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Nutrient relations in calcareous grassland under elevated CO₂

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Abstract Plant nutrient responses to 4 years of CO₂ enrichment were investigated in situ in calcareous grassland. Beginning in year 2, plant aboveground C:N ratios were increased by 9% to 22% at elevated CO₂ ($P < 0.01$), depending on year. Total amounts of N removed in biomass harvests during the first 4 years were not affected by elevated CO₂ (19.9 ± 1.3 and 21.1 ± 1.3 g N m⁻² at ambient and elevated CO₂), indicating that the observed plant biomass increases were solely attained by dilution of nutrients. Total aboveground P and tissue N:P ratios also were not altered by CO₂ enrichment (12.5 ± 2 g N g⁻¹ P in both treatments). In contrast to non-legumes (>98% of community aboveground biomass), legume C/N was not reduced at elevated CO₂ and legume N:P was slightly increased. We attribute the less reduced N concentration in legumes at elevated CO₂ to the fact that virtually all legume N originated from symbiotic N₂ fixation (%N_{difa} ≈ 90%), and thus legume growth was not limited by soil N. While total plant N was not affected by elevated CO₂, microbial N pools increased by +18% under CO₂ enrichment ($P = 0.04$) and plant available soil N decreased. Hence, there was a net increase in the overall biotic N pool, largely due increases in the microbial N pool. In order to assess the effects of legumes for ecosystem CO₂ responses and to estimate the degree to which plant growth was P-limited, two greenhouse experiments were conducted, using firstly undisturbed grassland monoliths from the field site, and secondly designed 'microcosm' communities on natural soil. Half the microcosms were planted with legumes and half were planted without. Both monoliths and microcosms were exposed to elevated CO₂ and P fertilization in a factored design. After two seasons, plant N pools in both unfertilized monoliths and microcosm communities were

unaffected by CO₂ enrichment, similar to what was found in the field. However, when P was added total plant N pools increased at elevated CO₂. This community-level effect originated almost solely from legume stimulation. The results suggest a complex interaction between atmospheric CO₂ concentrations, N and P supply. Overall ecosystem productivity is N-limited, whereas CO₂ effects on legume growth and their N₂ fixation are limited by P.

Key words Dinitrogen fixation · Plant functional types · legumes · Nutrient limitation · Phosphorus

Introduction

Most studies of vascular plant responses to atmospheric CO₂ enrichment show an enhancement of growth when carried out under sufficient nutrient supply. In natural environments, plant productivity is often limited by the availability of mineral nutrients such as nitrogen and phosphorus (Vitousek and Howarth 1991) which in turn may limit the stimulation of plant growth by CO₂ enrichment. This underlines the importance of considering plant nutrient status in understanding their growth responses to increased CO₂ concentrations (Woodward et al. 1991; Overdieck 1993; Körner 1995b). However, a simple approach via Liebig's law (von Liebig 1840) to this problem is inadequate for several reasons.

1. A number of studies in intact ecosystems have shown that nutrient demand per unit of biomass is reduced at elevated CO₂ (e.g. Curtis et al. 1989; Owensby et al. 1993; Körner and Miglietta 1994), so plants can produce more biomass with a given amount of mineral nutrients by increasing the carbon-to-nutrient ratio.
2. Nutrient acquisition by plants may be enhanced by CO₂ fertilization via enhanced nutrient foraging of roots and mycorrhiza or enhanced symbiotic N₂ fixation (Norby 1987; Arnone and Gordon 1990; Vogel and Curtis 1995; Zanetti et al. 1996).

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3. Rates of nutrient cycling may be indirectly altered by elevated CO₂ in a number of ways including changes in herbivory, soil moisture, rhizodeposition affecting e.g. microbial nutrient mineralization and immobilization, litter decomposition (Gorisson 1994).

In the long term, such ecosystem-level feedbacks may affect nutrient availability and distribution within the ecosystem and thus strongly determine plant responses to elevated CO₂. Diaz et al. (1993) and Hättenschwiler and Körner (1998) observed decreased plant growth and symptoms of N deficiency in plants grown at elevated CO₂ in microcosms and attributed these effects to increased N immobilization by soil microbes, which resulted in reduced plant N availability. In contrast, N mineralization rates may increase at elevated CO₂ because microbial activity may be primed through enhanced rhizodeposition at elevated CO₂ (Zak et al. 1993; Körner and Arnone 1992). However, all these studies were carried out in systems where nutrient cycles were most likely not in equilibrium. Such pronounced short-term effects of CO₂ enrichment on plant-microbe interactions have not been observed in natural systems in which microbial activity may be co-limited by mineral nutrients and organic carbon supply (Rice et al. 1994; Niklaus and Körner 1996; Niklaus 1998).

