* plants also did not alter PLFA patterns (Rønn *et*

al., 2002). In contrast, Montealegre *et al.* (2002) detected changes in bulk soil PLFA profiles of white clover in response to CO_2-enrichment, which were statistically significant at $P<0.1$.

In most studies measuring PLFA, no significant responses to elevated CO_2 were found. PLFA profiles have shown to be sensitive to soil type and management (Bossio *et al.*, 1998) and heavy metal contamination (Frostegård *et al.*, 1993), which represent major impacts on the soil habitat. In comparison, possible effects of CO_2-enrichment on the soil environment and hence on its microbial community are likely to be much smaller. Since many microbial groups contribute to individual PLFA the resolution of PLFA profiles is limited. So, even if responses of microbial communities to elevated CO_2 exist, they are possibly too subtle to be easily detected by PLFA analysis.

We found DNA fingerprints of elevated and ambient CO_2 treatments to be only different in minor, low-intensity bands. It must be noted, however, that these low-intensity bands still account for pre-dominant bacterial populations within the microbial community, as only predominant species (minimum 1% of the total bacterial DNA) will appear as band in DNA fingerprints produced using DGGE (Myzer *et al.*, 1993; Myzer and Smalla, 1998).

One possible cause for the detected effects on the bacterial community structure is altered rhizodeposition at elevated CO_2, as the top soil is densely explored by roots. The quantity of rhizodeposition was probably unaltered in this calcareous grassland, as demonstrated by C-isotope studies (Niklaus *et al.* 2001a, Niklaus *et al.* 2001b), but effects on rhizodeposit quality are unknown. Experimental information on the chemistry of rhizodeposits under elevated CO_2, especially in undisturbed soils, is generally lacking (Cardon, 1996; Darrah, 1996). Hodge *et al.* (1998) examined exudates from the roots of *Lolium perenne* grown in sterile sand mesocosms for 21 days. Under elevated CO_2, smaller amounts of phenolic acids and total sugars were released from the seedlings. Indirect evidence of rhizodeposition in soil systems can be gained by studies using Biolog microplates or similar approaches. Substrate utilisation of microbial communities associated with *Gutierza sarothrae* roots responded to CO_2 enrichment indicating changes in rhizodeposition (Rillig *et al.*, 1997). Grayston *et al.* (1998) found effects of elevated CO_2 in sole carbon utilisation patterns of microbial communities from the rhizosphere of *Danthoia richardsonii* and concluded that under elevated CO_2 compounds with a higher C:N ratio were released. Rhizodeposition differs from species to species (Uren, 2001), and plant responses to elevated CO_2 is species-specific (Rogers *et al.*, 1994). Therefore, it is difficult to scale up from results of experiments with monocultures at elevated CO_2 to the overall rhizodeposition of diverse plant communities. In a study by Mayr *et al.* (1999), community-level physiological profiles of soil from a open-top chamber experiment in alpine grassland differed between ambient and elevated CO_2 treatments. However,

because the potential utilisation of all substrate groups increased the quality of rhizodeposits was probably not altered. Besides altered rhizodeposition of individual species, changes in the overall rhizodeposition of the plant community could also result from altered plant community structures. In the grassland studied, individual species responses to elevated CO_2 were not statistically significant except for two subdominant sedges (*Carex flacca* and *C. caryophyllea*). This, plus a (at the species-level) non-significant increase in the biomass of low-abundance species led to higher species evenness in elevated CO_2 (Niklaus and Körner, 2004). If CO_2-effects on individual plant rhizodeposition and/or these small effects on plant community composition resulted in altered overall quality of rhizodeposits in this calcareous grassland is uncertain, because direct measurements are lacking, but the effects detected in bacterial community structure might be an evidence for it.

Because fungal biomass has a higher C:N ratio than the bacterial biomass and fungal hyphae are able to translocate nutrients across relatively large distances, increased C input at elevated CO_2 may favour fungi over bacteria in N limited systems (Hu *et al.*, 2001). Long-term CO_2 enrichment in two Californian grassland stimulated the fungal food chain more strongly than the bacterial food chain (Rillig *et al.*, 1999). As the PLFA 18:2ω6, used as indicator fatty acid for fungal biomass, did not respond to elevated CO_2, this hypothesis was not confirmed by the results of this study. The proportions of other fatty acids which are also related to fungi, like 18:3ω3 and 18:1ω9 (Zak *et al.*, 2000a), also did not change under CO_2 exposure.

We did not detect any significant differences between screened ambient CO_2 plots and unscreened control plots in DNA fingerprints or in PLFA profiles. We conclude that the screening itself did not cause considerable changes in microbial community structure. This was noticeable in terms of the importance of altered soil moisture at elevated CO_2. Elevated CO_2 plots and control plots behaved similarly in terms of soil moisture. Both treatments are generally slightly higher in soil water content than ambient CO_2 plots, especially at intermediate soil moisture levels and the amplitude of diurnal soil moisture cycle was reduced (Niklaus *et al.*, 1998; 2003). Because we could not detect any windscreen effect, we conclude that these altered soil moisture conditions did not affect the soil microbial community structure in the long term. However, it must be noted that at the time of the two samplings, soil moisture did not differ significantly between treatments (see Table D1). This is because soils had recently been saturated and the CO_2-effect on evapotranspiration had not yet expanded to a significant difference in soil moisture

(Ebersberger *et al.*, 2003). Possibly, effects of altered soil moisture on community structure might be detectable at drier conditions.

Conclusion

Overall, CO_2 enrichment in calcareous grassland caused only small detectable changes in microbial community structure. Other evidence from the same CO_2 enrichment experiment suggests that substrate availability to microorganisms did not change considerably under elevated CO_2 (Niklaus *et al.*, 2003). Hence, possible changes in substrate quality were not distinct enough to profoundly affect soil microbial community structure. However, considering the vast diversity of the soil microbial community, the PLFA and 16S rDNA-DGGE approaches we used are broad scale methods with only limited resolution. Possibly, there were effects on sub-community or single species level that could not be detected by these methods.

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References

Allen, M. F., Andrews, J. A., Finzi, A. C., Matamala, R., Richter, D. D., and Schlesinger, W. H. (2000). Effects of free-air CO_2 enrichment (FACE) on belowground processes in a *Pinus taeda* forest. *Ecological Applications* **10**, 437-448.

Bardgett, R. D., Hobbs, P. J., and Frostegård, A. (1996). Changes in soil fungal:bacterial biomass ratios following reductions in the intensity of management of an upland grassland. *Biology and Fertility of Soils* **22**, 261-264.

Basile, F., Voorhees, K. J., and Hadfield, T. L. (1995). Microorganism gram-type differentiation based on pyrolysis-mass spectrometry of bacterial fatty acid methyl ester extracts. *Applied and Environmental Microbiology* **61**, 1534-1539.

Berntson, G. M., and Bazzaz, F. A. (1996). Belowground positive and negative feedbacks on CO_2 growth enhancement. *Plant and Soil* **187**, 119-131.

Bligh, E. G., and Dyer, W. J. (1959). A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemical and Physiology* **37**, 911-917.

Bossio, D. A., Scow, K. M., Gunapala, N., and Graham, K. J. (1998). Determinants of soil microbial communities: effects of agricultural management, season, and soil type on phospholipid fatty acids profiles. *Microbial Ecology* **36**, 1-12.

Bruce, K. D., Jones, T. H., Bezemer, T. M., Thompson, L. J., and Ritchie, D. A. (2000). The effect of elevated atmospheric carbon dioxide levels on soil bacterial communities. *Global Change Biology* **6**, 427-434.

Cardon, Z. G. (1996). Influence of rhizodeposition under elevated CO_2 on plant nutrition and soil organic matter. *Plant and Soil* **187**, 277-288.

Darrah, P. R. (1996). Rhizodeposition under ambient and elevated CO_2 levels. *Plant and Soil* **187**, 265-275.

Diaz, S., Grime, J. P., Harris, J., and McPherson, E. (1993). Evidence of a feedback mechanism limiting plant response to elevated carbon dioxide. *Nature* **364**, 616-617.

Ebersberger, D., Niklaus, P. A., and Kandeler, E. (2003). Long-term CO_2 enrichment stimulates N-mineralisation and enzyme activities in calcareous grassland. *Soil Biology and Biochemistry* **35**, 965-972.

Frostegård, A., and Bååth, E. (1996). The use of phospholipid fatty acid analysis to estimate bacterial and fungal biomass in soil. *Biology and Fertility of Soils* **22**, 59-65.

Frostegård, A., Bååth, E., and Tunlid, A. (1993). Phospholipid fatty acid composition, biomass and activity of microbial communities from two soil types experimentally

exposed to different heavy metals. *Applied and Environmental Microbiology* **59**, 3605-3617.

Grayston, S. J., Cambell, C. D., Lutze, J. L., and Gifford, R. M. (1998). Impact of elevated CO_2 on the metabolic diversity of microbial communities in N-limited grass swards. *Plant and Soil* **203**, 289-300.

Griffiths, B. S., Ritz, K., Ebblewhite, N., Paterson, E., and Kilham, K. (1998). Ryegrass rhizosphere microbial community structure under elevated carbon dioxide concentrations with observation on wheat rhizosphere. *Soil Biology and Biochemistry* **30**, 315-321.

Heuer, H., Krsek, M., Baker, P., Smalla, K., and Wellington, E. M. H. (1997). Analysis of actinomycete communities by specific amplification of genes encoding 16S rRNA and gel-electrophoretic separation in denaturing gradients. *Applied and Environmental Microbiology* **63**, 3233-3241.

Hodge, A., Paterson, E., Grayston, S. J., Campbell, C. D., Ord, B. G., and Killham, K. (1998). Characterisation and microbial utilisation of exudate material from the rhizosphere of *Lolium perenne* grown under CO_2 enrichment. *Soil Biology and Biochemistry* **30**, 1033-1043.

Hu, S., Chapin III, F. S., Firestone, M. K., Field, C. B., and Chiariello, N. R. (2001). Nitrogen limitation of microbial decomposition in a grassland under elevated CO_2. *Nature* **409**, 188-191.

Hu, S., Firestone, M. K., and Chapin III, F. S. (1999). Soil microbial feedbacks to atmospheric CO_2 enrichment. *Trends in Ecology & Evolution* **14**, 433-437.

Insam, H., Bååth, E., Berreck, M., Frostegård, A., Gerzabeck, M. H., Kraft, A., Schinner, F., Schweiger, P., and Tschuggnall, G. (1999). Responses of the soil microbiota to elevated CO_2 in an artificial tropical ecosystem. *Journal of Microbiological Methods* **36**, 45-54.

Klironomos, J. N., Rillig, M. C., and Allen, M. F. (1996). Belowground microbial and microfaunal responses to *Artemisia tridentata* grown under elevated atmospheric CO_2. *Functional Ecology* **10**, 527-534.

Leadley, P. W., Niklaus, P., Stocker, R., and Körner, C. (1997). Screen-aided CO_2 control (SACC): a middle-ground between FACE and open-top chamber. *Acta Oecologia* **18**, 207-219.

Leadley, P. W., Niklaus, P. A., Stocker, R., and Körner, C. (1999). A field study of the effects of elevated CO_2 on plant biomass and community structure in a calcareous grassland. *Oecologia* **118**, 39-49.

Marilley, L., Hartwig, U. A., and Aragno, M. (1999). Influence of an elevated atmospheric CO_2 content on soil and rhizosphere bacterial communities beneath *Lolium perenne* and *Trifolium repens* under field conditions. *Microbial Ecology* **38**, 39-49.

Marschner P., Kandeler E., Marschner B. (2003). Structure and function of the soil microbial community in a long-term fertilizer experiment. *Soil Biology & Biochemistry* **35**, 453-461.

Mayr, C., Miller, M., and Insam, H. (1999). Elevated CO_2 alters commmunity-level physiological profiles and enzymes activities in alpine grassland. *Journal of Microbiological Methods* **36**, 35-43.

Montealegre, C. M., Van Kessel, C., Blumentahl, J. M., Hur, H.-G., Hartwig, U. A., and Sadowsky, M. J. (2000). Elevated atmospheric CO_2 alters microbial population structure in a pasture ecosystem. Global Change Biology 6, 475-482.

Montealegre, C. M., van Kessel, C., Ruselle, M. P., and Sadowsky, M. J. (2002). Changes in microbial activity and composition in a pasture ecosystem exposed to elevated atmospheric carbon dioxide. *Plant and Soil* **243**, 197-207.

Morgan, J. A., Pataki, D. E., Körner, C., Clark, H., Del Grosso, S. J., Grünzweig, J. M., Knapp, A. K., Mosier, A. R., Newton, P. C. D., Niklaus, P. A., Nippert, J. B., Nowak, R. S., Parton, W. J., Polley, H. W., and Shaw, M. R. (2004). The role of water relations in grassland and desert ecosystem responses to rising atmospheric CO_2. *Oecologia*, **in press**.

Myzer, G., de Waal, E. C., and Uitterlinden, A. G. (1993). Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes encoding for 16S rRNA. *Applied and Environmental Microbiology* **59**, 695-700.

Myzer, G., and Smalla, K. (1998). Application of denaturing gradient gel electrophoresis (DGGE) and temperatue gradient gel electrophoresis (TGGE) in microbial ecology. *Antonie van Leeuwenhock* **73**, 127-141.

Niklaus, P. A. (1998). Effects of elevated atmospheric CO_2 on soil microbiota in calcareous grassland. *Global Change Biology* **4**, 451-458.

