

Does the response of perennial ryegrass to elevated CO₂ concentration depend on the form of the supplied nitrogen?

V. GLOSER*, M. FREHNER, A. LÜSCHER, J. NÖSBERGER and U.A. HARTWIG

Institute of Plant Sciences, ETH-Zurich, CH-8092 Zurich, Switzerland

Abstract

To test whether different nitrogen form (nitrate or ammonium) in substrate can alter the response to elevated partial pressure of CO₂ (pCO₂) plants of perennial ryegrass (*Lolium perenne* cv. Bastion) were grown from seeds in growth chambers under pCO₂ of either 35 Pa (ambient, CA) or 70 Pa (elevated, CE) in a hydroponic system (with nutrient and pH control) for 24 d. Nitrogen was supplied as ammonium, nitrate or an equimolar mixture of both N forms. Under CE plants grew faster than their counterparts under CA during the first 14 d but after 23 d of cultivation stimulation disappeared. Despite the strong positive effect of mixed forms of N on plant growth, the beneficial effect of CE was similar to that in the other two N treatments. However, the almost alike final growth response to CE had different underlying mechanisms in different N treatments. Plants supplied with nitrate as a sole source of nitrogen had lower leaf mass ratio but much higher specific leaf area compared to plants supplied with ammonium. The decrease in the content of leaf organic N (per unit of structural dry mass) under CE was found only in leaves of plants supplied with ammonium on day 14. Nevertheless, the available form of N evidently contributes to changes of leaf N content under CE. The high levels of N and non-structural saccharides in plants supplied with ammonium at CE suggest that the CO₂-response of these plants was controlled by factors other than amount of available carbon and nitrogen.

Additional key words: ammonium, *Lolium perenne*, nitrate, nitrogen source.

Introduction

An increase in the partial pressure of atmospheric CO₂ (pCO₂) usually results in enhanced photosynthesis and subsequently, in an increased production of plant biomass (Bazzaz 1990, Bowes 1993). The growth response of plants to elevated pCO₂ under natural conditions, however, may be significantly influenced by other environmental factors (Bowes 1993).

The growth rate of plants is often positively correlated with the content of nitrogen in the tissues (Ingestad 1979, Hirose 1988, Garnier and Vancaeyzeele 1994). In plants grown at elevated atmospheric pCO₂ (CE), the nitrogen content is usually significantly lowered (Sinclair 1992, Schenk *et al.* 1995, Bowler and Press 1996, Poorter *et al.* 1997, Zanetti *et al.* 1997). The results of the first three years of the Swiss Free Air Carbon Dioxide

Enrichment (FACE) experiment indicate that in spite of greater efficiency in using nitrogen and the high fertiliser input, the above-ground growth of *Lolium perenne* is limited by insufficient supply of N under CE (Zanetti *et al.* 1997). This interpretation is supported by the fact that the legume *Trifolium repens* produced considerably more above-ground biomass (Hebeisen *et al.* 1997), presumably due to its ability to symbiotically fix N₂ (Hartwig *et al.* 1996, Zanetti *et al.* 1996, Zanetti and Hartwig 1997, Lüscher *et al.* 2000).

Several hypothesis have been proposed to explain a reduced growth response to CE due to insufficient N-supply. The hypothesis assume the possible effect of limiting capacity of soil transport (Conroy 1992), the immobilisation of mineral N by rhizospheric microflora

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Abbreviations: d.m. - dry mass; LMR - leaf mass ratio; NP - nitrogen productivity; pCO₂ - partial pressure of CO₂; PPF - photosynthetic photon flux; RGR - relative growth rate; RMR - root mass ratio; Rubisco - ribulose 1,5-bisphosphate carboxylase-oxygenase; SAR - specific absorption rate; TNS - total non-structural saccharides.

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*Corresponding author (present address): Department of Plant Physiology, Faculty of Science, Masaryk University, Kotlářská 2, CZ-61137 Brno, Czech Republic; fax: (+420) 5 412 112 14, e-mail: vitgloser@sci.muni.cz

(Díaz *et al.* 1993, Hartwig *et al.* 1996), and the regulatory effect of CE on the specific rate of N uptake (Jackson and Reynolds 1996). However, only little attention was paid to form in which N is available. In the majority of experiments conducted to investigate plant growth under CE, nitrogen was supplied as nitrate (Hocking and Meyer 1991, Ryle *et al.* 1992, McKee and Woodward 1994, Roumet *et al.* 1996). Ammonium ions, either alone or in combination with nitrate, were used less frequently (Griffin *et al.* 1993, Bowler and Press 1996, Nijs and Impens 1997). Therefore, information about possible interactions between the type of N nutrition and CE on plant growth is lacking.