The N status of an ecosystem and therefore its response to CO₂ enrichment may be improved at elevated CO₂ by increasing symbiotic N₂ fixation (Hartwig et al. 1996) or by decreasing N losses through leaching, denitrification or herbivory. Species associated with symbiotic N₂ fixers may be particularly responsive to CO₂ fertilization because excess carbon assimilated at high CO₂ becomes available for energy-demanding symbiotic N₂ reduction. Estimated total plant carbon costs per fixed N₂ range from 3 to 19 mg C mg⁻¹ N₂ (Schulze et al. 1994) and up to one-third of the carbon fixed may be used for energy needs of N₂ reduction (Pate 1976; Ta et al. 1987). However, these costs have to be compared to the alternative needs for soil exploration and active ion uptake which also may be high in N-limited environments (Lambers 1985). Furthermore, the C expenses per unit of assimilated mineral N increase dramatically under enhanced competition for increasingly limiting soil N at elevated CO₂. Accordingly, stimulation of legume growth under elevated CO₂ has been found in a number of experiments (e.g. Wilson et al. 1933; Masterson and Sherwood 1978; Finn and Brun 1982; Murphy 1986; Overdieck and Reining 1986a, b; Norby 1987; Ta et al. 1987; Vogel and Curtis 1995; Zanetti et al. 1996). In the long term, legume stimulation together with transfer of legume N to non-N₂-fixing species via legume litter decomposition, exudation and mycorrhizal hyphal interconnections between legumes and non-legumes also increases overall plant productivity (Rao and Giller 1993; Farnham and George 1993; Seresinhe et al. 1994; Laidlaw et al. 1996). However, all these experiments were carried out under fertile, horticultural or agronomic conditions not representative for natural

plant communities where other nutrients such as P may limit legume growth. In contrast Navas et al. (1995) working with unfertilized intact mediterranean grassland sods found no CO₂ response of legumes.

The century-old nutrient-poor calcareous grasslands we used in our study are ideally suited to test effects of CO₂ enrichment on nutrient relations in systems where vegetation and soils are in quasi stable state, and where plant assemblages have been established through natural selection. Biomass production can be expected to be very sensitive to effects of CO₂ enrichment on nutrient cycling. We found an increase in community aboveground biomass of +25% after 4 years of CO₂ enrichment in the calcareous grassland system studied in the present paper. There was no effect in the first year, but the effect became stronger over time. Thus, we tested the following hypotheses:

1. The observed biomass stimulation at elevated CO₂ resulted solely from a widening of the C/N and C/P ratio, with no increase in N or P uptake per unit land area. Thus, the total amount of plant nutrients removed by the regular biomass harvest is assumed to be the same under both treatments.
2. Symbiotic N₂ fixation by legumes is enhanced at elevated CO₂ only if soil P supply is not limiting.
3. Enhanced plant-plant and plant-microbe competition for limiting soil N leads to reduced soil N availability at elevated CO₂.
4. Increased soil carbon inputs at elevated CO₂ result in a net immobilization of nutrients by soil microbes, leading to an overall increase in biotic N pools, but no change in the plant nutrient pool.

Not all the hypotheses stated above were directly testable in the field. Therefore, the field study was complemented with microcosm studies in the greenhouse as is illustrated in Fig. 1 (Stöcklin et al. 1998; J. Stöcklin and Ch. Körner, unpublished work). In these controlled environments effects of natural *versus* enhanced P supply and of presence *versus* absence of legumes could be tested. Together with data from other studies the present investigation presents an in-depth and synthetic view of the complex carbon and nutrient interactions described above.

Materials and methods

Study ecosystem and experimental design

A field experiment was carried out in an undisturbed calcareous grassland in northwest Switzerland near the village of Nenzlingen (47°33'N 7°34'E, 520 m a.s.l.; Leadley and Körner 1996). This species-rich, low productivity pasture (peak season aboveground biomass *c.* 300 g m⁻² a⁻¹) is dominated by *Bromus erectus* Huds. and has been extensively grazed by cattle for as long as records are available (Huovinen-Hufschmid and Körner 1998). The shallow topsoil (*c.* 15 cm) is a neutral to slightly basic (pH ≈ 7–8) transitional rendzina underlain by calcareous debris.

A total of 24 plots (1.27 m² area each) were selected and CO₂ treatments randomly assigned within 8 blocks. The experiment