Niklaus, P. A., Spinnler, D., and Körner, C. (1998). Soil moisture dynamics of calcareous grassland under elevated CO_2. *Oecologia* **117**, 201-208.

Niklaus, P. A., Wohlfender, M., Siegwolf, R., and Körner, C. (2001). Effects of six years atmospheric CO_2 enrichment on plant, soil, and soil microbial C of a calcareous grassland. *Plant and Soil* **233**, 189-202.

Niklaus, P. A., Alphei, J., Ebersberger, D., Kampichler, C., Kandeler, E., and Tscherko, D. (2003). Six years of *in situ* CO_2 enrichment evoke changes in soil structure and soil biota of nutrient-poor grassland. *Global Change Biology* **9**, 585-600.

Niklaus, P. A. and Körner, C. (2004). Synthesis of a six-year study of calcareous grassland responses to *in situ* CO_2 enrichment. *Ecological Monographs,* **in press**.

Ogermann, P., Spycher, B., Schaub, D., and Sollberger, R. (1994). Die Landschaftsstruktur im Raum Nenzlingen - geoökologisch gesehen. *Regio Basiliensis* **35**, 91-100.

Paul, E. A., and Clark, F. E. (1996). "Soil Microbiology and Biochemistry," Academic Press, San Diego, CA.

Rillig, M. C., Field, C. B., and Allen, M. F. (1999). Soil biota responses to long-term atmospheric CO_2 enrichment in two California annual grasslands. *Oecologia* **119**, 572-577.

Rillig, M. C., Scow, K. M., Klironomos, J. N., and Allen, M. F. (1997). Microbial carbon-substrate utilization in the rhizosphere of *Gutierrezia sarothrae* grown in elevated atmospheric carbon dioxide. *Soil Biology and Biochemistry* **29**, 1387-1394.

Ringelberg, D. B., Stair, J. O., Almeida, J., Norby, R. J., O'Neill, E. G., and White, D. C. (1997). Consequences of rising atmospheric carbon dioxide levels for the belowground microbiota associated with white oak. *Journal of Environmental Quality* **26**, 495-503.

Rogers, H. H., Runion, G. B., and Krupa, S. V. (1994). Plant responses to atmospheric CO_2 enrichment with emphasis on roots and the rhizosphere. *Environmental Pollution* **83**, 155-189.

Rønn, R., Gavito, M., Larsen, J., Jakobsen, I., Frederiksen, H., and Christensen, S. (2002). Response of free-living soil protozoa and microorganisms to elevated atmospheric CO_2 and presence of mycorrhiza. *Soil Biology & Biochemistry* **34**, 923-932.

Schortemeyer, M., Hartwig, U. A., Hendrey, G. R., and Sadowsky, M. J. (1996). Microbial community changes in the rhizospheres of white clover and perennial ryegrass exposed to free air carbon dioxide enrichment (FACE). *Soil Biology and Biochemistry* **28**, 1717-1724.

ter Braak, C. J. F., and Prentice, I. C. (1988). A theory of gradient analysis. *Advances in Ecological Research* **18**, 271-317.

ter Braak, C. J. F., and Smilauer, P. (1998). "CANOCO Reference Manual and User's Guide to Canoco for Windows: Software for Canonical Community Ordination (version 4)," Microcomputer Power, Ithaca, NY, USA.

Uren, N. C. (2001). Types, amounts, and possible functions of compounds released into the rhizosphere by soil-grown plants. *In* "The rhizosphere Biochemistry and organic substances at the soil-plant interface" (E. Pinton, Z. Varanini and P. Nannipieri, eds.), pp. 19-40. Marcel Dekker, Inc., New York, Basel.

White, D. C., Davis, W. M., Nickels, J. S., King, J. C., and Bobbie, R. J. (1979). Determination of the sedimentary microbial biomass by extractable lipid phosphate. *Oecologia* **40**, 51-62.

Wiemken, V., Laczko, E., Ineichen, K., and Boller, T. (2001). Effects of elevated carbon dioxide and nitrogen fertilization on mycorrhizal fine roots and the soil microbial community in beech-spruce ecosystems on siliceous and calcareous soil. *Microbial Ecology* **42**, 126-135.

Williams, M. A., Rice, C. W., and Owensby, C. E. (2000). Carbon dynamics and microbial activity in tallgrass prairie exposed to elevated CO_2 for 8 years. *Plant and Soil* **227**, 127-137.

Zak, D. R., Pregitzer, K. S., Curtis, P. S., and Holmes, W. E. (2000a). Atmospheric CO_2 and the composition and function of soil microbial communities. *Ecological Applications* **10**, 44-59.

Zak, D. R., Pregitzer, K. S., Curtis, P. S., Teeri, J. A., Fogel, R., and Randlett , D. L. (1993). Elevated atmospheric CO_2 and feedback between carbon and nitrogen cycles. *Plant and Soil* **151**, 105-117.

Zak, D. R., Pregitzer, K. S., King, J. S., and Holmes, W. E. (2000b). Elevated atmospheric CO_2, fine roots and the response of soil microorganisms: a review and hypothesis. *New Phytologist* **147**, 201-222.

Zak, D. R., Ringelberg, D. B., Pregitzer, K. S., Randlett , D. L., White, D. C., and Curtis, P. S. (1996). Soil microbial communities beneath *Populus grandientata* grown under elevated CO_2. *Ecological Applications* **6**, 257-262.

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Six years of *in situ* CO_2 enrichment evoke changes in soil structure and soil biota of nutrient-poor grassland

P. A. NIKLAUS*, J. ALPHEI†, D. EBERSBERGER‡, C. KAMPICHLER§, E. KANDELER‡ and D. TSCHERKO‡

**Institute of Botany, Schönbeinstrasse 6, University of Basel, CH-4056 Basel, Switzerland, †Institute of Zoology, Berliner Strasse 28, D-37073 Göttingen, Germany, ‡Institute of Soil Science, University of Hohenheim, D-70599 Stuttgart, Germany, §Institute of Biology, Grunewaldstrasse 34, Freie Universität Berlin, D-12165 Berlin, Germany*

Abstract

Nutrient-poor grassland on a silty clay loam overlying calcareous debris was exposed to elevated CO_2 for six growing seasons. The CO_2 exchange and productivity were persistently increased throughout the experiment, suggesting increases in soil C inputs. At the same time, elevated CO_2 lead to increased soil moisture due to reduced evapotransporation. Measurements related to soil microflora did not indicate increased soil C fluxes under elevated CO_2. Microbial biomass, soil basal respiration, and the metabolic quotient for CO_2 (qCO_2) were not altered significantly. PLFA analysis indicated no significant shift in the ratio of fungi to bacteria. 0.5 M KCl extractable organic C and N, indicators of changed DOC and DON concentrations, also remained unaltered. Microbial grazer populations (protozoa, bacterivorous and fungivorous nematodes, acari and collembola) and root feeding nematodes were not affected by elevated CO_2. However, total nematode numbers averaged slightly lower under elevated CO_2 (−16%, ns) and nematode mass was significantly reduced (−43%, $P = 0.06$). This reduction reflected a reduction in large-diameter nematodes classified as omnivorous and predacious. Elevated CO_2 resulted in a shift towards smaller aggregate sizes at both micro- and macro-aggregate scales; this was caused by higher soil moisture under elevated CO_2. Reduced aggregate sizes result in reduced pore neck diameters. Locomotion of large-diameter nematodes depends on the presence of large enough pores; the reduction in aggregate sizes under elevated CO_2 may therefore account for the decrease in large nematodes. These animals are relatively high up the soil food web; this decline could therefore trigger top-down effects on the soil food web. The CO_2 enrichment also affected the nitrogen cycle. The N stocks in living plants and surface litter increased at elevated CO_2, but N in soil organic matter and microbes remained unaltered. Nitrogen mineralization increased markedly, but microbial N did not differ between CO_2 treatments, indicating that net N immobilization rates were unaltered. In summary, this study did not provide evidence that soils and soil microbial communities are affected by increased soil C inputs under elevated CO_2. On the contrary, available data (^{13}C tracer data, minirhizotron observations, root ingrowth cores) suggests that soil C inputs did not increase substantially. However, we provide first evidence that elevated CO_2 can reduce soil aggregation at the scale from μm to mm scale, and that this can affect soil microfaunal populations.

Keywords: carbon and nitrogen dynamics, protozoa, soil aggregates, soil bacteria and fungi, soil food web, soil microfauna

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Correspondence: P. A. Niklaus, Institute of Botany, Schönbeinstrasse 6, CH-4056 Basel, Switzerland, fax + 41 61 267 3504, e-mail: pascal.niklaus@unibas.ch

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Introduction

Atmospheric CO_2 concentration increased from 280 to 360 μL L^{-1} since preindustrial times (Amthor, 1995) and is expected to double before the end of the 21st century (IPCC, 1995). Experimental exposure of plants to elevated CO_2 concentrations has shown that photosynthesis and plant productivity generally increase under these conditions, although large variation among different species and ecosystems was found (Körner, 1996; Poorter *et al*., 1996). In many grasslands, growth stimulation seems in part to reflect soil moisture savings (cf. Jackson *et al*., 1998; Niklaus *et al*., 1998b; Owensby *et al*., 1999; Volk *et al*., 2000). If increases in grassland net primary productivity (NPP) persist over time, soil C inputs will ultimately increase via enhanced plant biomass turnover and/or root carbon (C) losses. Organic C supply limits or colimits soil microbial activity in most grasslands (Smith & Paul, 1990; van de Geijn & van Veen, 1993), and profound changes in soil C cycling and energetics can therefore be expected.

Natural or seminatural grassland cover a significant fraction of the world's terrestrial surface (Houghton, 1995; Parton *et al*., 1995); however, only few studies have investigated C fluxes under elevated CO_2 in such systems (Ross *et al*., 1995; Hungate *et al*., 1997; Niklaus *et al*., 2001a). This is in part due to methodological difficulties with assessment of soil C balances and plant-soil C fluxes (Hilbert *et al*., 1987; Darrah, 1996; Hungate *et al*., 1996; Lund *et al*., 1999; Niklaus *et al*., 2000). Exudation is a major component of soil C inputs, but there is virtually no data available on flux rates and chemical composition of exudates under field conditions. In hydroponic culture, large increases in exudation rates have been detected at elevated CO_2. These effects, however, are much smaller or absent in the field due to soil matrix-effects and resorption of organic compounds exuded by plants (cf. Darrah, 1996; Jones & Darrah, 1996). Experimental evidence on fine root turnover, another major source of soil C inputs, also is controversial (Fitter *et al*., 1996; Arnone *et al*., 2000; Pregitzer *et al*., 2000; Niklaus *et al*., 2001a); several studies even indicate a decrease in fine root turnover at elevated CO_2, sometimes associated with an increase in root longevity. Even less is known on the chemical quality of soil C inputs. The C : N ratio of green leaves generally increases at elevated CO_2, and the content of phenolic compounds and secondary metabolites sometimes also increases (Penuelas & Estiarte, 1998), which could slow down decomposition. However, tissue quality-effects often vanish during leaf senescence and are not any longer found in surface litter. Accordingly, rates of decomposition of naturally senesced litter often are not affected (grassland leaf litter: Hirschel *et al*., 1997; wheat root litter: Van Vuuren *et al*., 2000; but see Cotrufo & Ineson, 1996; Ball & Drake, 1997). Virtually nothing is known of the *in situ* decomposition of root litter or of effects of elevated CO_2 on the chemistry of root exudates. Thus, soil C inputs are likely to increase under elevated CO_2 (because plant biomass increases), but the extent of this increase and the pathways by which extra C enters the soil are currently unknown. Even if we knew the increase in soil C input under elevated CO_2, predictions of the response of the soil biota to this change would be complicated by a number of indirect effects and feedback mechanisms:

1. The C and N cycle are intimately coupled in natural ecosystems, and N availability colimits microbial biomass in many natural ecosystems (Merckx *et al*., 1987; Liljeroth *et al*., 1990; van de Geijn & van Veen, 1993). Microbial responses to elevated CO_2-induced extra C fluxes could therefore be limited. On the other hand, increased microbial growth under elevated CO_2 could lead to increased immobilization of available N, in turn decreasing plant growth (Diaz *et al*., 1993). Extra C supply could also stimulate microbial decomposition of native soil organic matter, thus improving plant N availability (Zak *et al*., 1993). These mechanisms are not exclusive (Hungate, 1999), and large changes in N availability may primarily occur in systems in which nutrient cycles are far from equilibrium (Niklaus & Körner, 1996; Hu *et al*., 1999).
2. The soil biomass (microflora plus soil fauna) is in itself a complex, highly dynamic subsystem, which consist of a wide variety of organisms organized at several trophic levels. Most studies of soil microbial responses to elevated CO_2 have been restricted to measurements of total microbial biomass, which is in essence a test for changes in the mass of the microflora (Hu *et al*., 1999 for a review). However, organisms at higher trophic levels such as protozoa, nematodes, and microarthropods exert important controls on population size and turnover rates of the microflora. Assessment of total microbial biomass alone may therefore miss ecologically important changes in C dynamics within the soil food web (cf. Kampichler *et al*., 1998). For example, extra C supplies at elevated CO_2 might stimulate growth of the microflora, but no extra biomass may accumulate, because it is grazed by soil microfauna, resulting in increased C turnover (and increased metabolic quotient of CO_2). Grazer populations may increase due to the extra food available.

