Soil mineral nitrogen availability was unaffected by elevated atmospheric pCO$_2$ in a four year old field experiment (Swiss FACE)

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Abstract

The effect of elevated (60 Pa) atmospheric carbon dioxide partial pressure (p$_{CO2}$) and N fertilisation on the availability of mineral N and on N transformation in the soil of a *Lolium perenne* L. monoculture was investigated in the Swiss FACE (*Free Air Carbon dioxide Enrichment*) experiment. The apparent availability of nitrate and ammonium for plants was estimated during a representative, vegetative re-growth period at weekly intervals from the sorption of the minerals to mixed-bed ion-exchange resin bags at a soil depth of 5 cm. N mineralisation was measured using sequential coring and *in situ* exposure of soil cores in the top 10 cm of the soil before and after the first cut in spring 1997. High amounts of mineral N were bound to the ion exchange resin during the first week of re-growth. This was probably the combined result of the fertiliser application, the weak demand for N by the newly cut sward and presumably high rates of root decay and exudation after cutting the sward. During the first 2 weeks after the application of fertiliser N at the first cut, there was a dramatic reduction in available N; N remained low during the subsequent weeks of re-growth in all treatments. Overall, nitrate was the predominant form of mineral N that bound to the resin for the duration of the experiment. Apparently, there was always more nitrate than ammonium available to the plants in the high N fertilisation treatment for the whole re-growth period. Apparent N availability was affected significantly by elevated p$_{CO2}$ only in the first week after the cut; under high N fertilisation, elevated p$_{CO2}$ increased the amount of mineral N that was apparently available to the plants. Elevated p$_{CO2}$ did not affect apparent net transformation of N, loss of N or uptake of N by plants. The present data are consistent with earlier results and suggest that the amount of N available to plants from soil resources does not generally increase under elevated atmospheric p$_{CO2}$. Thus, a possible limiting effect of N on primary production could become more stringent under elevated atmospheric p$_{CO2}$ as the demand of the plant for N increases.

Introduction

It is widely accepted that elevated atmospheric partial pressure of CO$_2$ (p$_{CO2}$) stimulates plant growth. The extent of the growth response to elevated atmospheric p$_{CO2}$ however, depends on the availability of other resources required for plant growth, namely light, water and mineral nutrients. In fertile soils, nitrogen often limits plant growth in natural and managed grassland.

Therefore, when plants grow under elevated p$_{CO2}$, the availability of mineral N may be the key factor which controls the plants' response to elevated atmospheric p$_{CO2}$.

Several experiments showed that *Lolium perenne* above-ground biomass does not respond as strongly to CO$_2$ as was expected, even under high N fertilisation (Hebeisen et al., 1997a, b; Lüscher et al., 1996, 1998; Soussana et al., 1996). There is substantial evidence showing that insufficient N nutrition prevents a stronger response to CO$_2$ with respect to above-ground biomass (Fischer et al., 1997; Isopp et

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al., 2000; Lüscher et al., 2000; Soussana and Hartwig, 1996; Soussana et al., 1996; Zanetti and Hartwig, 1997; Zanetti et al., 1996, 1997).

The amount of N available to plants depends on N cycling, i.e. on the rate of N uptake by plants and soil organisms, on the rate of release of inorganic N due to mineralisation processes as well as on the amount of N lost from the system (leaching, denitrification, volatilisation) and on N fertilisation. The effect of elevated atmospheric p_{CO2} on the availability of soil N is under debate: Some researchers (Zak et al., 1993) suggest an increased mineralisation, others a decreased availability of N due to a higher rate of N immobilisation (Diaz et al., 1993; Hartwig et al., 1996). Hungate et al. (1997) and Ineson et al. (1998) presented results supporting the view that elevated p_{CO2} may alter N cycling in terrestrial ecosystems. Such changes could indeed change the amount of N available to plants.

The form of available N is also important for plant growth, because the rate of uptake and assimilation of ammonium (NH_4^+) and nitrate (NO_3^-) is not usually the same for all plants (Pilbeam and Kirkby, 1992; Ulrich, 1992); grown in the exclusive presence of either NH_4^+ or NO_3^- have different morphology and chemistry, both of which may affect the plants' response to elevated p_{CO2}.