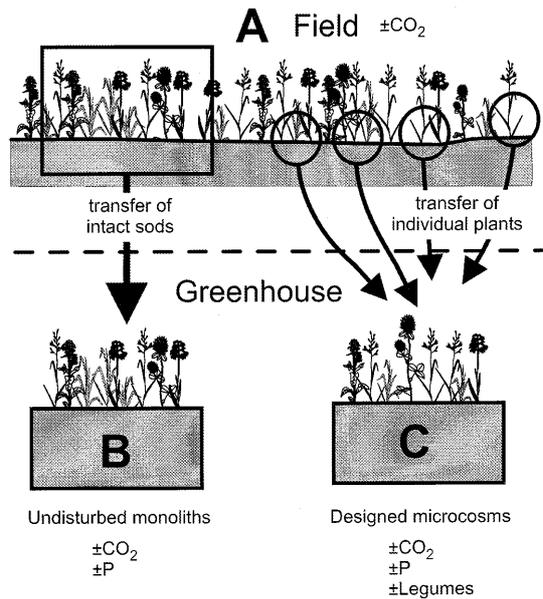


Fig. 1 Experimental design. **A** 24 plots were exposed to ambient or elevated atmospheric CO_2 in situ ($\pm\text{CO}_2$; Leadley et al. 1998). **B** 24 intact sods ("monoliths") were transferred to the greenhouse, where they were exposed to ambient or elevated CO_2 ($\pm\text{CO}_2$) alone or in combination with a low dosage phosphorus fertilization ($\pm\text{P}$; Stöcklin et al. 1998). **C** Plantlets were planted in 32 microcosms filled with field-derived sieved soil. Treatments were ambient or elevated CO_2 ($\pm\text{CO}_2$), phosphorus addition ($\pm\text{P}$) and presence or absence of legumes ($\pm\text{Legumes}$; J. Stöcklin and Ch. Körner, unpublished work)

included eight unscreened ambient air control plots (C, $356 \mu\text{l CO}_2 \text{ l}^{-1}$), eight screened ambient air plots (A, $356 \mu\text{l CO}_2 \text{ l}^{-1}$) and eight plots in which atmospheric CO_2 concentration was increased (E, $600 \mu\text{l CO}_2 \text{ l}^{-1}$) using a novel CO_2 enrichment system (SACC, screen aided CO_2 control; Leadley et al. 1997) which minimizes microclimatic impacts but generates stable CO_2 concentrations under both windy and calm conditions. The CO_2 enrichment was maintained 24 h a day and was shut down during mid winter only (December–February).

Symbiotic dinitrogen fixation

In order to quantify symbiotic N_2 fixation, the field plots received addition of mineral ^{15}N on 14 March, 29 April and 30 June 1995 equivalent to a total of $52 \text{ mg } ^{15}\text{N m}^{-2}$, which is less than 2% of the total annual atmospheric N deposition at the site (*c.* $2.5 \text{ g N m}^{-2} \text{ a}^{-1}$; B. Spycher, personal communication). Double-labelled ammonium nitrate ($^{15}\text{NH}_4^{15}\text{NO}_3$, >98% ^{15}N , Cambridge Isotopes Laboratories, Andover, Mass., USA) was dissolved in 10 l tap-water per plot and evenly sprayed over the plot. An extra 20 l of water was applied in order to minimize direct uptake of the label by leaves and to wash the label into the soil.

Plant material harvested in the field in June and October 1995 was analysed for ^{15}N abundance by isotope-ratio mass-spectrometry (Finnigan MAT delta S IRMS coupled to a Finnigan MAT CHN, Finnigan MAT, Bremen, Germany). The fraction of total legume N that originated from symbiotic N_2 fixation was calculated as

$$\%N_{\text{dfa}} = \frac{(^{15}\text{N}_{\text{legume}} - ^{15}\text{N}_{\text{non-legumes}})}{(^{15}\text{N}_{\text{non-legumes}} - ^{15}\text{N}_{\text{atmosphere}})}$$

where $^{15}\text{N}_{\text{legume}}$, $^{15}\text{N}_{\text{non-legumes}}$ and $^{15}\text{N}_{\text{atmosphere}}$ are the ^{15}N -mole fractions of the total N in legumes, all non-legume plants present in the plot and atmospheric N_2 , respectively (Wood and McNeill 1993).

Test of P-limitation of legume responses to elevated CO_2

In order to test whether legumes were important for the CO_2 response of the system and whether legume responses to CO_2 enrichment were P-limited, we combined the field work with two types of greenhouse experiments which included a P-fertilization treatment (Fig. 1). First, monoliths consisting of intact calcareous grassland sods of $26 \times 36 \times 26 \text{ cm}$ size (width \times length \times height) were placed in plastic containers and exposed to either ambient or elevated atmospheric CO_2 ($630 \mu\text{l l}^{-1}$) in daylight greenhouses (Stöcklin et al. 1998). The CO_2 treatment was factorially combined with a low dose P fertilization ($1 \text{ g P m}^{-2} \text{ a}^{-1}$ applied as triple superphosphate 40%, Landor AG, Birsfelden, Switzerland). Second, microcosms were designed which contained a legume presence *versus* absence treatment (the proportion of planted individuals was either 60:40:0 or 60:20:20 graminoids:non-legume forbs:legumes with the number of species and individuals maintained constant among treatments; J. Stöcklin and Ch. Körner, unpublished work). Plantlets were planted in sieved and homogenized soil from a similar grassland site near Nenzlingen. CO_2 and P fertilization treatments were identical to the monolith experiment.

Plant sampling

In order to maintain the natural structure and species composition all communities in the field and greenhouse were clipped at a height of 5 cm above the ground in June and October. In June, plants were sorted into functional types: graminoids, non-legume forbs and legumes. In the field, 12 legume species were present (*Anthyllis vulneraria* L., *Chamaespartium sagittale* L., *Hippocrepis comosa* L., *Lathyrus pratensis* L., *Lotus corniculatus* L., *Medicago lupulina* L., *Trifolium campestre* Schreb., *T. medium* L., *T. montanum* L., *T. ochroleucon* Huds., *T. pratense* L., *T. repens* L., *Vicia sativa* L.). In the monoliths excavated at the field site, the same species were found than in the field except *L. pratensis*, *T. campestre* and *V. sativa* which were missing. In the designed microcosms four of the legumes most abundant in the field were planted (*L. corniculatus*, *H. comosa*, *T. medium*, *T. repens*).