   Despite the variety of organisms involved in and the complexity of the soil food web, the fluxes of C and energy are compartmentalized (Moore *et al*., 1993). Many soil species can be classified as grazing

primarily on bacterial biomass (e.g. protozoa and certain groups of nematodes), whereas others graze predominantly on fungi (other nematodes and microarthropods). Species within the same compartment share similar properties and are thus likely to respond as a unit to environmental changes. For example, species in the bacterial food chain in general have higher turnover rates and are more susceptible to desiccation than species depending on fungi.

3. Elevated CO_2 can affect the soil biota indirectly by altering soil physical conditions. In many experiments, elevated CO_2 increased soil moisture (Jackson *et al*., 1994; Owensby *et al*., 1997; Niklaus *et al*., 1998b), the effect being driven by reduced leaf conductance (Morison, 1985). These water savings can be compensated by increased leaf area, especially in communities with low ground cover. Large-scale atmospheric feedback could reduce these water savings (Amthor, 1999), but general circulation models (GCMs) predict more humid conditions for temperate latitudes so that (on average) future plant growth conditions will include increased CO_2 concentrations and moister soils (Coughenour & Chen, 1997; Neilson & Drapek, 1998).
4. Soil structure is an important determinant of plant productivity and microbial dynamics, primarily, because it affects water and nutrient fluxes and the niches of specific soil microbes. Consequently, indirect effects of elevated CO_2 on soil structure could strongly modify the responses of plants and soil microbes to elevated CO_2. Association of mineral particles with organic matter forms aggregates of different sizes and stability (Tisdall & Oades, 1982), the different size fractions harbouring distinct groups of soil microorganisms and organic matter transformations (Sessitsch *et al*., 2001). Domains of individual, parallel-orientated clay particles ($< 1\,\mu m$ thick) are held together by physical (electrostatic) forces, organic molecules, and cations. Several such domains, held together by polysaccharides and other organic compounds, plant, fungal and bacterial debris, form mechanically relatively stable microaggregates. Microaggregates in turn bind to larger and less stable macroaggregates, which are stabilized primarily by roots and hyphae. Elevated CO_2 has been shown to alter soil moisture and plant productivity. Thus, the amount of soil solution and the concentration of binding agents in soil solution may change, thereby affecting the processes controlling aggregation. Indeed, such effects have been reported in two-field (Rillig *et al*., 1999b; Niklaus *et al*., 2001c) and one greenhouse study (Niklaus *et al*., 2001a).

In this paper, we investigate effects of six years of CO_2 enrichment on soil aggregate structure, nitrogen dynamics, and composition of the soil biota of nutrient-poor calcareous grassland. The CO_2 exchange (Stocker *et al*., 1997) and productivity (Leadley *et al*., 1999) increased at elevated CO_2, suggesting increased soil C inputs. Soil moisture levels were persistently increased under elevated CO_2 (Niklaus *et al*., 1998b) due to reduced stomatal conductance (Lauber & Körner, 1997) and evapotranspiration (Stocker *et al*., 1997).

Specifically, we tested whether (1) soil aggregate size distribution was affected by elevated CO_2; (2) whether elevated CO_2 induced an increase in microbial immobilization and/or N mineralization, thus decreasing and/or increasing plant N availability, respectively; (3) microbial biomass or microbial grazer populations increased as consequence of the presumed extra C supply, and (4) this response, if there was any, occurred within the fungal or bacterial compartment of the soil food web. Results of these analyses are discussed in relation to a range of previously published data from the same trial.

Materials and methods

Study site

The field site is located in a nutrient-poor, south-facing species-rich calcareous grassland in northwestern Switzerland (47°33′N 7°34′E, 520 m asl, 20° slope). This pasture has been used for extensive grazing for at least a hundred years (Schläpfer *et al*., 1998). The plant community, characterized as *Mesobromion* community, contains over 90 plant species and is dominated by *Bromus erectus* Huds. which accounts for *ca.* half of the above-ground community biomass. A detailed description of the vegetation is given in Huovinen-Hufschmid & Körner (1998). These temperate grasslands are largely created by man, and are sustained by biomass removal through livestock grazing or mowing, which prevents dominance of rank grass and scrub. In our study, cattle grazing was replaced by extensive mowing, which is frequent practice. These grasslands once were common throughout central Europe, but today are restricted to remnants, primarily due to land use intensification and abandonment of unproductive sites. Plant communities are natural in the sense that, except for biomass removal, they evolved naturally; soils are not disturbed. The Rendzina-type soil, which is typical for these habitats, consists of a 10–15 cm neutral to slightly basic ($pH \approx 7,8$) silty clay loam top-soil and is underlain with calcareous debris. In the top 10 cm, the horizon, where most of the fine roots occur, organic C and N contents are *ca.* 3.9% and 0.33%, respectively (Niklaus, 1998).

Experimental design

A total of 24 plots (1.27 m^2 each) containing natural undisturbed vegetation and soil were selected. The plots were organized in a randomized complete block design with four pairs of blocks orientated perpendicular to the slope. Treatments, which were assigned randomly within each block, were unscreened ambient control plots ('C', 360 μL CO_2 L^{-1}, no screen), screened ambient CO_2 plots ('A', 360 μL L^{-1}, screened) and screened elevated CO_2 plots ('E', 600 μL L^{-1}, screened). The CO_2 enrichment was applied using a novel CO_2 enrichment technique utilizing small windscreens reaching a height of 50 cm above-ground and leaving a 7-cm gap at the bottom to allow free convection of air and movement of animals. This *screen aided CO_2 control (SACC)* allowed accurate control of CO_2 enrichment while still minimizing microclimatic impacts and permitting high spatial replication (Leadley *et al*., 1997). The CO_2 enrichment began in March 1994 and operated around the clock except during mid-winter (December–February) when it was shut down. In order to maintain the high species diversity common to these plant communities, each year plots were clipped at a height of 5 cm in June and October

Soil sampling

When the experiment was destructively harvested (June 22–August 1, 1999), soil samples (0–10 cm) were taken concurrently with removal of the plant material These samples were used for microbial biomass determination, extraction of microarthropods and nematodes, analysis of enzyme activities, extraction of soluble organic C, and soil physical fractionation. Plots were harvested blockwise, and samples were packed on ice until transport back to the lab. Soil samples have been taken on April 20 for enzyme analysis and on April 26 for microbial biomass determination by substrate-induced respiration (SIR).

Soil moisture

Soil moisture was determined gravimetrically in soil cores probing 0–10 cm, and the result converted to volumetric soil moisture using independent bulk density measurements (Niklaus *et al*., 1998b).

Soil microbial biomass and activity

Soil microbial biomass C and N was determined by chloroform fumigation–extraction (Brookes *et al*., 1985; Vance *et al*., 1987) and by substrate induced respiration (SIR; Anderson & Domsch, 1978). Soil basal respiration, a measure of respiratory activity of the microbe/soil system in the absence of exogenous C sources, was determined by O_2 consumption. The metabolic quotient for CO_2 (qCO_2) was calculated by dividing basal respiration by microbial biomass measurements by SIR.

For chloroform fumigation–extraction soils were sieved (2 mm mesh size), homogenized, and remaining roots carefully removed with tweezers. One of two field-moist subsamples (water content of 40–50% water capacity, weight corresponding to 20 g dry weight) was immediately extracted for 30 min with 100 mL 0.5 M K_2SO_4 (table shaker at 200 rpm). The second sample was fumigated with ethanol-free $CHCl_3$ for 24 h and $CHCl_3$ removed by repeated evacuation. The evacuation time was kept to a minimum in order to minimize losses of volatile nitrogenous compounds (i.e. ammonia and organic amines). After removal of the chloroform, fumigated samples were extracted in the same way as the unfumigated soils. All extracts were centrifuged (10 min at 1000 g), filtered (No. 589 3½, Schleicher und Schüll, Dassel, Germany) and kept frozen until further analysis. Organic C and N in the extracts were determined using an automated TOC/TON analyser (DIMA TOC-100 with TN_b extension, Dimatec, Essen, Germany) and microbial biomass C (C_{mic}) calculated as $C_{mic} = (C_{fumigated} - C_{unfumigated})/k_{EC}$, where k_{EC} is the extraction efficiency for microbial C ($k_{EC} = 0.29$; obtained by calibration against SIR measurements) and $C_{fumigated}$ and $C_{unfumigated}$ are the organic C contents of the extracts of fumigated and unfumigated soils, respectively. Microbial biomass N was similarly calculated using $k_{EN} = 0.54$ (Brookes *et al*., 1985).

For determination of microbial biomass by SIR, sieved fresh soil (2 mm mesh size) was adjusted to a water content of 50% of its water holding capacity and preincubated for 3 days at 22 °C. Thereafter samples were amended with a glucose/talcum mixture, substrate-induced respiration measured for 6 h in an automated, custom-built multichannel device equipped with an infrared gas analyser, and SIR converted to the plot's microbial biomass C following the procedure outlined in Anderson & Domsch (1978). Basal respiration and qCO_2 were determined similarly using a slightly different system. Soil samples were preincubated as described above, but for 5 days at 20 °C. Then, respiration of samples was measured in an automated system measuring O_2 consumption by electrolytic O_2 microcompensation (thus accounting only for aerobic activity, see Scheu, 1992 for a detailed description). Basal respiration was determined as average O_2 consumption between 15 and 20 h, and the metabolic quotient for CO_2 (qCO_2, h^{-1}) was calculated by dividing basal respiration by the microbial biomass of the same samples (which was measured by SIR in the same apparatus).

Microarthropods

Soil microarthropods were extracted from soil using a Macfayden canister extractor (Edwards, 1991). Briefly, soil samples were inserted upside down into the sample holder, and animals extracted by heating the soil samples from the top thus driving soil animals downwards were they were collected in a trap containing saturated aqueous picric acid. Temperatures applied for extraction were 3 days at 30 °C, followed by (in this order) 2 days at 35 °C, 1 day at 40 °C, 1 day at 45 °C and finally three days at 50 °C. Thereafter, the animals were transferred to a 75% aqueous ethanol in which they remained stored until counting.

Nematodes

Soil samples were sieved (4 mm) and nematodes extracted using a modified O'Connor wet extraction apparatus. Forty hours after the beginning of the extraction, temperature was increased from 20 °C to 45 °C over 6 h, while trapping vessels were cooled to 13–15 °C (Alphei, 1995). Subsequently, nematodes were killed by heat and fixed in 4% formaldehyde solution. Total numbers of nematodes were counted under a dissection microscope. Out of each sample, 100 specimens were picked at random and mounted on slides for identification and biomass determination. Nematodes were sorted into six trophic groups (bacterial feeders, fungal feeders, root hair feeders, omnivores, plant parasites, predators) according to Yeates *et al*. (1993). Individual fresh weights were calculated using the formula of Andrássy (1956). Dry weights were calculated as 22.5% of fresh weights (cf. Buecher & Hansen, 1971; Sohlenius, 1979).

PLFA

Phospholipid fatty acids (PLFA) were analysed following the procedure used by Frostegård *et al*. (1993). Briefly, 1 g of moist soil was incubated for 2 h in a single-phase chloroform–methanol–citrate buffer mixture. Then, the solution was split into two phases by further addition of chloroform and citrate buffer and the samples centrifuged. The organic phase containing the microbial lipids was dried at 40 °C under a nitrogen stream. The lipids isolated were then fractionated into neutral, glyco- and polar-lipids including phospholipids using Si-columns (Varian, Harbor City, USA). Then, the phospholipid fraction was dried under a stream of N_2 and methylnonadecanoate added as internal standard to the samples. The phospholipids were converted to fatty acid methyl esters by mild alkaline methanolysis and analysed by capillary gas chromatography (Autosystem XL, Perkin-Elmer, Norwalk, CT, USA, fitted with a 50 m capillary column [HP-5, Agilent, Palo Alto, CA, USA] and a flame ionisation detector).

Following Frostegård & Bååth (1996), the ratio of the fungal 18 : 2**ω**6–10 bacterial PLFA was used as indicator of fungal to bacterial biomass ratios (the bacterial PLFA i15 : 0, a15 : 0, 15 : 0, i16 : 0, i17 : 0, a17 : 0, 17 : 0, cy17 : 0, 18 : 1**ω**7, cy19 : 0 were used in the calculation; Frostegård and Bååth also included 16 : 1**ω**5 and 16 : 1**ω**9, but these could not be determined with our equipment). A ratio of 20 : 4**ω**5 to total PLFA was used as indicator of protozoan biomass fraction (Paul & Clark, 1996).

Soluble organic C

Fresh soil samples corresponding to 25 g dry matter were extracted for 20 min in 80 mL 0.5 M KCl. Suspensions were filtered (No. 589 3½, Schleicher und Schüll, Dassel, Germany), acidified with HCl to remove inorganic carbonate, and analysed for total organic C and N using an automated TOC/TON analyser (as above).

Soil aggregate size distribution

Soil physical structure was determined using two methods: (1) mild chemical dispersion followed by wet-sieving, yielding macroaggregates, and (2) determination of the microaggregate size distribution after soil disintegration using soil organic matter oxidation and chemical dispersion:

Fresh soil samples corresponding to 150 g dry matter were suspended in 170 mL of 0.5% sodium hexametaphosphate and gently shaken for 2 h. Hexametaphosphate improves dispersion and binds Ca^{2+} ions, which otherwise would precipitate with the density separation agent (polytungstate). The suspension was washed through a stack of sieves (2000, 1000, 500, 250 and 125 μm) with 3 L of water. A few macroaggregates, held together by plant root systems, were larger than 2000 μm and remained on the top sieve. These aggregates were disintegrated by forcing them through the top-most sieve. The fraction < 125 μm was centrifuged for 10 min at 2000 g and the supernatant discarded. The obtained size fractions were stirred into 100 mL aqueous sodium polytungstate (SOMETU, Falkenried, Germany) solution with a density of 1.7 g cm^{-3}. The suspension was allowed to settle for 10 min. Then, the low-density macroorganic supernatant was removed and the high-density organomineral fraction ($\rho > 1.7$ g cm^{-3}) was vacuum-filtered through a glass fibre filter (GF9, Schleicher und Schüll, Dassel, Germany), washed with distilled water and dried at 80 °C for 48 h.