In order to investigate possible effects of elevated atmospheric p_{CO2} on components of N cycling and on the apparent availability of mineral N to plants, we measured the following in the soil below a *Lolium perenne* sward under ambient and elevated p_{CO2}: (i) the availability of NO_3^- and NH_4^+ during one representative, vegetative re-growth period and (ii) the rates of apparent N uptake by the sward as well as N mineralisation, N immobilisation nd N losses before and after the cut. The results allow the discussion of contrasting hpotheses (Díaz et al., 1993; Hartwig et al., 1996; Zak et al., 1993) about CO_2 effects on availability of soil mineral N.

Materials and methods

Experimental site, growing conditions and N fertilisation

The experimental site is located at Eschikon (8° 41′ E, 47° 12′ N), 20 km north-east of Zürich at an altitude of 550 m above sea level. Climatic data were provided by a meteorological station close to the experimental site. The soil was classified as a fertile, eutric cambisol (FAO, 19-) with pH values (in water of the (0... cm) topsoil? between 6.5 and 7.6, containing 28% clay, 33% silt and 36% sand and with a high availability of P and K. The organic matter content was 2.9 and 5.1%.

Plots (2.8 × 1.9 m) were arranged in three blocks. Since May 1993, monoculture swards of *Lolium perenne* L. were grown at two levels of p_{CO2} (35 Pa, ambient and 60 Pa, elevated) in the field using the *Free Air Carbon dioxide Enrichment* (FACE) technology (Lewin et al., 1994) in the Swiss FACE experiment (Daepp et al., 2000; Hebeisen et al., 1997a, b; Lüscher et al., 1998; Zanetti et al., 1996). Since 1996, the swards have been cut five times per year at 5 cm above ground level. N fertiliser was applied as dissolved N_HNO_3 (14 g N m^{-2} per year for the low level of fertilisation and 56 g N m^{-2} per year for the high level of fertilisation). The fertiliser was applied according to the principle used in Zanetti et al. (1996): 2.8 g N m^{-2} for the low level of fertilisation and 11.2 g N m^{-2} for the high level of fertilisation at the particular cut in May 1997.

The experimental plots in the present study were feflillsed at the beginning of the growing season (April 4th 1997) and 2 days after the first cut (May 13th, 1997).

Ion exchange resin bag method (in situ)

Ion exchange resin (IER) was used to measure apparent N availability during one re-growth of *L. perenne* (weekly from May 15th until June 16th, 1997). IER bags, buried in the soil accumulate ions by exchange processes (Binkley and Matson, 1983; Gibson et al., 1985). The extent of accumulation of NO_3^- and NH_4^+ enables to estimate relative effects of experimental treatments on mineral N pools that have been mineralised but not immediately taken up by plant roots (Binkley et al., 1986). The IER bags were prepared by placing 12 mL of wet mixed-bed resin (Amberlit IRN-150, Sigma Chemical Co., St. Luis, MO, USA, 1:1 mixture of cation and anion exchanger with a total ion exchange capacity of 0.55 meq mL^{-1}) in nylon mesh bags (6.5 × 60 HC, mesh size 42 μm, 30% openings). During the first week, the IER bags were buried 2 days after the cut and a few hours after the N fertilisation.

The soil within a grass tussock was slit with a trowel, and an IER bag was placed horizontally at a depth of about 5 cm. Five resin bags were buried in each plot and exposed for 1 week. After each weekly

exposure, all five bags were removed (and replaced by five fresh bags) and washed with de-ionised water, taking care to remove soil particles that adhered to them, and frozen until extraction and determination.