In the field experiment, three 6.5-cm-diameter subsamples (largely stubbles) were harvested to ground level in March and June every year to estimate total standing aboveground biomass. We also took three or four 2.5-cm-diameter soil cores to 8 cm depth in March, June and October to estimate root biomass in the top soil layer (the stone-free major rooting zone). The field study will continue through 1999, but the greenhouse experiments were terminated in October 1996 and harvested. All plant samples were dried for 24 h at 80°C before they were weighed.

Plant carbon, nitrogen and phosphorus

Plant samples were powdered and analyzed for C and N contents by dry combustion using an automated C-H-N analyzer (LECO CHN-900, LECO Corporation, St. Joseph, Mich., USA). P contents were measured by a micro-Kjeldahl type wet digestion (1:1 v/v mixture of H_2SO_4 conc. and 30% H_2O_2 at 400°C) and subsequent colorimetric determination of phosphate in the digests (Watanabe and Olsen 1965). The P contents were calculated based on KH_2PO_4 standards processed with every batch of samples.

Soil inorganic nitrogen concentrations

We used two techniques to assess soil ammonium and nitrate levels in the field. First, we directly extracted soil samples, and second, we incubated ion exchange resins in situ which permitted an integrated, though indirect, measurement of soil N availability.

Soil samples taken in June and October 1995 were extracted with 0.5 M K_2SO_4 (table shaker, 200 rpm, 30 min) and resulting soil suspensions were filtered. Ammonium in the extracts was

determined conductometrically (Wescan Model 360 Ammonia Analyzer, Alltech Associates, Deerfield, Ill., USA). Nitrate concentrations were determined by first reducing nitrate to ammonium in basic solution using a flow-through reduction cartridge filled with zinc granules and then analyzing for ammonium concentrations as described above.

Ion exchange resin bags consisted of cylindrical nylon mesh bags (100- μm mesh size) of 7 mm diameter and 7.5 cm length and were filled with either anion or cation exchange resin beads (Dowex 1X8 and Dowex 50W-X8, respectively). One resin bag of each ion exchange resin type was inserted vertically into the ground of each plot and incubated in situ from 3 May to 29 June 1995. A second set of resin bags was positioned equally but incubated from 10 July to 20 October, 1995. After retrieving the bags from the field, adhered soil was removed by washing with a small amount of distilled water and the resins were extracted with 0.5 M KCl solution for 24 h using a table shaker. Ammonium and nitrate concentrations were determined as described above for the soil extracts.

Statistical analysis

Data were analysed by analysis of variance. When necessary data were first *log*-transformed to meet the assumption of normal distribution of data and homogeneity of variances. All model terms were fitted as in type III ANOVA models in SAS (SAS Institute 1993). Factors were block, SACC treatment alone and combined with CO₂ enrichment for the field experiment. In the greenhouse experiments factors were CO₂ treatment, P fertilization, and, in the planted systems, legume presence. A priori linear contrasts were used to test for effects of elevated CO₂ within fertilizer levels. Effects with $P \leq 0.05$ are referred to as significant, effects with $P \leq 0.1$ as marginally significant. All error estimates given in the text and error bars in figures are standard errors.

Results

Community-level nutrient pools

In the first experimental season (1994), when no plant biomass response was observed, the C/N ratio in harvested aboveground biomass also did not differ significantly between ambient and elevated CO₂ (+6%, n.s.). However, C/N ratios in biomass were generally higher at elevated CO₂ in the following years (+12% in 1995, +9% in 1996 and +22% in 1997, all $P < 0.01$). Therefore, relative effects of CO₂ enrichment on total harvested N were much smaller than effects on harvested mass or were even absent (Fig. 2). Consequently, the total amount of N removed from the ecosystem by harvest during the first 4 years did not differ between CO₂ treatments (19.9 ± 1.3 at ambient and 21.1 ± 1.3 g N m⁻² at elevated CO₂). N inventories were also calculated for total aboveground biomass in March (before significant growth occurs) and in the stubble layer left after the June harvest but no significant differences between CO₂ treatments were detected (data not shown).

Total plant P in harvested aboveground vegetation remained unaltered or was slightly higher at elevated CO₂ (+7% in 1995 and +15% in 1996, n.s., Fig. 2). Consequently, plant aboveground N to P ratios were not affected by CO₂ enrichment (12.5 ± 0.2 in June 1995 and 13.7 ± 0.2 g N g⁻¹ P in June 1996).