The distribution of microaggregates was determined using samples corresponding to 10 g dry weight. Organic matter was destroyed by amending moist soils with 60 mL H_2O_2 (15% volume) and heating to 90 °C. Further

H_2O_2 was added until gas development ceased. Then, soils were dispersed by adding 200 mL 0.0125 M $Na_4P_2O_7$ and shaking end-over-end for 16 h. The sand size fraction (> 63 μm) was separated from the resulting suspension by wet sieving. The remaining fractions were further separated into silt and clay fractions by sedimentation. All samples were dried overnight at 105 °C, weighed, and the result corrected for the mass of $Na_4P_2O_7$ contained in the samples. This procedure does *not* yield the fundamental mineral particles – there is still some level of aggregation, which is what we were interested in (see Results for details).

Statistical analysis

All data were log-transformed prior to analysis of variance (ANOVA). According to the experimental design, the ANOVA model included the factors Block and SACC/CO_2 treatment. To test for effects of SACC and CO_2 alone, *a priori* linear contrasts were used to compare the corresponding treatment within the SACC/CO_2 factor. For analysis of subplot data (e.g. different aggregate size-fractions within each plot), a hierarchical design containing the following additional terms was used: Plot (CO_2), Size, CO_2·Size. The main effects of CO_2 were tested against Plot (CO_2). Size- and CO_2·Size-effects were tested against the residual. All model terms were fitted sequentially as in Type I ANOVA models. The CO_2 effects within size classes were tested using linear contrasts (LMATRIX subcommand of the UNIANOVA procedure in SPSS 9.0; SPSS, Chicago, IL). Repeated measures analysis was done with the multivariate repeated measures ANOVA procedure of SPSS. Effects with $P \leq 0.05$ are considered as significant, effects with $P \leq 0.1$ as marginally significant. Error estimates given in the text and error bars in figures are standard errors of the means. In figures (*) indicates $P \leq 0.1$, *$P \leq 0.05$ and **$P \leq 0.01$, ***$P \leq 0.001$.

Results

Soil moisture

Soil moisture generally increased under elevated CO_2 ($P < 0.001$; Fig. 1). For a detailed description of CO_2-effects on soil water relations see Niklaus *et al.* (1998b), where seasonal and diurnal dynamics of soil moisture are presented and the interplay between leaf area index (LAI), stomatal conductance, and canopy temperature is reported. Screened ambient CO_2 plots were generally drier than unscreened control plots, primarily because of increased air movement due to the blowers used for CO_2 disposal. Soil moisture under elevated CO_2 was approximately equal to that in unscreened control plots, i.e., CO_2 enrichment and screening of plots had opposite effects on soil water of approximately equal magnitude. It should be noted, however, that the differences in soil moisture between CO_2 treatments, though systematic, were only a small fraction of the changes in soil moisture occurring during regular soil drying cycles and that inter-annual variability also is considerable.

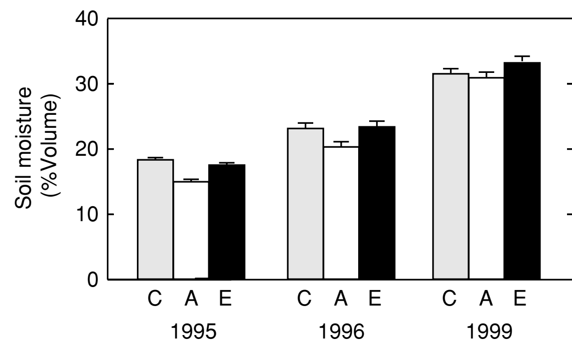

Fig. 1 Peak biomass (June) water content in top 10 cm of soil. The effects of elevated CO_2 and plot screening shown are typical for soil moisture conditions throughout the experiment and significant at $P < 0.01$ (repeated measures analysis; C = unscreened control plots, A = ambient CO_2 plots, E = elevated CO_2 plots).

Soil physical structure

The CO_2 enrichment caused a shift in soil organomineral aggregate distribution towards smaller sizes ($P = 0.004$; Fig. 2a). There was a lower mass of macroaggregates > 125 μm in diameter ($P = 0.03$), and consequently a greater mass < 125 μm. Screening of plots caused a similar effect, but opposite in direction.

Microaggregate size distribution also revealed a shift towards smaller sizes at elevated CO_2 (Fig. 2b; $P = 0.01$ for the CO_2-size interaction). Again, an opposite effect of plot screening was detected ($P = 0.02$ for the SACC-size interaction).

X-ray diffraction measurements, which were run test-wise on a limited number of samples, did not reveal differences in clay mineral composition between CO_2 treatments. The dominant clay minerals present were illite, kaolinite, and chlorite. Quartz and calcite were not present in the samples.

Extractable organic C and N

Increased plant root exudation or fine root turnover would increase microbial C availability and could be reflected in increased dissolved or extractable organic C levels. However, no such increase was found (Fig. 3). Total extractable N, comprising both dissolved and extractable organic N as well as inorganic N also did not change under elevated CO_2.

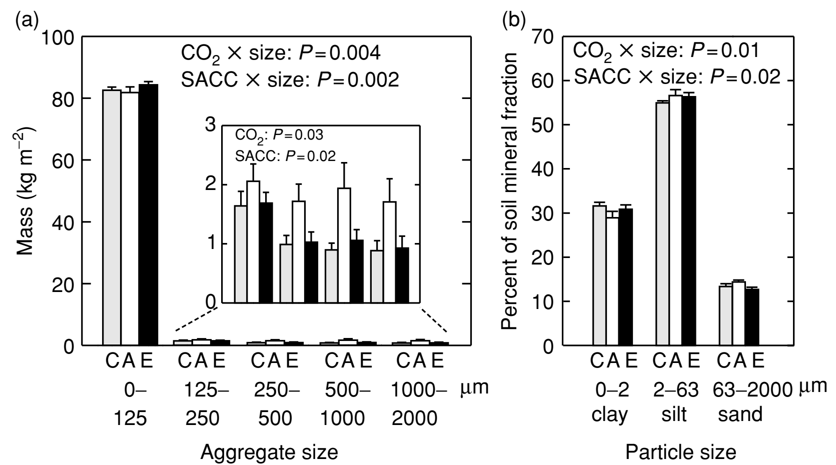

Fig. 2 Effects of elevated CO_2 on soil structure. (a) Organomineral aggregate sizes and (b) microaggregate size distribution after oxidation of soil organic matter with hydrogen peroxide and dispersion with pyrophosphate. (Note that dispersion is still incomplete. After further dispersion and organic matter oxidation, 7% of the minerals were recovered in the sand size fraction, 52% as silt, and 41% as clay; C = unscreened control plots, A = ambient CO_2 plots, E = elevated CO_2 plots).

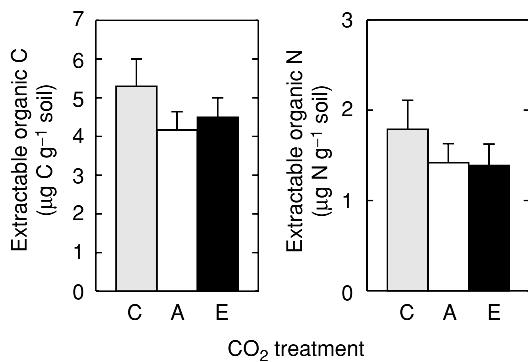

Fig. 3 Soil organic C and total N extractable with 0.5 M KCl (C = unscreened control plots, A = screened ambient CO_2 plots, E = screened elevated CO_2 plots).

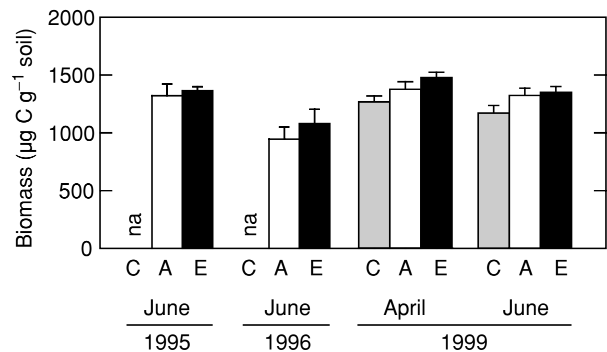

Fig. 4 Biomass of soil microflora as determined by substrate-induced respiration (SIR). Differences between ambient and elevated CO_2 are not significant. Peak season data from pervious years (June 1995 and 1996; Niklaus *et al*., 1998b) is also included in the figure. Due to the limited number of samples that could be handled parallel and the absence of windscreen effect in previous harvests (cf. Niklaus *et al*., 1998b), SIR of unscreened controls was not assessed in June 1995 and 1996. (C = unscreened control plots, A = ambient CO_2 plots, E = elevated CO_2 plots; na = not assessed).

Microbial biomass

Soil microbial biomass C measurements made by chloroform fumigation–extraction, and by SIR were significantly correlated ($r^2 = 0.45$, $P < 0.001$). Elevated CO_2 did not alter microbial C throughout the six years of CO_2 treatment, regardless of the method used (−1%, ns, when measured by fumigation–extraction; +6%, ns, when measured by SIR/O_2 consumption; +2%, ns, when measured by SIR/CO_2 release, see Fig. 4 and Table 1). Also, microbial N and C/N were not significantly altered by elevated CO_2 (Table 1). Repeated measures analysis covering all measurements made during the experiment also did not reveal any effect of elevated CO_2 (measurements in Fig. 4 plus data from the first three years of treatment previously published in Niklaus, 1998, i.e., additional measurements in October 1994, March 1995, October 1995, March 1996 and October 1996).

Screening of plots caused no effect on microbial biomass as measured by $CHCl_3$ fumigation–extraction (+6%, ns). Plot screening caused a marginally significantly decrease in SIR when measured by CO_2 release (−11%, $P = 0.07$), but no change when measured by O_2 consumption (+7%, ns), possibly indicating an increase in mass or activity of aerobe relative to anaerobe organisms.

Basal respiration and qCO_2

Elevated CO_2 altered neither basal respiration (+10%, ns) nor qCO_2 [+3.8%, ns $(3.0 \pm 0.3) \cdot 10^{-3}\,h^{-1}$ and $(3.1 \pm 0.3) \cdot 10^{-3}\,h^{-1}$ at ambient and elevated CO_2, respectively]. Screening of plots reduced basal respiration (−22%, $P = 0.02$) and consequently marginally significantly reduced qCO_2 [−18%, $P = 0.07$ $(3.7 \pm 0.3) \cdot 10^{-3}\,h^{-1}$ in the unscreened control plots].

Protozoa, bacteria and fungi

PLFA data did not reveal significant shifts in the ratio of protozoa, bacteria, and fungi.

Soil nematodes

Total nematode numbers averaged lower under elevated CO_2 (−16%, ns; Fig. 5a) and total nematode weight was significant reduced (−43%; $P = 0.06$). This reduction was primarily driven by a decline of large nematodes under elevated CO_2 (omnivores and predators), whereas mass and numbers of other nematodes trophic groups did not change significantly (Fig. 6). Screening of plots generally had opposite effects, so that nematode numbers and weights in elevated CO_2 were equal to numbers in unscreened control plots.

Soil microarthropods

Not one of acari (−1.5%, ns), collembola (+7%, ns) or total microarthropod individual numbers (+0.4%, ns) were altered by elevated CO_2 (Fig. 5b). Screening of plots, however, decreased the numbers of microarthropods (−25% for acari, −40% for collembola, and −30% for total microarthropods, $P < 0.01$ for all).

Nitrogen dynamics and partitioning within the ecosystem

At peak biomass, microbes contained approximately the same amount of N as did plant shoots plus roots (Fig. 7). The N stocks in living plants and surface litter increased, but the amount in soil organic matter and microbes remained unaltered. Nitrogen mineralization, as assessed in soil incubations, increased markedly in April (+30%; $P = 0.05$) but not significantly in June (Table 2).

Discussion

During the six years of treatment, above-ground plant biomass accumulation (> 5 cm layer) increased in response to CO_2 alone between 5 and 32% (average response of 21%). Below-ground biomass showed substantial spatial variability (Leadley *et al*., 1999) and short-term (up to several weeks) turnover of fine root biomass may be a more important component of below-ground NPP than are seasonal growth (spring) and die-back (autumn) of roots. Nevertheless, increased root biomass at the end of the experiment, and increased above-ground growth both suggest increases in soil C inputs under elevated CO_2, although the magnitude of these fluxes remains uncertain.