It is well known, that plants grown with ammonium as a sole source of nitrogen have usually different morphological traits compared to plants grown with nitrate; ammonium increases the biomass of leaves per unit leaf area and causes a significant decrease in the allocation of biomass to roots (Cramer and Lewis 1993, Troelstra *et al.* 1995). A high rate of NH_4^+ uptake leads to

a high content of organic N (Clarkson *et al.* 1986, Hecht and Mohr 1990, Ourry *et al.* 1996) while NO_3^- -fed plants store nitrate in vacuoles (Cramer and Lewis 1993). The assimilation of NH_4^+ ions occurs in the roots and takes place right after uptake to prevent the intracellular accumulation of toxic NH_4^+ . Therefore, the fast uptake and assimilation of NH_4^+ may serve to overcome the N limitation of plant growth under elevated pCO_2 . Moreover, a high rate of ammonium assimilation increases the demand of the root for carbon skeletons and stimulates the transport of saccharides from the shoot to the root. The higher demand for saccharides in the roots may prevent the excessive accumulation of saccharides in the leaves, which is known to down-regulate the rate of photosynthesis in plants grown at elevated pCO_2 (Stitt 1991, Drake *et al.* 1997). The aim of this experiment was to investigate whether an increased supply of nitrogen in the form of ammonium ions will result in stronger growth response of *Lolium perenne* to elevated pCO_2 along with increased N content in biomass.

Materials and methods

Plants and cultivation: Caryopses of *Lolium perenne* (L.) cv. Bastion were germinated on quartz sand moistened with half-strength nutrient solution (as used for hydroponic cultivation; see below); nitrogen was provided as 500 μM NH_4NO_3 . Seven days after germination at constant temperature (20 °C), the seedlings were placed in a hydroponic culture system. The nutrient solution contained: 500 μM KH_2PO_4 , 500 μM K_2SO_4 , 1500 μM CaSO_4 , 1000 μM MgSO_4 , 1000 μM KCl , 0.2 μM NaCl , 4.6 μM MnSO_4 , 0.4 μM ZnSO_4 , 0.16 μM CuSO_4 , 230 μM H_3BO_3 , 0.05 μM H_2MoO_4 and 50 μM FeNa-EDTA . To this N-free basic nutrient solution, nitrogen was added as $\text{Ca}(\text{NO}_3)_2$ (NO_3^- treatment) or $(\text{NH}_4)_2\text{SO}_4$ (NH_4^+ treatment) or as an equimolar mixture of both N forms (MIX treatment). The total nitrogen content in all treatments was 1 mM. The nutrient solution was changed every three days, and the pH was adjusted twice a day to 5.5 ± 0.5 with 0.5 M H_2SO_4 or with 0.5 M KOH. Aeration and circulation of the solution within the containers was achieved so that the air bubbles did not affect the roots. CO_2 fumigation was begun right after the plants were transferred to the hydroponic system. The mean atmospheric partial pressure of CO_2 in the climate chamber was 36.7 ± 0.2 Pa (CA) and 69.7 ± 0.3 Pa (CE). The pCO_2 was controlled by using an infrared gas analyser *WMA2* (*PP-Systems*, Hitchin, UK). The photosynthetic photon flux (PPF) was $540 \pm 20 \mu\text{mol m}^{-2} \text{s}^{-1}$ for 16 h. At the beginning and end of photoperiod, the PPF was increased/decreased at regular intervals over a 2-h period. Light was provided by fluorescent tubes (215 W, *Sylvania*, Danvers, USA) and incandescent lamps (100 W, *Philips*, Zurich, Switzerland)

at a ratio 7:11. The relative humidity was 70 % and temperatures were 20 ± 1 °C (day) and 13 ± 1 °C (night). Plants were rotated every three days within the growth chamber and once a week between chambers.

Growth analysis: There were four harvests: an initial harvest when the seedlings were put into the hydroponic system and three harvests 14, 19, and 23 d later. Each plant was divided into roots, "stems" (= leaf basis + leaf sheaths), and "leaves" (= leaf blades). The leaf area was determined by using a leaf area meter *LI-3100* (*Li-Cor*, Lincoln, USA). Plant roots were washed with deionized water before excision. The samples were frozen on dry-ice immediately after the destruction and freeze-dried later.