Nutrient pools in plant functional types

In the first 3 years of treatment, nutrient pools were assessed at the level of functional types. Beginning in year 2, non-legume forb and grass C/N ratios were significantly increased at elevated CO₂ (Fig. 3). This effect was not present in legumes (which represent only 1.5% of biomass), resulting in a marginally significant func-

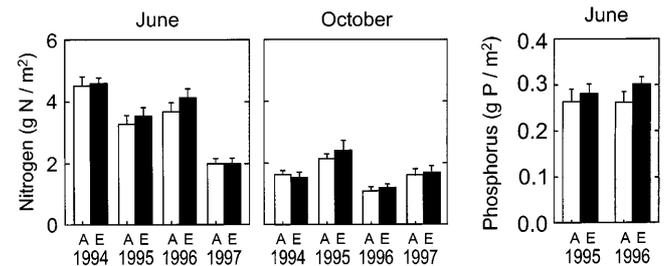


Fig. 2 Plant aboveground N and P in the field as function of CO₂ concentration and season (A ambient, E elevated CO₂). The corresponding biomass significantly increased at elevated CO₂ beginning in 1995 (community aboveground biomass stimulation of +20% to +30%, $P = 0.02$ –0.1 depending on date; see Leadley et al. 1998)

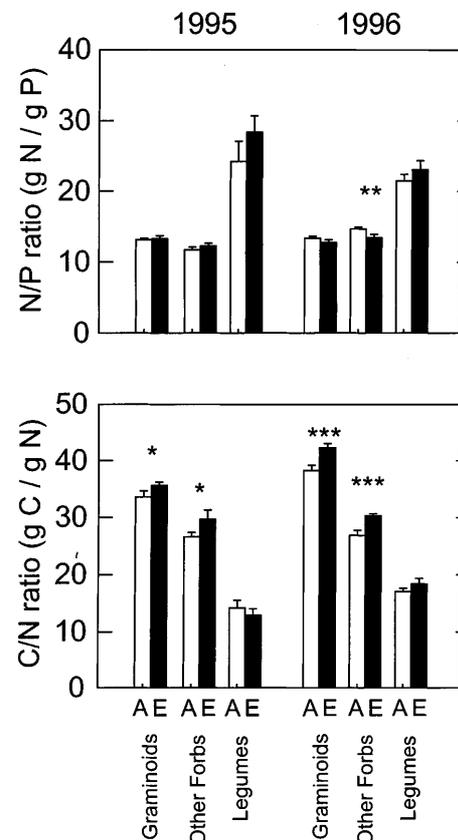


Fig. 3 Field peak biomass aboveground N:P and C:N ratios as function of plant functional type (graminoids, non-legume forbs and legumes), CO₂ treatment (A ambient, E elevated CO₂) and year. The functional group \times CO₂ interaction is significant for both C/N and N/P ($P = 0.06$ and $P = 0.07$, repeated measures analysis including both years)

tional type \times CO₂ interaction ($P = 0.056$). In contrast to N, concentration of P decreased in both legumes and non-legumes. This resulted in a marginally significant functional type \times CO₂ interaction on N:P ratios ($P = 0.07$). These observations are compatible with the hypothesis that due to their ability to fix atmospheric N₂, legume growth is P-limited while non-legumes are primarily N-limited.

Symbiotic dinitrogen fixation

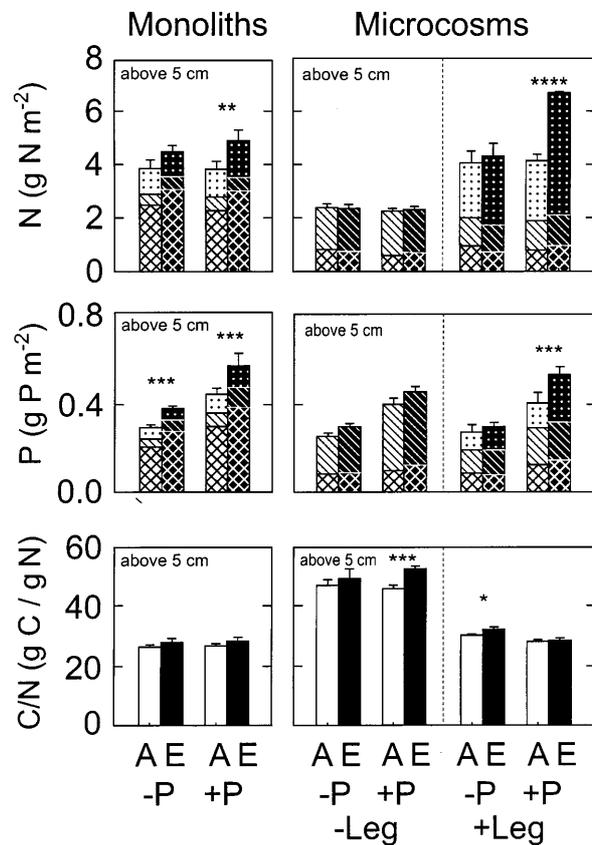
In the field experiment, the fraction of legume tissue N that derived from the atmosphere (%N_{dfa}) averaged 89% \pm 1% in June and 91% \pm 2% in October 1995 but was not influenced by elevated CO₂ (Table 1). In June 1995, %N_{dfa} was calculated per genus for the species that were present in more than half of the plots. The fraction of N that the six *Trifolium* species and *Lotus corniculatus* derived from the atmosphere was large in both genera (87.4% \pm 1.3% in *Trifolium* and 95% \pm 0.5% in *Lotus*). Hence, atmospheric N₂ was the dominant N source of legumes. Since total legume N pools were not altered at elevated CO₂, total N₂ fixed per ground area did not change after 4 years of in situ CO₂ enrichment.