Table 1 Microbial C and N as determined by chloroform fumigation–extraction (June 1999)

	CO_2 treatment Control	Ambient CO_2	Elevated CO_2	Relative effect of CO_2
Microbial C (μg C (g soil)$^{-1}$)	1238 ± 52	1319 ± 46	1307 ± 31	−1%, ns
Microbial N (μg N (g soil)$^{-1}$)	133 ± 9	154 ± 8	150 ± 7	−2%, ns
Microbial C : N (μg C (μg N)$^{-1}$)	9.5 ± 0.3	8.6 ± 0.2	8.8 ± 0.2	+2%, ns

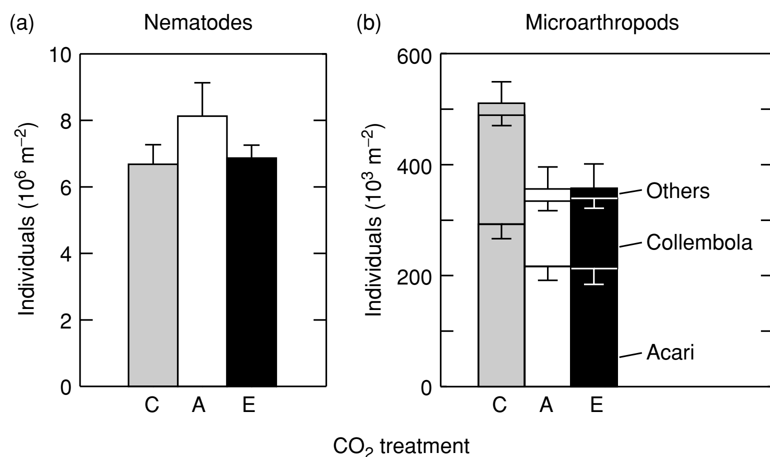

Fig. 5 (a) Number of nematode individuals and (b) number of collembola, acari and total microarthropod individuals in experimental treatments (C = unscreened control plots, A = ambient CO_2 plots, E = elevated CO_2 plots).

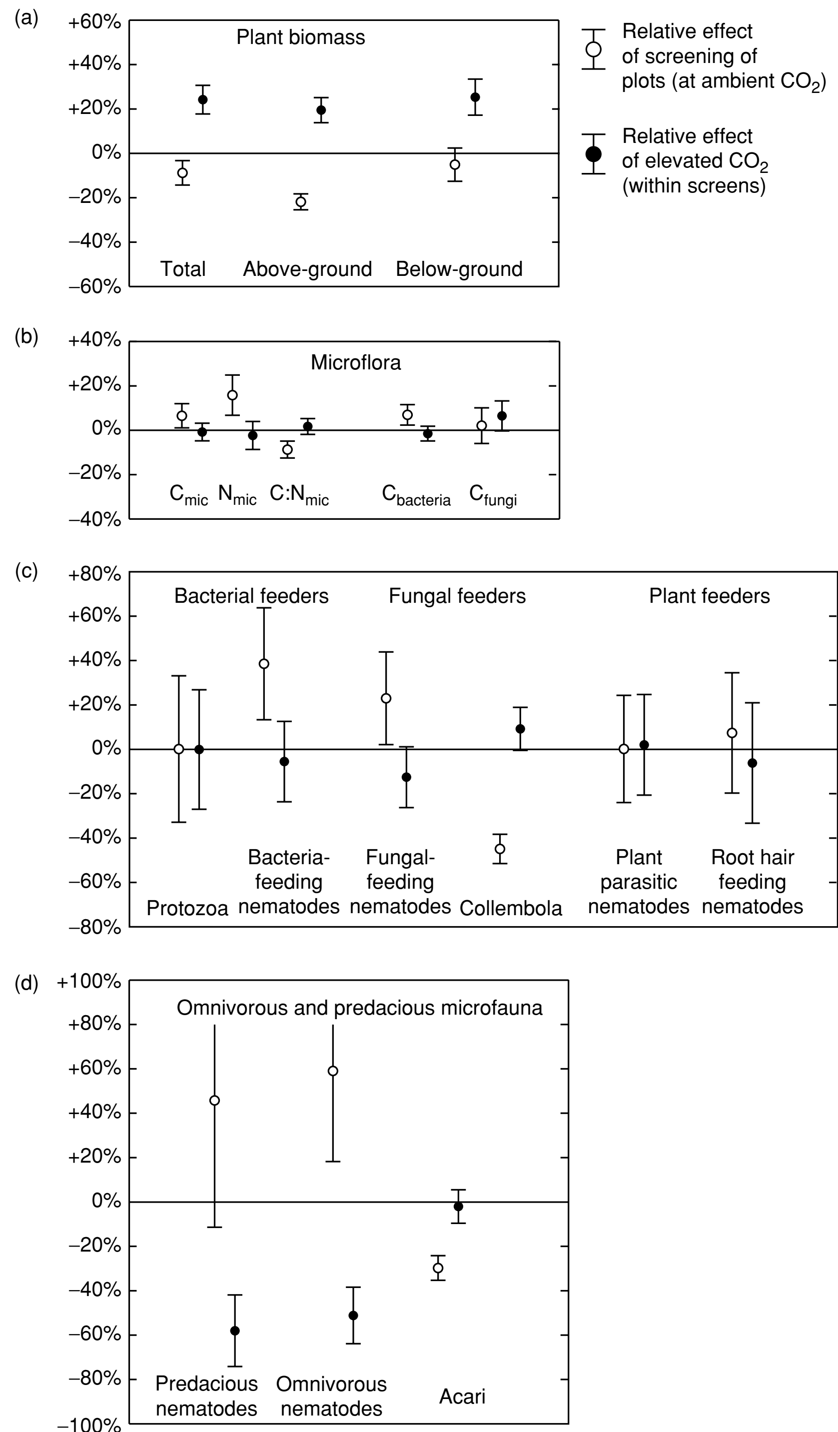

Fig. 6 Relative effects of windscreens (at ambient CO_2) and CO_2 enrichment (within windscreens) on soil biota components. (a) Effects on plant biomass, above- and below-ground, are given for comparison. (b) Effects on total soil microflora and on the bacterial and fungal fraction. The bacteria/fungi-separation is based on characteristic bacterial and fungal PLFA. (c) Effects on soil microfauna, tentatively grouped into bacterial and fungal grazers and organisms feeding predominantly on plants. (d) Effects on omnivorous or predacious organisms, or groups of organisms not sorted to species and containing predacious or omnivorous animals (mites). All data are weight based, with the exception of microarthropods for which no weight data was available and effects are based on the number of individuals.

Elevated CO_2 did affect soil community structure and nutrient dynamics. The observed effects appear to rely on three mechanisms: (1) increased soil moisture availability under elevated CO_2; (2) reduced soil aggregate sizes, which may be related to effects on soil moisture; (3) plant productivity-related effects of elevated CO_2.

Plant productivity-driven effects of elevated CO_2 can be separated from soil-moisture driven effects on soil communities by factorially combining the CO_2 treatment with a soil moisture/irrigation treatment (cf. Volk *et al.*, 2000). In the present study, this was tentatively possible because (1) the windscreen-effect on soil moisture was

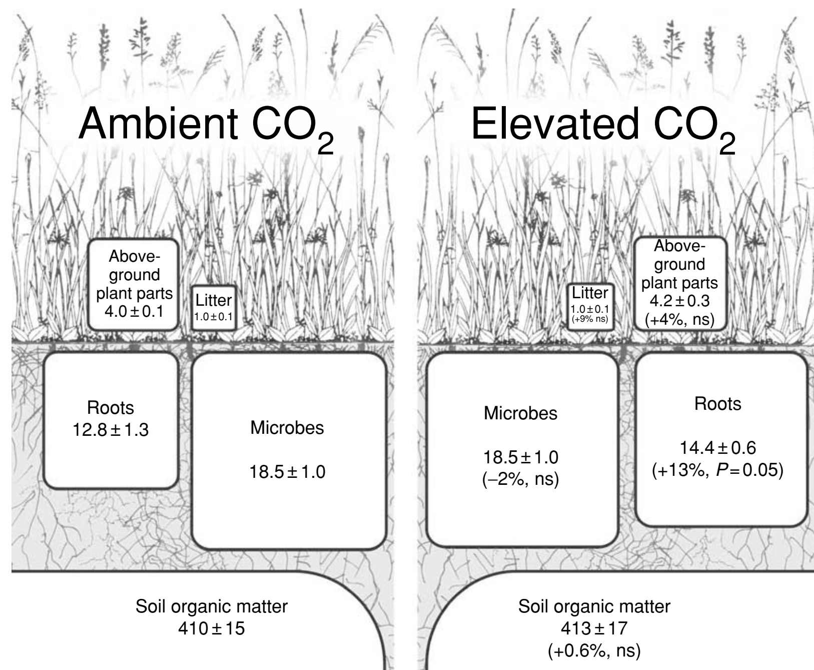

Fig. 7 Peak biomass nitrogen partitioning within the ecosystem. Data refer to 1999 when the experiment was terminated after six growing seasons. All data are in $g\,N\,m^{-2}$ ± standard error of the mean.

Table 2 Nitrogen mineralization rates determined in lab-incubated soil samples

Date	CO_2 treatment Control	Ambient CO_2 ($\mu g\,N\,g^{-1}\,soil\,d^{-1}$)	Elevated CO_2	Relative effect of screening	Relative effect of CO_2
April 1999	14.7 ± 1.0	13.3 ± 1.6	17.3 ± 1.4	−9%, ns	+30%, $P = 0.02$
June 1999	8.7 ± 0.7	8.9 ± 0.8	9.1 ± 0.4	+2%, ns	+3%, ns

almost exactly compensated for by the elevated CO_2-induced reduction in evapotranspiration (cf. also Fig. 1), and (2) by and large plant growth responded to CO_2 enrichment, but not to screening (averaged over all years of treatment: screening effect: −6%, ns; CO_2-effect: +21%, $P < 0.01$). This indicates that at least part of the observed effect of elevated CO_2 on productivity did not depend on soil water savings. Overall, the comparison of unscreened control and screened ambient treatment roughly tests for soil moisture effect alone, whereas the comparison on screened ambient and screened elevated CO_2 plots tests for the effect of elevated CO_2 including the alteration of soil H_2O.

Soil structure

Elevated CO_2 caused a shift towards smaller aggregate sizes. Interestingly, this shift was also found in microaggregates containing only few mineral particles (Lal, 2000). The CO_2-effects observed in these microaggregates suggest that the aggregate size shift occurred over the entire size range of μm to mm.

Treatment effects on aggregate sizes and on soil moisture exhibited similar patterns, suggesting that soil water savings at elevated CO_2 drove the changes in soil physical structure. Soil aggregates are created by shrinkage on drying and stabilization by microbial debris, fungal hyphae, roots and polysaccharides. More pronounced drying–wetting cycles in screened ambient CO_2 plots and increased concentrations of binding agents at lower soil moisture may have improved aggregation (J. M. Oades, pers. comm.; Oades, 1978; 1984).

It is noteworthy that Rillig *et al*. (1999b) observed the opposite effect, i.e., increased aggregate sizes in two Mediterranean grassland ecosystems under elevated CO_2. Root and fungal biomass were increased, which is probably the driver of these changes (Rillig *et al*., 1999a). Rillig *et al*. further speculated that increased mycorrhizal secretion of glomalin in elevated CO_2 plots was a key in increased soil aggregation. Another potential explanation for the discrepancy to our study might be that Rillig *et al*. used a much more gentle soil fractionation technique than we did (immersion of soil in H_2O, whereas in the present study soils were very gently

shaken for several hours in 1% hexametaphosphate). It may therefore well be that both methods simply assess a different hierarchical level of soil aggregation.

The consequences of these particle size shifts are yet largely unclear. Since soil texture is an important determinant of gas diffusivity and redox potential, it appears likely that the release of radiatively active trace gases such as CH_4 and N_2O could increase under elevated CO_2. Indeed, this has been observed in several studies (Megonigal & Schlesinger, 1997; Arnone & Bohlen, 1998; Ineson *et al*., 1998; Phillips *et al*., 2001a, b).

Carbon and nitrogen dynamics

Total soil microbial biomass did not increase at elevated CO_2, even after six years of treatment. It is noteworthy that a disparity between microbial biomass responses and the stimulation of root system size has also been observed in most other studies. This suggests either that soil C inputs do not scale proportionally with root mass (e.g. because the ratio of coarse to fine roots changes, possibly for purely allometric reasons), or that soil microbes respond less than proportionally to increased C inputs. Reasons for the latter may include strongly delayed responses due to long turnover times of related soil pools and nutrient limitations.

We have previously speculated that the lack of effect on total microbial biomass was related to N-limitation of microbial growth (Niklaus, 1998). The basis for this reasoning was that short-term (one week) laboratory incubations with extra C supplied only induced substantial microbial growth when samples were amended with extra mineral N as well. Phosphorus, on the other hand, was readily acquired from soil organic matter. These limitations correspond to the limitations observed on plant growth: overall productivity of this grass-dominated ecosystem is N-limited; legumes fix atmospheric N but comprise only $<5\%$ of peak community biomass, because their growth is *P*-limited (J. Stöcklin, pers. comm.; Niklaus *et al*., 1998a). Another possible explanation for the lack of microbial biomass response to elevated CO_2, however, is that soil C inputs did not increase as much as we initially believed, based on photosynthetic responses. This suspicion is supported by the lack of increase in DOC concentrations; by ^{13}C pulse labelling experiments (Niklaus *et al*., 2001a) which did not show any statistically significant increases in microbial biomass labelling and turnover at elevated CO_2; by soil fractionation experiments which did not reveal any increases in low-density macro-organic matter (Niklaus *et al*., 2001c); and by minirhizotron observations which did not show any increase in fine root turnover under elevated CO_2 (Arnone *et al*., 2000).