Method of sequential soil coring

N transformation was investigated in the soil of the *L. perenne* swards for two time periods (from April 21st to May 11th 1997 and from May 15th to June 12th, 1997) before and after the cutting of shoot material 5 cm above ground (May 13th). Rates of total N mineralisation, nitrification and ammonification as well as losses of NO_3^- by leaching and rates of apparent N uptake by plants were estimated by sequential soil coring and by *in situ* exposure of undisturbed soil cores in PVC tubes (2.6 cm in diameter; see also Berendse et al., 1994; Jacot et al., 2000; Raison, 1987). N transformation was calculated from changes in the mineral N content in the tubes relative to changes in the bare soil (see below). Three sets of soil cores with four cores in each set (total 12 cores) were taken from each plot at the beginning of each of the two exposure periods. One soil core in each set (total 3 cores) was taken at the beginning and was extracted within 24 h (initial value of mineral N). Two cores in each set were taken and put back immediately into the plot again and exposed in the PVC tubes in the top 10 cm of the soil. One of these two tubes of each set was capped on both sides to prevent any flow of the soil solution; the tubes were aerated through four holes in the side walls of the tube just above ground level. The second of these two tubes in each set was open at the top but was covered with a nylon mesh (42 μm mesh size, 30% openings) at the bottom to prevent in-growth of roots and at the same time to allow water to flow to and from the tube. The fourth core of each set of soil cores was taken at the end of each exposure period. This procedure resulted in a total of 12 samples per plot and measurement period.

Determination of NO_3^- and NH_4^+

The resin from the five bags per plot was pooled and placed in a 20 mL extraction column fitted with stopcock and extracted with 100 mL of 2 M NaCI in 0.1 M HCl (Giblin et al., 1994). The content of NH_4^+ and NO_3^- ions in the soil samples was determined within 24 h after collection. Soil samples (20 g fresh weight) were shaken for 1 h with 50 mL of 1 M KCl, and the extract was filtered through a No. 589[2] ashless filter paper (Schleicher and Schuell, Dassel, Germany).

Concentration of NO_3^- and NH_4^+ in both soil and resin extracts was determined colorimetrically with a segment flow analyser (Aliance Instruments, USA). Soil was dried at 60 °C for 48 h.

Calculations

The rates of net mineralisation (N_{min}), net ammonification (N_{nit}) and net ammonification (N_{amm}) were calculated from the difference between the concentrations of the respective N forms in the soil of the initial soil samples ($N_{b(t0)}$) and from the soil samples exposed in the closed tubes ($N_{e(t1)c}$) during the period of exposure Δt ($t0$ = start of exposure; $t1$ = end of exposure; b = bare soil; e = exposed in tubes; c = closed tube):

i) $N_{nit} = (NO_3^-{}_{e(t1)c} - NO_3^-{}_{b(t0)})/\Delta t$
(mg $NO_3^- - N$ kg soil^{-1} DW d^{-1})

ii) $N_{amm} = (NH_4^+{}_{e(t1)c} - NH_4^+{}_{b(t0)})/\Delta t$
(mg $NH_4^+ - N$ kg soil^{-1} DW d^{-1})

iii) $N_{min} = N_{nit} + N_{amm}$
(mg N kg soil^{-1} DW d^{-1})

Rates of mineral N losses (N_L) through leaching from the soil were calculated by comparing the concentration of mineral N in the closed ($N_{e(t1)c}$) and open ($N_{e(t1)o}$) tubes at the end of the exposure period for the time period Δt. The mineral N deficit in the soil cores exposed in open tubes represents an upper limit for leaching losses of mineral N because the roots inside the cores did not take up water and mineral N (Raison et al., 1987); the potential loss of N as gas was not considered (o = open tube):

iv) $N_L = (N_{min(c)} - N_{min(o)})/\Delta t$
(mg N kg soil^{-1} DW d^{-1})

The rate of apparent mineral N uptake by plants (N_u was calculated from the difference between the mineral N concentration in the soil samples exposed in the open tubes without any plant roots ($N_{e(t1)o}$) and in the samples in bare soil ($N_{b(t1)}$) at the end of the exposure period. This calculation is based on the assumption that the net rates of N mineralisation were the same in the soil inand around the tubes:

v) $N_U = (N_{e(t1)o} - N_{b(t1)})/\Delta t$
(mg N kg soil^{-1} DW d^{-1})

Table 1. Harvested biomass and N concentration of the 1st and 2nd cut of *Lolium perenne* monoculture as affected by atmospheric pCO_2 and N fertilisation. Means of 3 replicates and SE are shown