P limitation and legume-effects on the communities' CO₂-response

In undisturbed monoliths, effects of elevated CO₂ depended on P-fertilization. Similarly to the field data, ecosystem aboveground N was not significantly increased at elevated CO₂ in unfertilized systems. However when P-fertilized, community aboveground N was significantly increased by CO₂ enrichment (+28%, $P = 0.03$; Fig. 4). This community-level CO₂ \times P interaction was caused by a larger increase of legume N under CO₂ enrichment when P was added (+32%, n.s., versus -3%, n.s., in unfertilized monoliths).

In the designed communities all microcosms contained the same number of species and individuals, but half of the communities did not contain any legumes. This allowed us to explore the effect of legume presence on community N pools. In the legume-free communities, N pools in plant aboveground biomass in June of the second year of treatment were nearly independent of CO₂ concentration and P-fertilization (2.26–2.37 g N m⁻²; Fig. 4), and so was biomass (229–

269 g m⁻²). Introduction of legumes strongly increased aboveground plant N inventories (+77%, $P < 0.0001$) while biomass increased by +15% only. This increase in N pools was almost exclusively due to the higher N concentration in legume tissue compared to non-legume tissue and most likely originated from symbiotic N₂ fixation. Non-legume forb N (+34% expressed per number of planted individuals, $P = 0.003$) and graminoid N (+20%, n.s.) also showed an increase at elevated CO₂ in the legume-containing communities which indicates greater N availability to non-legumes when legume N was stimulated. In the legume-containing communities, addition of P alone or CO₂ enrichment



Functional types:

□ Legumes ▨ Other forbs ▩ Graminoids

Fig. 4 Model ecosystem plant aboveground N and P as well as C:N ratios as function of plant functional type (N and P stocks only; graminoids, legumes and non-legume forbs), CO₂ treatment (A ambient, E elevated CO₂) and P fertilization (-P unfertilized; +P = 1 g P m⁻² a⁻¹) in monoliths and microcosm communities (-Leg = legume-free; +Leg = legume-containing communities). Data are for 1996, the 2nd year of treatment. In monoliths, legume biomass was not significantly affected by elevated CO₂. Non-legume biomass increased by +24% ($P = 0.003$) and +34% ($P = 0.05$) in unfertilized and fertilized monoliths (Stöcklin et al. 1998). In microcosms, elevated CO₂ stimulated legume biomass by +109% ($P = 0.01$) and non-legumes by +25% ($P = 0.04$) when P-fertilized. CO₂ had no effect in the unfertilized systems (J. Stöcklin and Ch. Körner, unpublished work)

Table 1 Percentage of legume N which originated from symbiotic N₂ fixation (%N_{dfa}) at ambient (A) and elevated (E) CO₂ treatment in field grown plants (mean \pm SE)

Treatment	June 1995	October 1995
Ambient CO ₂ (A)	90.6 \pm 1.1	92.9 \pm 1.4
Elevated CO ₂ (E)	88.6 \pm 1.4	91.0 \pm 3.6

alone did not change legume or ecosystem aboveground biomass (J. Stöcklin and Ch. Körner, unpublished work) and N pools. However, when P fertilizer and CO₂ were added in combination, community aboveground biomass and N pools increased by +64% and +61%, respectively ($P < 0.0001$ for both). This increase was mainly due to increases in the N-rich legume fraction (+103% increase in biomass and +107% increase in total N, both $P < 0.0001$; Fig. 4),

Estimates of total plant N (including roots) at the end of the experiment in October showed the same treatment effects as were found in June for aboveground biomass. Total plant N pools remain unaffected by either P-fertilization or CO₂ enrichment when legumes were absent (Fig. 5). Introduction of legumes into the communities on average increased community N by +96% ($P < 0.0001$). In the presence of legumes, effects of elevated CO₂ on total plant N were significant only when the systems were P-fertilized (+19%, $P = 0.02$). Community N was significantly increased by P fertilization at ambient (+18%, $P = 0.05$) but even more at elevated CO₂ (+26%, $P = 0.005$).

Soil inorganic nitrogen

Amounts of ammonium and nitrate extracted from field soil samples in June 1995 were reduced at elevated CO₂ by 42% and 36%, respectively, but due to large variation differences were not statistically significant (Fig. 6). The patterns found for nitrate and ammonium collected on ion exchange resins were similar to those in soil extracts, but were statistically significant for soil nitrate which was reduced by 40% in June ($P = 0.04$) and by 17% in October 1995 (n.s.). Hence, taken together, both

assays indicate a reduction in free soil nitrate under elevated CO₂, but little change in ammonium.