At peak biomass, microbes contained approximately the same amount of N than plant shoots plus roots did (cf. Fig. 7). Almost all vascular plant species in this grassland are perennial. Plants cover part of their N needs during growth from retranslocation from root and rhizomes to the shoot; nevertheless, a large part of the N required is taken up from the soil. Immobilization of extra N in microbial biomass could therefore critically reduce plant N availability and limit growth. This limitation would be most severe when nutrient assimilation by plants and enhanced input of easily available C into the soil coincide, i.e., during periods of vigorous shoot (high N demand) and root growth (high soil C inputs). In our grassland, this corresponds to the mid-spring period (cf. root and shoot biomass data in Leadley *et al*., 1999 and Niklaus *et al*., 2001b). Microbial biomass N, however, did not differ between CO_2 treatments in April 1999, indicating that net N immobilization rates did not differ. However, soil N mineralization was substantially increased under elevated CO_2 (+30%, but no effect of windscreens; cf. Table 2). Taken together, our data supports the feedback mechanism proposed by Zak *et al*. (1993) rather than the one demonstrated by Diaz *et al*. (1993), although these are not mutually exclusive.

Soil food web

Animals inhabiting the litter layer or living very close to the soil surface (collembola and mites) were affected by plot screening; their numbers were reduced, presumably due to the increased air movement. Soil microbial C and plant growth, however, were not affected; and nematode numbers increased in the drier, screened plots (and decreased in elevated CO_2). Nematodes presumably did not suffer from drier soils because they live within an aggregates surface water film, which does not dry out until the soil matrix potential gets very low. Locomotion of nematodes is even more effective in thin water films (Wallace, 1958). Experiments (G. Yeates, pers. comm; Yeates *et al*., 2002 and references therein) demonstrated that nematode activity including reproduction occurs in water films as thin as 1 μm (which is a fraction of the nematodes body diameters of up to ~50 μm). Yeates *et al*. argue that the concentration of soil nutrients would increase in thin water films and that grazing of surface-bound microbes would also become more efficient. This mechanism is compatible with our finding that nematode numbers decreased under elevated CO_2 (higher soil H_2O), despite the anticipated extra food available.

Nematode migration is limited by pore neck sizes (cf. Yeates *et al*., 2002). The reduction in soil aggregate sizes under elevated CO_2 will also have reduced pores sizes, providing an alternative explanation for the reduced nematode numbers. This effect would be most

Table 3 Elevated CO_2 studies reporting microfaunal effects (a) Experiments with planted ecosystems, conducted in pots and microcosms (b) Studies in natural or seminatural ecosystems

Ecosystem	CO_2 treatment (μmol CO_2 mol^{-1})	Experimental duration	Plant responses		Soil microbial responses						Reference
			Shoots	Roots	Microflora	Fungi	Bacteria	Protozoa	Nematodes	Microarthropods	
(a) Pot and microcosm studies											
Artemisia tridentata	700, low N	12 weeks	+11%, ns	+41%	+16%, ns	+32%	+6%, ns		+32%	+14%, ns	Klironomos *et al.*, 1996
	700, High N	12 weeks	+23%, ns	+41%	+70%, ns	+100%	+44%		+88%	+47%	
Brassica x euramericana	700, low N	23 weeks			−15%, ns†			+548%†		+38%†	Lussenhop *et al.*, 1998
	700, high N	23 weeks			−34%, ns†			+151%†		−24%†	
Brassica nigra	700	4 weeks	−11%, ns	+2%, ns			−7%, ns	+6%, ns			Treonis & Lussenhop, 1997
Mixtures of *Cardamine hirsuta*, *Poa annua*, *Senecio vulgaris* and	560	4.5 months		−46%*	ns	ns	ns			52%	Kampichler *et al.*, 1998
spergula arvensis	560	9 months		88%*	ns	ns	ns			ns	Jones *et al.*, 1998
(b) Naturally established ecosystems											
Perennial pasture	700	420 days	+10%, ns	77%	ns				+11%		Yeates *et al.*, 1997 Ross *et al.*, 1996
Annual grassland on	700	4 growing seasons				ns	ns	ns	ns		Hungate *et al.*, 2000
sandstone soil	700	6 growing seasons		+23%, ns		+68%	−8%	+8%		+109%	Rillig *et al.*, 1999a
Annual grassland on	700	4 growing seasons				ns	ns	ns	ns		Hungate *et al.*, 2000
sand stone soil	700	6 growing seasons		+49%		+93%	+17%	−15%	ns	+39%	Rillig *et al.*, 1999a
Perennial low-fertility grassland	600	6 growing seasons	+19%	+25%	ns	ns	ns	ns	−43%	ns	This study

*0–10 cm, no significant response in 10–20 cm layer.
†rhizo sphere soil.

pronounced in large animals; indeed, the largest reductions in animal numbers were observed in omnivorous and predator nematodes (Fig. 6), whereas smaller nematodes showed no such effect. These nematodes are at relatively high trophic levels; therefore, the effect of CO_2 enrichment on soil structure could trigger a soil-structure mediated top-down effect on the soil food web.

Microfaunal effects of elevated CO_2 have only been studied in a limited number of studies (Table 3). There are probably too few experiments to draw any generalizations. Nevertheless, we speculate that effects occur primarily when root biomass is stimulated by elevated CO_2 and that the bacterial part of the food web responds more than the fungal part when rapid root growth occurs (which favours exudation and sloughing of root tissue) and when elevated CO_2 increases soil moisture. Indeed, increased protozoan numbers have been observed in rhizosphere soil of *Populus x euramericana* hybrids grown at elevated CO_2 and were associated with a decrease in microbial biomass (Lussenhop *et al*., 1998). In this experiment, root biomass and fine root turnover increased strongly under elevated CO_2. No effects on total protozoa were detected in annual grassland (Hungate *et al*., 2000) or in *Brassica nigra* pot systems (Treonis & Lussenhop, 1997; but plant growth did not increase under elevated CO_2). We did not detect any effects on protozoan biomass in this study; however, estimates were based on characteristic protozoan PLFA and statistical power was relatively low (Ebersberger *et al.* unpublished). A general problem with effects on organisms turning over quickly is that huge population changes can occur after relatively minor disturbances, triggering a series of successive population peaks starting with bacteria, followed by protozoan population growth, in turn stimulating protozoan predators, etc. (Clarholm, 1994). Any CO_2 effects detected could therefore be transitional, and many repeated observations are required to obtain a good measure of average populations.

Effects on the fungal compartment were detected in *Artemisia tridentata* shrub microcosms (Klironomos *et al*., 1996) in which fungi and microarthropods increased markedly. This effect was associated with relatively strong effects on root mass (+41%); effects on bacteria were smaller. Hungate *et al*. (2000) and Rillig *et al*. (1999a) observed strong seasonal dynamics of microbial measurements in the grassland they investigated, most likely because of its annual character. Fungal biomass and microarthropod numbers increased in the harvest in year six of treatment (Rillig *et al*., 1999a) but were not increased in the two harvests in year four (Hungate *et al*., 2000).

Seasonal dynamics of microbial measurements are probably less pronounced in perennial ecosystems (e.g. Newton *et al*., 1996; this experiment). Ross *et al*. (1996) did not find increases in microbial biomass in temperate pasture, despite a 77% increase in root biomass; nematode numbers increased by 11% (Yeates *et al*., 1999).

Conclusions

Elevated CO_2 did not alter total soil microbial biomass, even after six years of increased plant productivity. Our initial hypothesis that either soil microbial biomass or grazer populations would increase due to better substrate availability could not be corroborated. In contrast to other studies reporting a shift from the fungal to the bacterial part of the food web, we failed to find any indication of such a change. However, elevated CO_2 decreased soil aggregate sizes, most likely due to increased soil moisture in elevated CO_2 plots. This change in soil structure may have far-reaching consequences for water and nutrient fluxes and organic matter binding. Trace gas production may also be affected; N_2O production occurs in the anaerobic centre of aggregates, and a reduction in aggregate diameter is likely to reduce the proportion of anaerobic–aerobic sites. This effect may be counteracted by the overall increase in soil moisture found. Reduced aggregate sizes and pore neck diameters are probably responsible for the reduction in larger-diameter soil nematodes we observed. Because these nematodes are relatively high up the soil food web, this could trigger as top-down effect on the soil food web.

Increased mineralization of soil organic matter N under elevated CO_2 occurred in spring when plant growth was rapid and soils were moist. This extra N available may have allowed for faster growth under elevated CO_2; however, total N removed in plant mass over the course of the experiment did not differ between CO_2 treatments (unpublished results), suggesting that this effect was only transitory. Microbial N pools also did not change, indicating that elevated CO_2 did not stimulate net microbial immobilization of N which could have imposed a negative feedback on plant growth as reported in other studies.

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References

Alphei J (1995) Die freilebenden Nematoden von Buchenwäldern mit unterschiedlicher Humusform: Struktur

der Gemeinschaften und Funktion in der Rhizosphäre der Krautvegetation. *Berichte Des Forschungszentrums Waldökosysteme Göttingen*, **125**, 1–165.

Amthor JS (1995) Terrestrial higher-plant response to increasing atmospheric [CO_2] in relation to the global carbon cycle. *Global Change Biology*, **1**, 243–274.

Amthor JS (1999) Increasing atmospheric CO_2, water use, and water stress: scaling up from the plant to the landscape. In: *Carbon Dioxide and Environmental Stress* (eds Luo Y, Mooney HA), pp. 33–59. Academic Press, San Diego.

Anderson JPE, Domsch KH (1978) A physiological method for the quantitative measurement of microbial biomass in soils. *Soil Biology and Biochemistry*, **10**, 215–221.

Andrássy I (1956) Die Rauminhalts-und Gewichtsbestimmung der Fadenwürmer (Nematoda). *Acta Zoologica Hungarica*, **2**, 1–15.

Arnone JAI, Bohlen PJ (1998) Stimulated N_2O flux from intact grassland monoliths after two growing seasons under elevated CO_2. *Oecologia*, **116**, 331–335.

Arnone JA, Zaller JG, Spehn E *et al.* (2000) Dynamics of roots systems in intact native grasslands: effects of elevated atmospheric CO_2. *New Phytologist*, **147**, 73–85.

Ball AS, Drake BG (1997) Short-term decomposition of litter produced by plants grown in ambient and elevated atmospheric CO_2 concentrations. *Global Change Biology*, **3**, 29–35.

Brookes PC, Landman A, Pruden G *et al.* (1985) Chloroform fumigation and the release of soil nitrogen: a rapid direct extraction method to measure microbial biomass nitrogen in soil. *Soil Biology and Biochemistry*, **17**, 837–842.

Buecher EJ, Hansen EL (1971) Mass culture of axenic nematodes using continuous aeration. *Journal of Nematology*, **3**, 199–200.

Clarholm M (1994) The microbial loop in soil. In: *Beyond the Biomass: Compositional and Functional Analysis of Soil Microbial Communities* (eds Ritz K, Dighton J, Giller KE), pp. 221–230. John Wiley, Chichester.

Cotrufo MF, Ineson P (1996) Elevated CO_2 reduces field decomposition rates of *Betula pendula* (Roth.) leaf litter. *Oecologia*, **106**, 525–530.

Coughenour MB, Chen DX (1997) Assessment of grassland ecosystem responses to atmospheric change using linked plant-soil process models. *Ecological Applications*, **7**, 802–827.

Darrah PR (1996) Rhizodeposition under ambient and elevated CO_2 levels. *Plant and Soil*, **187**, 265–275.

Diaz S, Grime JP, Harris J *et al.* (1993) Evidence of a feedback mechanism limiting plant response to elevated carbon dioxide. *Nature*, **364**, 616–617.

Edwards CA (1991) The assessment of populations of soil-inhabiting invertebrates. *Agriculture, Ecosystems and Environment*, **34**, 145–176.

Fitter AH, Self GK, Wolfenden J *et al.* (1996) Root production and mortality under elevated atmospheric carbon dioxide. *Plant and Soil*, **187**, 299–306.

Frostegård A, Bååth E (1996) The use of phospholipid fatty acid analysis to estimate bacterial and fungal biomass in soil. *Biology and Fertility of Soils*, **22**, 59–65.

Frostegård A, Bååth E, Tunlid A (1993) Shifts in the structure of soil microbial communities in limed forests as revealed by phospholipid fatty acid analysis. *Soil Biology and Biochemistry*, **25**, 723–730.

van de Geijn SC, van Veen JA (1993) Implications of increased carbon dioxide levels for carbon input and turnover in soils. *Vegetatio*, **104/105**, 283–292.

Hilbert DW, Prudhomme TI, Oechel WC (1987) Response of tussock tundra to elevated carbon dioxide regimes: analysis of ecosystem CO_2 flux through nonlinear modelling. *Oecologia*, **72**, 466–472.

Hirschel G, Körner C, Arnone JA (1997) Will rising atmospheric CO_2 affect leaf litter quality and *in situ* decomposition rates in native plant communities. *Oecologia*, **110**, 387–392.

Houghton RA (1995) Land-use change and the carbon cycle. *Global Change Biology*, **1**, 275–287.

Hu S, Firestone MK, Chapin FS (1999) Soil microbial feedbacks to atmospheric CO_2 enrichment. *Trends in Ecology and Evolution*, **14**, 433–437.

Hungate BA (1999) Ecosystem responses to rising atmospheric CO_2: feedbacks through the nitrogen cycle. In: *Carbon Dioxide and Environmental Stress* (eds Luo Y, Mooney HA), pp. 265–285. Academic Press, San Diego.

Hungate BA, Holland EA, Jackson RB *et al.* (1997) The fate of carbon in grasslands under carbon dioxide enrichment. *Nature*, **388**, 576–579.