		1st cut		2nd cut	
		DM	[N]	DM	[N]
		(g m^{-2})	(mg g^{-1})	(g m^{-2})	(mg g^{-1})
35 Pa CO$_2$	low N	181	20.1	300	13.7
	high N	385	39.3	529	25.8
60 Pa CO$_2$	low N	138	16.7	351	10.2
	high N	429	30.0	652	17.7
SE		*23*	*1.0*	*20*	*1.1*

Statistical evaluation

The experimental design was a split-plot (three replicates) with p_{CO2} as the main plot factor and N supply as the subplot factor. Statistical analyses were carried out using the GLM (general linear model) procedure of the SAS statistical analysis package (SAS Institute, Cary, NC, USA). Normal distribution of the data and their homogeneity of variance were checked.

Results

Biomass and N yield L. perenne *as affected by atmospheric* p_{CO2} *and N fertilisation at the first two cuts in 1997*

When analysed over the two harvests, high N fertilisation increased *L. perenne* yields strongly (ANOVA N $p < 0.0001$) and elevated atmospheric p_{CO2} tended to increase DM yield (ANOVA p_{CO2} p=0.067) (Table 1). A positive yield response, however, was measured only in the high N treatment (ANOVA $p_{CO2} \times$ N $p < 0.05$). Likewise, N concentrations in the harvested tissue were strongly increased by high N fertilisation (ANOVA N $p < 0.0001$), elevated atmospheric p_{CO2} led to a significant decrease in N concentration (ANOVA p_{CO2} $p < 0.001$). As a result, under both N fertilisation treatments, less N was harvested under elevated atmospheric p_{CO2} compared to ambient conditions (ANOVA p_{CO2} $p < 0.05$).

Apparent N availability

No overall effect of p_{CO2} on the accumulation of NO$_3^-$ ions in IER was found (Table 2, Figure 1).
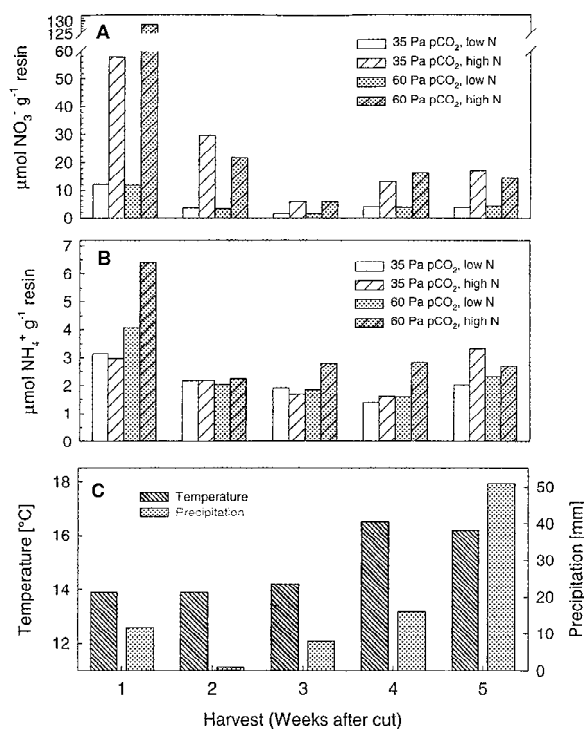

Figure 1. Accumulation of nitrate (A) and ammonium (B) ions in ion exchange resin (IER) bags and mean air temperature and sum of precipitation (C) during one re-growth period of a *Lolium perenne* sward in weekly intervals. The measurements were made from May 15th to June 16th, 1997; the sward was cut on May 13th. Means of three replicates are shown; for statistics see Table 2.

Apparent NO$_3^-$ availability, however, changed with time after the application of N fertiliser at cutting and was strongly affected by the amount of N fertiliser applied (Table 2, Figure 1). The largest amount of accumulated NO$_3^-$ was found during the first week of re-growth in the plots with high N fertilisation and exposed to elevated atmospheric p_{CO2}. Thereafter, no effect of elevated atmospheric p_{CO2} on the apparent NO$_3^-$ availability was found.