Nitrogen partitioning between plants and microbes

In June of the 3rd year of field CO₂ enrichment, total plant N (aboveground plus roots in the 0–8 cm soil

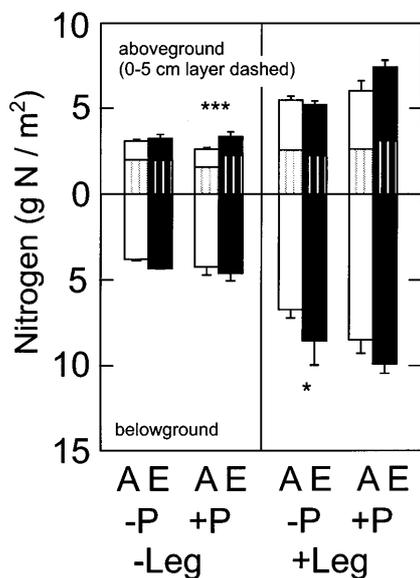


Fig. 5 Total plant N in microcosms at the final harvest in October 1996. See Fig. 4 legend for treatment abbreviations

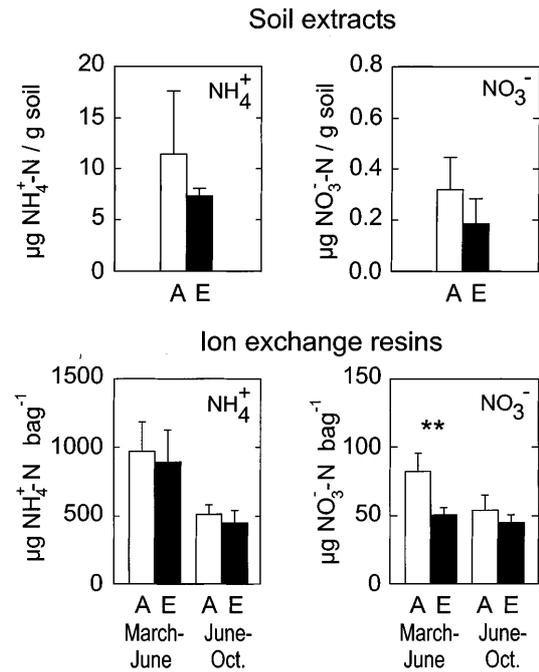


Fig. 6 Ammonium and nitrate recovered by soil extraction with 0.5 M K₂SO₄ and in situ incubation of ion exchange resin bags in the second year of CO₂ enrichment (A ambient, E elevated CO₂)

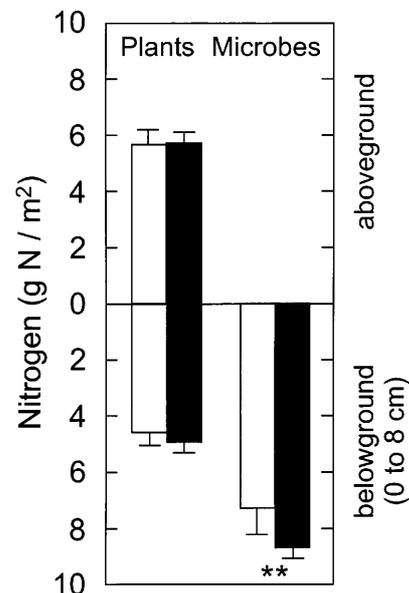


Fig. 7 Above- and belowground land-area-based plant and microbial N pools (data are for June 1996, the 3rd year of treatment)

layer) remained unaffected by elevated CO₂ (Fig. 7). In contrast, microbial biomass N pools averaged 18% higher at elevated CO₂ than under ambient conditions ($P = 0.04$; Niklaus 1998).

Discussion

The combined investigation of plants, microbes and soils in the field and in stepwise simplified model ecosystems in the greenhouse allowed us to disentangle the interactions between C, N and P relations in near natural plant assemblages exposed to elevated CO₂. For the sake of clarity, we first discuss effects of CO₂ enrichment and P supply on plant nutrient status and then address effects of elevated CO₂ on soil processes and microbes.

Plant nutrient status

N concentrations in harvested biomass were reduced at elevated CO₂. Similar effects have been reported in other natural ecosystems (prairie: Owensby et al. 1993; mediterranean grassland: Körner and Miglietta 1994; alpine grassland: Schächli and Körner 1997; salt marsh: Curtis et al. 1989). This appears to be an universal response of plants to elevated CO₂. In situ, RuBP carboxylase contents of leaves from the dominant grass *Bromus erectus* were found to be reduced by 45% on a leaf area basis (Sage et al. 1997) and the reduction was even larger on a leaf dry weight basis. This, plus dilution of nutrients by structural and non-structural carbohydrates at elevated CO₂ (Körner 1995a) explain the observed overall decrease in N concentrations. As a consequence, total amounts of N harvested in the field at ambient and elevated CO₂ did not significantly differ despite significant differences in biomass (hypothesis 1). Thus, the increase in productivity at elevated CO₂ was solely achieved by nutrient dilution, and the management did not impinge differently on systems under contrasting CO₂ regimes because equal amounts of N and P were withdrawn from the ecosystem by mowing (unlike what was found in fertile systems by Overdieck and Reining 1986a,b). The dilution effect by mobile carbohydrates also explains the reduction in tissue P concentrations observed under CO₂ enrichment. Reduced tissue quality in terms of N and P contents and the increased carbohydrate levels at elevated CO₂ will have implications for herbivores (see the review by Lincoln et al. 1993).