Hungate BA, Jackson RB, Field CB *et al.* (1996) Detecting changes in soil carbon in CO_2 enrichment experiments. *Plant and Soil*, **187**, 135–145.

Hungate BA, Jaeger CH, Gamara G *et al.* (2000) Soil microbiota in two annual grasslands: responses to elevated atmospheric CO_2. *Oecologia*, **124**, 589–598.

Huovinen-Hufschmid C, Körner C (1998) Microscale patterns of plant species distribution, biomass and leaf tissue quality in calcareous grassland. *Botanica Helvetica*, **108**, 69–83.

Ineson P, Coward PA, Hartwig UA (1998) Soil gas fluxes of N_2O, CH_4 and CO_2 beneath *Lolium perenne* under elevated CO_2: The Swiss free air carbon dioxide enrichment experiment. *Plant and Soil*, **198**, 89–95.

IPCC (1995) *Radiative Forcing of Climate Change and an Evaluation of the IPCC 1992 IS92 Emission Scenarios* (eds Houghton JT, Meira Filho LG, Hoesung Lee BJ, Callander BA, Haites E, Harris N, Maskell K), p. 339. Cambridge University Press, Cambridge.

Jackson RB, Sala OE, Field CB *et al.* (1994) CO_2 alters water use, carbon gain, and yield for the dominant species in a natural grassland. *Oecologia*, **98**, 257–262.

Jackson RB, Sala OE, Paruelo JM *et al.* (1998) Ecosystem water fluxes for two grasslands in elevated CO_2: a modelling analysis. *Oecologia*, **113**, 537–546.

Jones DL, Darrah PR (1996) Resorption of organic compounds by roots of *Zea mays* L. and its consequences in the rhizosphere. *Plant and Soil*, **178**, 153–160.

Jones TH, Thompson LJ, Lawton JH *et al.* (1998) Impacts of rising atmospheric carbon dioxide on model terrestrial ecosystems. *Science*, **280**, 441–443.

Kampichler C, Kandeler E, Bardgett RD *et al.* (1998) Impact of elevated atmospheric CO_2 concentration on soil microbial biomass and activity in a complex, weedy field model ecosystem. *Global Change Biology*, **4**, 335–346.

Klironomos JN, Rillig MC, Aellen MF (1996) Below-ground microbial and microfaunal responses to *Artemisia tridentata* grown under elevated atmospheric CO_2. *Functional Ecology*, **10**, 527–534.

Körner C (1996) The response of complex multispecies systems to elevated CO_2. In: *Global Change and Terrestrial Ecosystems. IGBP Series No. 2* (eds Walker BH, Steffen WL), pp. 20–42. Cambridge University Press, Cambridge.

Lal R (2000) Soil aggregation and C sequestration. In: *Global Climate Change and Tropical Ecosystems* (eds Lal R, Kimble JM, Stewart BA), pp. 317–329. CRC Press, Boca Raton.

Lauber W, Körner C (1997) *In situ* stomatal responses to long-term CO_2 enrichment in calcareous grassland plants. *Acta Oecologica*, **18**, 221–229.

Leadley PW, Niklaus PA, Stocker R *et al.* (1997) Screen-aided CO_2 control (SACC): a middle ground between FACE and open-top chambers. *Acta Oecologica*, **18**, 207–219.

Leadley PW, Niklaus PA, Stocker R *et al.* (1999) A field study of the effects of elevated CO_2 on plant biomass and community structure in a calcareous grassland. *Oecologia*, **118**, 39–49.

Liljeroth E, van Veen JA, Miller HJ (1990) Assimilate translocation to the rhizosphere of two wheat lines and subsequent utilization by rhizosphere microorganisms at two soil nitrogen concentrations. *Soil Biology and Biochemistry*, **22**, 1015–1021.

Lund CP, Riley WJ, Pierce LL *et al.* (1999) The effects of chamber pressurization on soil-surface CO_2 efflux and the implications for NEE measurements under elevated CO_2. *Global Change Biology*, **5**, 269–281.

Lussenhop J, Treonis A, Curtis PS *et al.* (1998) Response of soil biota to elevated atmospheric CO_2 in poplar model systems. *Oecologia*, **113**, 247–251.

Megonigal JP, Schlesinger WH (1997) Enhanced CH_4 emissions from a wetland soil exposed to elevated CO_2. *Biogeochemistry*, **37**, 77–88.

Merckx R, Dijkstra A, den Hartog A *et al.* (1987) Production of root-derived material and associated microbial growth in soil at different nutrient levels. *Biology and Fertility of Soils*, **5**, 126–132.

Moore JC, de Ruiter PC, Hunt HW (1993) Influence of productivity on the stability of real and model ecosystems. *Science*, **261**, 906–908.

Morison JIL (1985) Sensitivity of stomata and water use efficiency to high CO_2. *Plant, Cell and Environment*, **8**, 467–474.

Neilson RP, Drapek RJ (1998) Potentially complex biosphere responses to transient global warming. *Global Change Biology*, **4**, 505–521.

Newton PCD, Clark H, Bell CC *et al.* (1996) Interaction of soil moisture and elevated CO_2 on the above-ground growth rate, root length density and gas exchange of turves from temperate pasture. *Journal of Experimental Botany*, **47**, 771–779.

Niklaus PA (1998) Effects of elevated atmospheric CO_2 on soil microbiota in calcareous grassland. *Global Change Biology*, **4**, 451–458.

Niklaus PA, Glöckler E, Siegwolf R *et al.* (2001a) Carbon allocation in calcareous grassland under elevated CO_2: a combined ^{13}C pulse-labelling/soil physical fractionation study. *Functional Ecology*, **15**, 43–50.

Niklaus PA, Körner C (1996) Responses of soil microbiota of a late successional alpine grasslands to long term CO_2 enrichment. *Plant and Soil*, **184**, 219–229.

Niklaus PA, Leadley PW, Schmid B *et al.* (2001b) A long-term field study on biodiversity × elevated CO_2 interactions in grassland. *Ecological Monographs*, **71**, 341–356.

Niklaus PA, Leadley PW, Stöcklin J *et al.* (1998a) Nutrient relations in calcareous grassland under elevated CO_2. *Oecologia*, **116**, 67–75.

Niklaus PA, Spinnler D, Körner C (1998b) Soil moisture dynamics of calcareous grassland under elevated CO_2. *Oecologia*, **117**, 201–208.

Niklaus PA, Stocker R, Körner C *et al.* (2000) CO_2 flux estimates tend to overestimate ecosystem carbon sequestration at elevated CO_2. *Functional Ecology*, **14**, 546–559.

Niklaus PA, Wohlfender M, Siegwolf R *et al.* (2001c) Effects of six years atmospheric CO_2 enrichment on plant, soil, and soil microbial C of a calcareous grassland. *Plant and Soil*, **233**, 189–202.

Oades JM (1978) Mucilages at the root surface. *Soil Science*, **29**, 1–16.

Oades JM (1984) Soil organic-matter and structural stability – mechanisms and implications for management. *Plant and Soil*, **76**, 319–337.

Owensby CE, Ham JM, Knapp AK *et al.* (1999) Biomass production and species composition change in a tallgrass prairie ecosystem after long-term exposure to elevated atmospheric CO_2. *Global Change Biology*, **5**, 497–506.

Owensby CE, Ham JM, Knapp AK *et al.* (1997) Water vapour fluxes and their impact under elevated CO_2 in a C_4-tallgrass prairie. *Global Change Biology*, **3**, 189–195.

Parton WJ, Scurlock JMO, Ojima DS *et al.* (1995) Impact of climate change on grassland production and soil carbon worldwide. *Global Change Biology*, **1**, 13–22.

Paul EA, Clark FE (1996) *Soil Microbiology and Biochemistry*, 2nd edn. Academic Press, San Diego.

Penuelas J, Estiarte M (1998) Can elevated CO_2 affect secondary metabolism and ecosystem function? *Trends in Ecology and Evolution*, **13**, 20–24.

Phillips RL, Whalen SC, Schlesinger WH (2001a) Influence of atmospheric CO_2 enrichment on methane consumption in a temperate forest soil. *Global Change Biology*, **7**, 557–563.

Phillips RL, Whalen SC, Schlesinger WH (2001b) Influence of atmospheric CO_2 enrichment on nitrous oxide flux in a temperate forest ecosystem. *Global Biogeochemical Cycles*, **15**, 741–752.

Poorter H, Roumet C, Campbell BD (1996) Interspecific variation in the growth response of plants to elevated CO_2: a search for functional types. In: *Carbon Dioxide, Populations and Communities* (eds Körner C, Bazzaz FA), pp. 375–412. Academic Press, San Diego.

Pregitzer KS, Zak DR, Maziasz J *et al.* (2000) Interactive effects of atmospheric CO_2 and soil-N availability on fine roots of *Populus tremuloides*. *Ecological Applications*, **10**, 18–33.

Rillig MC, Field CB, Allen MF (1999a) Soil biota responses to long-term atmospheric CO_2 enrichment in two California annual grasslands. *Oecologia*, **119**, 572–577.

Rillig MC, Wright SF, Allen MF *et al.* (1999b) Rise in carbon dioxide changes soil structure. *Nature*, **400**, 628.

Ross DJ, Saggar S, Tate KR *et al.* (1996) Elevated CO_2 effects on carbon and nitrogen cycling in grass/clover turves of a Psammaquent soil. *Plant and Soil*, **182**, 185–198.

Ross DJ, Tate KR, Newton PCD (1995) Elevated CO_2 and temperature effects on soil carbon and nitrogen cycling in ryegrass/white clover turves of an Endoaquept soil. *Plant and Soil*, **176**, 37–49.

Scheu S (1992) Automated measurement of the respiratory response of soil microcompartments – active microbial biomass in earthworm feces. *Soil Biology and Biochemistry*, **24**, 1113–1118.

Schläpfer M, Zoller H, Körner C (1998) Influences of mowing and grazing on plant species composition in calcareous grassland. *Botanica Helvetica*, **108**, 57–67.

Sessitsch A, Weilharter A, Gerzabek MH *et al.* (2001) Microbial population structures in soil particle size fractions of a long-term fertilizer field experiment. *Applied and Environmental Microbiology*, **67**, 4215–4224.

Smith JL, Paul EA (1990) The significance of soil microbial biomass estimations. In: *Soil Biochemistry* (eds Bollag J, Stotzky G), pp. 357–393. Marcel Dekker, New York.

Sohlenius B (1979) A carbon budget for nematodes, rotifers and tardigrades in a Swedish coniferous forest soil. *Holarctic Ecology*, **2**, 30–40.

Stocker R, Leadley PW, Körner C (1997) Carbon and water fluxes in a calcareous grassland under elevated CO_2. *Functional Ecology*, **11**, 222–230.

Tisdall JM, Oades JM (1982) Organic matter and water-stable aggregates in soils. *Journal of Soil Science*, **33**, 141–163.

Treonis AM, Lussenhop JF (1997) Rapid response of soil protozoa to elevated CO_2. *Biology and Fertility of Soils*, **25**, 60–62.

Van Vuuren MMI, Robinson D, Scrimgeour CM *et al.* (2000) Decomposition of C-13-labelled wheat root systems following growth at different CO_2 concentrations. *Soil Biology and Biochemistry*, **32**, 403–413.

Vance ED, Brookes PC, Jenkinson DS (1987) An extraction method for measuring soil microbial biomass C. *Soil Biology and Biochemistry*, **19**, 703–707.

Volk M, Niklaus PA, Körner C (2000) Soil moisture effects determine CO_2-responses of grassland species. *Oecologia*, **125**, 380–388.

Wallace HR (1958) Movement of eelworms. II. A comparative study of the movement in soil of Heterodera schachtii (Schmidt) and of Ditylenchus dipsaci (Kühn). *Annaly of Applied Biology*, **46**, 86–94.

Yeates GW, Bongers T, de Goede RGM *et al.* (1993) Feeding habits in soil nematode families and genera – an outline for soil ecologists. *Journal of Nematology*, **25**, 315–331.

Yeates GW, Dando JL, Shepherd TG (2002) Pressure plate studies to determine how moisture affects access of bacterial-feeding nematodes to food in soil. *European Journal of Soil Science*, **53**, 355–365.

Yeates GW, Newton PCD, Ross DJ (1999) Response of soil nematode fauna to naturally elevated CO_2 levels influenced by soil pattern. *Nematology*, **1**, 285–293.

Yeates GW, Tate KR, Newton PCD (1997) Response of the fauna of a grassland soil to doubling of atmospheric carbon dioxide concentration. *Biology and Fertility of Soils*, **25**, 307–315.

Zak DR, Pregitzer KS, Curtis PS *et al.* (1993) Elevated atmospheric CO_2 and feedback between carbon and nitrogen cycles. *Plant and Soil*, **151**, 105–117.

Summary

Terrestrial ecosystems generally respond to rising atmospheric carbon dioxide (CO_2) concentrations with increased net primary productivity and increased water use efficiency. This may change the amount and quality of organic substances entering the soil and fuelling microbial metabolism. Soil microorganisms and their activity might also be affected by increased soil moisture at elevated CO_2. This thesis was designed to analyse the response of the soil microbial community in a species-rich calcareous grassland in the Swiss Jura Mountains, which had been exposed to ambient and elevated CO_2 concentrations (365 and 600 µl l^{-1}) for six growing seasons.