Significantly more NO$_3^-$ was apparently available in plots with high N fertilisation is compared to plots with low N for the whole re-growth period (Figure 1, Table 2). The time course for NH$_4^+$ ions to adsorb in the IER bags was about the same as for NO$_3^-$ (Figure 1). There were 10–20 times fewer NH$_4^+$ ions, however, than there were NO$_3^-$ ions. The highest values for NH$_4^+$ accumulation in the IER bags were found during the first week after cutting in the plots with high N fertilisation under elevated p_{CO2}. Elevated atmospheric p_{CO2} also increased NH$_4^+$ accumulation

Table 2. Summary of analyses of variance of accumulation of nitrate and ammonium in ion exchange resin bags during a vegetative re-growth period of a *Lolium perenne* sward. Significance of impact of CO_2 partial pressure (p_{CO2}), nitrogen fertilisation (N) and time (weeks) which elapsed after the sward was cut and fertilised (sampling), and interactions of these factors over the whole experimental period (A) and for each sampling (B). Significance: (*) $p \leq 0.05$; (**) $p \leq 0.01$; (***) $p \leq 0.001$; (****) $p \leq 0.01$; n.s. not significant

A

Source		Main factors			Interactions		
N-form	p_{CO2}	N	Sampling	$p_{CO2} \times N$	$p_{CO2} \times$ Sampling	$N \times$ Sampling	$p_{CO2} \times N \times$ Sampling
NO_3^-	n.s.	****	****	n.s.	n.s.	n.s.	n.s.
NH_4^+	n.s.	n.s.	*	n.s.	*	n.s.	n.s.

B

N-form	Source	Sampling				
		1	2	3	4	5
NO_3^-	p_{CO2}	*	n.s.	n.s.	n.s.	n.s.
	N	**	**	**	**	**
	$p_{CO2} \times N$	*	n.s.	n.s.	n.s.	n.s.
NH_4^+	p_{CO2}	**	n.s.	n.s.	**	n.s.
	N	n.s.	n.s.	n.s.	*	n.s.
	$p_{CO2} \times N$	n.s.	n.s.	**	n.s.	**

in the resin bags in the third and fourth weeks under high N fertilisation.

Concentrations of NH_4^+ and of NO_3^- in the soil cores taken to measure N transformation 20 days before the cut, at the cut and 27 days after the cut were not affected by atmospheric p_{CO2} (data not shown) but did confirm the very strong effect of N fertilisation on NO_3^- and NH_4^+ availability (ANOVA, $p<0.0001$).

N transformation

No significant effect of elevated atmospheric p_{CO2} on apparent N uptake and N transformation was found in the periods monitored (Tables 3 and 4). Although mineralisation tended to be slightly lower under elevated atmospheric p_{CO2} during the 20 days before the cut (Table 3) and slightly higher during the 27 days after the cut (Table 4). There was a significant effect of N fertilisation on N transformation, mainly on nitrification, before and after the cut (Tables 3 and 4). A higher rate of net nitrification but also a higher rate of immobilisation of NH_4^+ ions (indicative from negat-

Table 3. Rates of net nitrification (N_{nit}), net ammonification (N_{amm}), net mineralisation (N_{min}), uptake rate (N_u) and losses (N_L) of mineral nitrogen in the soil of a *Lolium perenne* sward (mg N kg soil^{-1} d^{-1}) during the 20 days before the first cut. Summary of the analyses of variance is given in the lower part of the table. Significance: (*) $p \leq 0.05$; (**) $p \leq 0.01$; (***) $p \leq 0.001$; n.s. not significant

Treatment		N_{nit}	N_{amm}	N_{min}	N_u	N_L
35 Pa P_{CO2}	Low N	0.42	0.90	1.32	1.32	−0.28
	High N	1.83	0.93	2.77	2.49	0.38
60 Pa p_{CO2}	Low N	0.19	0.83	1.01	1.17	−0.41
	High N	1.34	0.94	2.28	2.11	0.16
Standard error		*0.32*	*0.10*	*0.33*	*0.24*	*0.37*
Sources of variance						
P_{CO2}		*	n.s.	n.s.	n.s.	n.s.
N		***	n.s.	***	***	n.s.
$P_{CO2} \times N$		n.s.	n.s.	n.s.	n.s.	n.s.