The functional type specific widening of C/N ratio in the field indicates that forage quality of legume and non-legume tissue was affected differently by CO₂ enrichment. We attribute this to the fact that legume growth is not N-limited at elevated CO₂ because of symbiotical N₂ fixation. Despite deriving virtually all their N from the atmosphere, legumes did not introduce significant amounts of N into the ecosystem because they account for only a small fraction of community aboveground

biomass in the field ($\approx 1.5\%$). The greenhouse experiments with monoliths and designed microcosms demonstrated that this situation changes as soon as P supply is enhanced together with CO₂. The observed strong stimulation of legume growth and N pools when P-fertilizer and elevated CO₂ were applied in combination suggests that legume growth and N₂ fixation are co-limited by P-supply and carbohydrate availability (hypotheses 2). Given sufficient P-supply, large additional amounts of N are symbiotically fixed by legumes at elevated CO₂ which will eventually become available to non-legumes (see Introduction). In the longer term, this transfer of N to non-legumes may lead to a new competitive equilibrium between legumes and non-legumes which is co-determined by the amount of available N, P and C-assimilates to plants. Low P is most likely also the reason for the obvious discrepancy among effects of CO₂ enrichment on legume growth reported in the literature (see Introduction).

At our field site, an ongoing fertilizer trial so far revealed that productivity is primarily N limited, which is a common observation in calcareous grasslands (Smith et al. 1971; Willems et al. 1993) although sometimes a co-limitation by N and P is found (Jeffrey and Pigott 1973; Morecroft et al. 1994). Addition of mineral N immediately stimulated non-legume aboveground biomass (which is >98% of the community aboveground biomass), while the addition of mineral P lead to a significant and selective increase in legume biomass by year 2 (M. Ryf, personal communication). This finding in the field corresponds to the results of our greenhouse experiments which also showed that enhanced P supply – at least when combined with increased atmospheric CO₂ concentrations – lead to a significant gain in legume abundance.

Taken together, our results suggest a complex interaction between atmospheric CO₂ concentration, N and P supply. Ecosystem productivity is N-limited, while the rate of symbiotic N₂ fixation by legumes is controlled by P availability. Therefore, legumes are the primary beneficiaries of P fertilization and their competitive advantage becomes enhanced at increased atmospheric CO₂ concentrations.

Partitioning of nutrients within the plant-soil-microbe system

The reduction in soil inorganic N concentrations at elevated CO₂ may have been caused by increased soil exploration by plants and their mycorrhizal partners and by increased net microbial N immobilization (hypotheses 3). The reduction in nitrate suggests potentially reduced N losses through leaching, but predictions are complicated by the fact that soil moisture is generally higher at elevated CO₂ and therefore run-off may also be enhanced (Niklaus and Ch. Körner, unpublished work). Nevertheless, reduced N losses could lead to a slow but sustained increase of ecosystem N at elevated CO₂.

Microbial N pools did increase at elevated CO₂ (hypothesis 4; Niklaus 1998). This was possibly facilitated by the substantial seasonal net carbon fluxes to the soil at elevated CO₂ (R. Stocker, unpublished work) and is in line with other studies which report that in N-limited environments carbohydrate amendment primarily increases microbial N immobilization, whereas N mineralization is stimulated by addition of mineral N (calcareous grassland: Morecroft et al. 1994; temperate perennial grassland: Jonasson et al. 1996; arctic tundra: Marion et al. 1982; Shaver and Chapin 1980; silviculture: Turner and Olson 1976). However, increased microbial N immobilization alone should reduce plant N pools, which was not observed in the present study. Therefore, we speculate that increased mineralization of soil organic matter or increased N retention of the ecosystem at elevated CO₂ account for the increased total biotic N pool (no change in plants but an increase in microbial N).

Conclusions

Our study indicates that increased aboveground biomass production in this grassland at elevated CO₂ is solely achieved by nutrient dilution. Increased plant biomass and increased net microbial N immobilization at elevated CO₂ appear to have increased competition for soil N, and thus reduced soil inorganic N concentrations. Tissue forage quality in terms of N and P concentrations was reduced at elevated CO₂ and this effect was smaller in legumes than in non-legumes because legumes were less affected by increasingly scarce soil N. However, because of P-limitation of symbiotic N₂ fixation and the growth of legumes, whole community productivity was not stimulated by increased symbiotic N₂ fixation, which is in contrast to earlier findings in more fertile and thus non-P-limited systems.

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