In the first study, laboratory incubation experiments were conducted to explore the relationship between litter quality under elevated carbon dioxide and enzymes involved in carbon cycling. By using naturally senescent, mixed litter from the long-term field experiment, the overall effect of litter quality at elevated CO_2 on enzyme activity could be examined, integrating changes in litter chemistry, litter morphology and species composition. Litter and soil material were incubated together, and samples taken after 10, 30 and 60 days. Soil samples were then obtained close to the litter layer using a microtome cutting device. Litter and soil samples were analysed for invertase and xylanase activity. The lower litter quality produced under elevated CO_2, i.e. wider C/N ratio, yielded lower invertase and xylanase activities of litter. Litter addition stimulated activities in adjacent soil. Invertase activities of adjacent soil were not affected by litter quality, while soil xylanase activity was higher in soil compartments adjacent to litter from elevated CO_2 plots. The reduced enzyme activities of litter produced under elevated CO_2 can slow decomposition, at least during the initial stages. Since the effects of litter quality on enzyme activities in adjacent soil were small, we conclude that CO_2-induced belowground C-inputs (e.g. increased root mass) and altered soil moisture conditions are more important controls of enzyme activities than altered litter quality.

In the second study, functional diversity of the soil microbial community was assessed by analysing N-mineralisation and activities of enzymes of the C-, N-, P- and S-cycle of soil samples taken in spring and summer 1999, in the 6th season of CO_2 exposure. In spring, N-mineralisation increased significantly by 30% at elevated CO_2, while there was no significant difference between treatments in summer (+3%). Soil enzymes mediate microbial decomposition and mineralisation. The response of soil enzymes to CO_2 enrichment was also more pronounced in spring, when alkaline phosphatase and urease activities were increased most strongly, by 32% and 21%,

respectively. In summer, activity differences between CO_2 treatments were greatest in the case of urease and protease (+21% and +17% at elevated CO_2). The significant stimulation of N-mineralisation and enzyme activities at elevated CO_2 was probably caused by higher soil moisture and/or increased root biomass. At the same time, microbial biomass did not respond. These results indicate that elevated CO_2 will enhance belowground N-cycling in temperate grasslands.

In the third study, soil samples of spring and summer 1999 were analysed with modern molecular methods to study the structure of the soil microbial community. Two different approaches were used: (1) phospholipid fatty acid (PLFA) profiles, because this technique yields quantitative information on community structure and allows a discrimination between fungi and bacteria and (2) DNA fingerprints of the bacterial community, obtained by Denaturing Gradient Gel Electrophoresis (DGGE) of 16S rDNA fragments amplified by the Polymerase Chain Reaction (PCR). Bacterial diversity was assessed based on Shannon diversity indices. PLFA profiles were not affected by elevated CO_2 and the ratio of fungal and bacterial PLFA did not change. Ordination analysis of DNA fingerprints revealed a significant relation between CO_2 enrichment and variation in DNA fingerprints. This variation must be attributed to low intensity bands. Dominant bands did not differ between treatments. Diversity of the bacterial community, as assessed by the number of bands in DNA fingerprints and Shannon diversity indices, was not affected. The observed minute, but significant changes in the structure of the soil bacterial community might be caused by changes in the quality of rhizodeposits at elevated CO_2. These could either result from altered rhizodeposition of individual plants or from altered species composition of the calcareous grassland.

The 4th part of the thesis synthesises data on soil microorganisms, soil fauna (protozoans, nematodes, acarians, collembolans), soil structure and nitrogen cycle of calcareous grassland after CO_2 exposure for six growing seasons. Microbial biomass, soil basal respiration and the metabolic quotient were not altered significantly. PLFA analysis revealed no significant shift in the ratio of fungi to bacteria. Microbial grazer populations (protozoans, bacterivorous and fungivorous nematodes, acarians, collembolans) and root-feeding nematodes were not affected by elevated CO_2. Total nematode numbers, however, averaged slightly lower (-16%) and nematode mass was significantly reduced (by 43%) due to fewer large-diameter nematodes classified as omnivorous and predacious. CO_2 exposure resulted in a shift towards smaller aggregate sizes at both micro- and macro-aggregate scales; this was caused by higher soil moisture. Reduced aggregate sizes result in reduced pore neck diameters. This can confine the locomotion of

large-diameter nematodes and possibly accounts for their decrease. The CO_2 enrichment also affected the nitrogen cycle. The N stocks in living plants and surface litter increased, but N in soil organic matter and microorganisms remained unaltered. N mineralisation increased considerably, but microbial N did not differ between treatments, indicating that net N immobilization rates were unaltered.

These studies proved that elevated atmospheric CO_2 influenced both function and composition of the soil microbial community in species-rich grassland. The driving mechanisms were probably altered belowground C-inputs into the soil, both in terms of quality and quantity, and increased soil moisture under CO_2 enrichment. Altered aboveground litter quality at elevated CO_2 may have only minor effects on soil microorganisms.

Zusammenfassung

Terrestrische Ökosysteme reagieren auf die gegenwärtige Erhöhung der atmosphärischen Kohlendioxidkonzentration mit Steigerung der Nettoprimärproduktion und erhöhter Effektivität der Wassernutzung. Dies kann dazu führen, dass sich Menge und Qualität der organischen Substanzen, die in den Boden gelangen und den heterotrophen Bodenmikroorganismen als Energiequelle dienen, verändern. Bodenmikroorganismen und ihre Aktivität können zudem durch die höhere Bodenfeuchte unter erhöhtem CO_2 beeinflusst werden. Diese Dissertation hatte zum Ziel, die Reaktion der bodenmikrobiellen Gemeinschaft in einem Kalkmagerrasen im Schweizer Jura, der über sechs Vegetationsperioden mit CO_2-angereicherter Luft (600 ppm) begast wurde, näher zu charakterisieren.

Im ersten Teil der Dissertation wurde ein Modellexperiment im Labor durchgeführt, um den Einfluss der veränderten Streuqualität unter erhöhtem CO_2 auf die Aktivität von Enzymen des C-Kreislaufes zu bestimmen. Hierzu wurde gemischte Streu aus der normal-CO_2 und erhöhtes-CO_2-Variante des Langzeit-CO_2-Versuches mit Boden bei 15 °C für 10, 30 und 60 Tagen inkubiert. Mit Hilfe eines Gefriermikrotoms wurden dann Bodenproben in unmittelbarer Nähe der Streu (0,250 - 14 mm) gewonnen. Die Invertase- und Xylanaseaktivitäten der Streu- und Bodenproben wurden bestimmt. Aufgrund der geringeren Qualität der Streu, die unter erhöhtem CO_2 produziert wurde (z.B. weiteres C/N-Verhältnis), waren ihre Invertase- und Xylanaseaktivität generell erniedrigt. Die Streuzugabe stimulierte die Enzymaktivitäten im angrenzenden Boden. Die Invertaseaktiviät im angrenzenden Boden wurde durch die Herkunft der Streu nicht beeinflusst, die Xylanaseaktivität wies jedoch höhere Werte bei Inkubation mit Streumaterial aus der erhöhten-CO_2-Variante auf. Die geminderten Enzymaktivitäten der Streu, die unter erhöhtem CO_2 produziert wurde, können geringere Abbauraten, zumindest im Initialstadium des Abbaus, zur Folge habe. Da die Streuqualität insgesamt nur geringen Einfluss auf die Enzymaktivitäten im direkt angrenzenden Boden hatte, sind CO_2-induzierte Änderungen der C-Einträge über die Wurzeln und das veränderte Bodenfeuchteregime vermutlich bedeutsamer für die Ausprägung der Bodenenzymaktivitäten unter erhöhtem CO_2 als die veränderte Streuqualität.

In der zweiten Studie wurde die funktionelle Diversität der bodenmikrobiellen Gemeinschaft durch die Bestimmung der N-Mineralisation und der Aktivitäten der Enzyme aus den C-, N-, P- und S-Kreisläufen charakterisiert. Hierzu wurden Proben im Frühjahr und Sommer 1999, in der sechsten Vegetationsperiode des in-situ CO_2-

Anreicherungsversuches, genommen. Unter erhöhtem CO_2 war die N-Mineralisation im Frühjahr um 30% erhöht, während im Sommer kein signifikanter Anstieg (+ 3%) zu verzeichnen war. Die Enzyme reagierten ebenfalls im Frühjahr stärker auf die CO_2-Anreicherung als im Sommer, wobei die Aktivitäten der Alkalischen Phosphatase und Urease unter erhöhtem CO_2 am stärksten gesteigert waren (+32% bzw. +21%). Im Sommer waren die Unterschiede zwischen den CO_2-Varianten für Urease (+21%) und Protease (+17%) am größten. Die signifikante Steigerung der N-Mineralisation und der Enzymaktivitäten kann man auf die höhere Bodenfeuchte und die größere Wurzelmasse unter erhöhtem CO_2 zurückführen. Die Ergebnisse lassen darauf schließen, dass die CO_2-Erhöhung zu einer Steigerung des N-Umsatzes in Grünlandboden führt.

Im dritten Teil der Arbeit wurden die Bodenproben aus dem Frühjahr und Sommer 1999 mit modernen molekularen Methoden analysiert, um detaillierten Einblick in die bodenmikrobielle Gemeinschaftsstruktur zu gewinnen. Hierbei wurden zwei verschiedene Methoden verwendet: (1) Phospholipidfettsäure(PLFA)-Muster, da diese Technik quantitative Information zur Gemeinschaftsstruktur liefert und erlaubt, zwischen bakterieller und pilzlicher Biomasse zu unterscheiden und (2) DNA-Profile, hergestellt durch Denaturierende Gradienten-Gel-Elektrophorese (DGGE) von 16S rDNA Fragmenten, die mittels der Polymerasen Kettenrektion (PCR) amplifiziert wurden. Diese Methode ermöglicht eine detaillierte Analyse der bakteriellen Gemeinschaft. Die bakterielle Diversität wurde durch den Shannon-Diversitäts-Index beschrieben. Die PLFA-Muster und das Pilz-Bakterien-Verhältnis wurden nicht durch die CO_2-Anreicherung beeinflusst. Ordinationsanalyse (Partielle Redundanzanalyse) der DNA-Profile zeigte, dass es im Fall der Sommer-Proben eine signifikante Beziehung zwischen CO_2-Anreicherung und Variation in den DNA-Profilen gab. Diese Variation muss allerdings Banden mit geringer Intensität zugeschrieben werden, da sich die dominanten Banden zwischen den Varianten nicht unterschieden haben. Die bakterielle Diversität, abgeschätzt durch die Anzahl der Banden und durch den Shannon-Index, wurde durch die CO_2-Anreicherung des Kalkmagerrasens nicht beeinflusst. Die geringen, aber signifikanten Änderungen in der bakteriellen Gemeinschaftsstruktur wurden möglicherweise durch qualitative Änderungen der Rhizodeposition unter erhöhtem CO_2 hervorgerufen. Diese können entweder aus veränderter Rhizodeposition einzelner Pflanzenarten oder aber aus der veränderten Artenzusammensetzung der Pflanzengesellschaft resultieren.

Der vierte Teil der Dissertation besteht aus einer Synthese von Daten zu Mikroorganismen, Bodenfauna, Bodenstruktur und N-Kreislauf nach 6 Jahren CO_2-Anreicherung des Kalkmagerrasens. Mikrobielle Biomasse, Basalatmung und

metabolischer Quotient zeigten keine signifikante Änderung unter erhöhtem CO_2. Es fand auch keine Verschiebung des Pilz/Bakterien-Verhältnisses statt. Bodentiere, die Mikroorganismen beweiden, wie Protozoen, bakterivore und fungivore Nematoden, Milben und Collembolen, und wurzelfressenden Nematoden wurden nicht durch die CO_2-Anreicherung beeinflusst. Die Gesamtindividuenzahl der Nematoden war allerdings etwas erniedrigt unter erhöhtem CO_2 (-16%), die Nematodenbiomasse war signifikant um 43% reduziert. Dies ist auf den Rückgang der Nematoden mit einem großen Körperdurchmesser, die als omnivor und räuberisch klassifiziert wurden, zurückzuführen. Die CO_2-Anreicherung bewirkte eine Verschiebung zu kleineren Aggregatgrößen, sowohl auf der Mikro- als auch der Makroaggregateebene, vermutlich verursacht durch die erhöhte Bodenfeuchte. Kleinere Aggregatgrößen bedingen geringere Porendurchmessern, was die Bewegung großer Nematoden einschränken kann, und somit eine Erklärung für ihren Rückgang darstellt. Die CO_2-Anreicherung beeinflusste auch den N-Kreislauf im Ökosystem. Die N-Vorräte in lebenden Pflanzen und Streu stiegen unter erhöhtem CO_2, während die N-Vorräte in der bodenorganischen Substanz und in den Mikroorganismen gleich blieben. Die N-Mineralisation war zeitweise stark erhöht, aber der mikrobielle Stickstoff unterlag keiner Veränderung, so dass die netto N-Immobilisierungsraten vermutlich unverändert blieben.

Die vorliegende Arbeit zeigt, dass eine Erhöhung der atmosphärischen Kohlendioxids sowohl die Funktion als auch die Struktur der bodenmikrobiellen Gemeinschaft beeinflussen kann, zumindest in einem artenreichen Kalkmagerrasen. Als wichtige Mechanismen haben sich hierbei Veränderungen der Menge und Qualität der Kohlenstoffeintrage in den Boden und die erhöhte Bodenfeuchte bei CO_2-Anreicherung erwiesen. Der veränderten Streuqualität unter erhöhtem CO_2 kommt hingegen vermutlich nur eine geringere Bedeutung zu.

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