Treatment		N$_{nit}$	N$_{amm}$	N$_{min}$	N$_u$	N$_L$
35 Pa PCO$_2$	Low N	0.52	0.12	0.64	0.74	0.29
	High N	1.19	−0.95	0.24	3.00	0.05
60 Pa pCO$_2$	Low N	0.44	0.41	0.85	0.35	0.55
	High N	1.92	−0.81	1.11	2.81	1.40
Standard error		*0.49*	*0.25*	*0.61*	*0.49*	*0.62*
Source of variance						
pCO$_2$		n.s.	n.s.	n.s.	n.s.	n.s.
N		*	****	n.s.	****	n.s.
pCO$_2$ × N		n.s.	n.s.	n.s.	n.s.	n.s.

ive ammonification; Table 4) were found in plots with high N fertilisation after the cut (Table 4).

Apparent N uptake by plants was increased at high N fertilisation during both time periods (Tables 3 and 4). The losses of N from soil cores was the same under atmospheric p$_{CO2}$ and under both levels of N fertilisation (Table 4).

Discussion

Is availability of mineral N in the soil under a grass sward affected by elevated atmospheric p$_{C02}$ There are many indications that N can limit growth of non-leguminous plants under elevated atmospheric p$_{CO2}$ (e.g. Fischer et al., 1997; Isopp et al., 2000; Lüscher et al., 2000; Soussana et al., 1996; Zanetti et al., 1997; Table 1). Thus, it is assumed that the cycling of N in grassland ecosystems changes under these conditions, especially during the first years after atmospheric p$_{CO2}$ was increased experimentally. There are various hypotheses about the effect of elevated atmospheric p$_{CO2}$ on components of the N cycle in the soil, one of which is that an increased C:N ratio of litter from plants grown under elevated atmospheric p$_{CO2}$, inhibits decomposition and, thus N mineralisation. However, this does not seem to be generally true; Franck et al. (1997) showed that the effect of the C:N ratio in litter on the rates of decomposition is very species-

specific. Moreover, Hartwig et al. (2000) showed that the C:N ratio of plant litter most likely changes only in legume-less grass monocultures. Others hypothesis that elevated atmospheric p$_{CO2}$ affects the amount of N available to plants by causing a rise in the transfer of labile C from the roots to the soil (Hart et al., 1994); thus, the large amounts of labile C would have a positive effect on microbial activity. This could lead either to a higher rate of decomposition of litter and soil organic mailer, including N mineralisation (Zak et al., 1993) or to an increasing immobilisation of N by the microbial biomass, thus restricting the amount of N available to the plants (Díaz et al., 1993; Hartwig et al., 1996).

Neither one of these hypotheses, which are to some extent contradictory, were confirmed by the present study. The absolute availability of mineral N in the soil seemed to be largely unaffected by elevated atmospheric p$_{CO2}$ which corresponds to results obtained by Ross et al. (1996) from an other system. We did not observe a significant CO$_2$ effect on N availability as estimated from IER adsorption (Figure 1). The same was true for N transformation (Tables 3 and 4). From these results we can conclude that the availability of mineral N for plants was largely unaffected by elevated atmospheric p$_{CO2}$. However, considering that more N was bound to stubble and roots under elevated atmospheric p$_{CO2}$ (Hartwig et al., 2000; Jongen et al., 1995; Soussana et al., 1996), then one can understand that less N was harvested under elevated atmospheric p$_{CO2}$ compared to ambient conditions (Table 1).

Which factors affect the apparent availability of mineral N in the soil?

Fluxes of N within the ecosystem can be substantially altered by N fertilisation. Indeed, we found significantly increased rates of nitrification under high N fertilisation compared to low N fertilisation. The high rate of N uptake (N$_U$) relative to N$_{min}$ in the period after the cut under high N fertilisation reflects the abundantly available N; in contrast, under low N fertilisation, N$_U$ plus N$_L$ are similar to N$_{min}$ (Table 4).

An increased soil moisture, resulting from reduced plant transpiration under elevated p$_{CO2}$, has been shown to increase the rate of mineralisation in water limited systems (Hungate et al., 1997). On the other hand, an increased rate of denitrification (Ineson et al., 1998) could also indicate an increased rate of mineralisation. A significant increase in soil moisture under elevated atmospheric p$_{CO2}$ however, was not

found during the experimental period in the present experiment (Hebeisen et al., unpublished data).

The high amount of mineral N found in the first week of re-growth, particularly under elevated atmospheric p_{CO2} and high N fertilisation, was probably the result of several, possibly cumulating, factors: (i) N fertiliser was recently applied, (ii) the decomposition of fine roots after the cut (Paterson and Sim, 1999) may be particularly high in the elevated CO_2 plots since the root biomass is known to increase strongly as compared to the ambient CO_2 plots (Hartwig et al., 2000; Hebeisen et al. 1997a, b; Jongen et al., 1995). Indeed, Gorissen et al. (1995) found a higher rate of decomposition of root litter of *L. perenne* plants which were previously grown under elevated atmospheric p_{CO2}. However, this effect lasted only during the first 2 days, after 8 days, the rate of decomposition slowed down strongly. (iii) The demand of the sward for N during the first week after the cut is low (Hartwig et al, 1994; Ourry et al., 1990) since the growth rate of plants is reduced after cutting. All these effects possibly cumulated in the first week after the cut.

The high accumulation of NO_3^- in IER bags under elevated atmospheric p_{CO2} indicates either higher rate of nitrification or a lower uptake rate of nitrate by plants. Jackson and Reynolds (1996) suggested that the uptake of NO_3^- ions is down-regulated under elevated atmospheric p_{CO2} but the uptake of NH_4^+ was unaffected by the CO_2 level. In the present experiment, the first hypothesis appears to be more likely, since we did not observe any effect of elevated atmospheric p_{CO2} on the apparent N uptake by plants using the soil core method (Tables 3 and 4). It is also possible that the availability of NH_4^+ measured by the resin bag method is underestimated because of the lower mobility of this ion compared to NO_3^-.

The increased availability of NO_3^- and NH_4^+ during the first week after the cut under elevated atmospheric p_{CO2} contrasts the observed annual yield responses of *L. perenne* to elevated atmospheric p_{CO2} (Fischer et al., 1997; Hebeisen et al., 1997a, b; Lüscher et al., 1998; Zanetti et al., 1997). However, since the absolute net rate of primary production usually is very low during the first days after a cut, therefore also the N demand, such increased N availability may not be relevant for primary production; thus, it can not bring about a strong CO_2 response with respect to biomass. However, this high availability of particularly nitrate could explain the increased tillering which was observed in *L. perenne* (Suter and Lüscher, unpublished data). In subsequent weeks,

when the absolute growth rate usually is high and, thus, ample available mineral N could support a strong CO_2 response, mineral N availability was low in all treatments, independent of atmospheric p_{CO2}.

Our results are consistent with the finding that under elevated atmospheric p_{CO2}, the uptake of mineral N from the soil by *Lolium perenne* apparently does not increase, although the N demand by the plants was presumably higher (Fischer et al., 1997; Isopp et al., 2000; Zanetti et al., 1997). Indeed, earlier studies showed that the percentage of N derived from symbiotic N_2 fixation – a very sensitive way of assessing the availability of mineral N in fertile grassland ecosystems (Hartwig, 1998) – in white clover increased at elevated p_{CO2} (Lüscher et al., 2000; Soussana and Hartwig, 1996; Zanetti and Hartwig, 1997; Zanetti et al., 1996). This did not occur as a result of elevated atmospheric p_{CO2} *per se* (Almeida et al., 2000; Hartwig, 1998; Schortemeyer et al., 1999; Zanetti et al., 1998) but is thought to be due to a relatively reduced availability of mineral N. In the present study, availability of soil N for plant growth and N uptake by plants did not change as a direct consequence of elevated atmospheric p_{CO2} thus we conclude that any limiting effect of mineral N for plant primary production becomes more stringent under elevated atmospheric p_{CO2} in plants which depend solely on mineral N.

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