The specific leaf area (SLA, leaf area divided by leaf dry mass), and the leaf and root mass ratios (LMR, RMR, *i.e.* organ d.m. divided by plant d.m.) were calculated using the structural d.m. The recalculation of total d.m. to structural d.m. was done according to equation:

$$M_{\text{TNS}} = (1 - \text{TNS}) \times M$$

where M_{TNS} = structural d.m. of plant part, TNS = content of total nonstructural saccharides in plant part [g g^{-1}], and M = total d.m. of plant part.

Chemical analysis: The plant material was analysed for total nitrogen, inorganic nitrogen and total non-structural saccharides (TNS). Organic nitrogen was calculated as the difference between total and inorganic nitrogen. Two to four single samples were pooled to provide a sufficient amount of material for chemical analysis. Total nitrogen

was determined using an elemental analyser *CHN-1000* (LECO Corp., St. Joseph, USA). Inorganic nitrogen was extracted in hot water and determined with a continuous-flow analyser evolution (*Alliance Instruments*, Mery-sur-Oise, France). For TNS analysis, 10 mg of sample were extracted with 1 cm³ water in a sonicator at 45 °C for 20 min. Starch was digested with dialysed *Termamyl 120 L* (a heat-stable α -amylase from *Bacillus licheniformis*, *Novo Industri A/S*, Copenhagen, Denmark) at 95 °C for 15 min. The hexose in the supernatant after centrifugation (10 000 g, 10 min) of the extract was determined in duplicate by means of the anthrone method modified by Fischer *et al.* (1997). The reaction conditions were optimised for the simultaneous, quantitative detection of glucose, fructose and other saccharides.

Specific absorption rate (SAR) and nitrogen productivity (NP): The specific absorption rate for nitrogen in respective treatment was calculated from changes in total N and root dry mass with time according to equation described by Clarkson *et al.* (1986):

Results

Dry mass production and biomass allocation: After 14-d exposure, the average dry mass of plants grown at CE (irrespective the nutrition) was by 17 % higher ($P < 0.01$) than that of plants grown at CA; however, by the end of the experiment, the difference in plant dry mass between the two CO₂ treatments was not significant (Fig. 1). The specific leaf area (SLA) was increased ($P < 0.01$), the leaf mass ratio (LMR) was reduced ($P < 0.001$) and root mass ratio (RMR) was increased ($P < 0.01$) in plants under CE (Fig. 2). No effect of CE on SLA, LMR and RMR was found on day 23 of cultivation.

Mixed ammonium and nitrate nutrition (MIX) had the strongest effect on plant growth in both CO₂ treatments (Fig. 1); the dry mass of plants supplied with ammonium (NH₄⁺) or nitrate (NO₃⁻) was not significantly different over the whole experimental period. LMR and SLA of

$SAR_N = [(N_{T_2} - N_{T_1})/(T_2 - T_1)] \times [(lnMr_2 - lnMr_1)/(Mr_2 - Mr_1)]$
where N_T = total N in the plant, T = time in days, and Mr = root dry mass.

Whole plant nitrogen productivity, a measure of the efficiency of utilization of nitrogen in plant growth (Ingestad 1979) was estimated as mean relative growth rate (RGR) over a defined period of time per unit of nitrogen in plant biomass (mean value for the same time period).

Statistics: The *SPSS v. 7.0* statistical package (SPSS Inc., Chicago, USA) was used to evaluate the results. The effects of experimental factors on plants were tested by means of the multifactorial analysis of variance. Main effects of both experimental factors and their interaction were evaluated as well as the multiple comparison of means based on the method of LSD contrasts. The homogeneity of variances was checked by Bartlett's and Cochran's tests, and heterogeneous sets of data were log-transformed before calculation.

plants in MIX treatment were similar to LMR and SLA of the ammonium treatment (Fig. 2). Plants supplied with nitrate showed a higher SLA compared with plants from the other two treatments (Fig. 2) but differences in LMR and RMR were significant only on day 14.

Chemical composition of the plant: After 14 d of growth, the content of total nitrogen in plant leaves and stems cultivated at CE was significantly lower than that in plants grown at CA only in plants in ammonium treatment ($P < 0.01$, Table 1). Likewise, the content of organic nitrogen was exclusively lower in leaves and stems of NH₄⁺-grown plants under CE (Table 1). The distribution of organic N in the plant organs was not influenced by the pCO₂ or by the N form (data not shown). There was no effect of CE on both total and

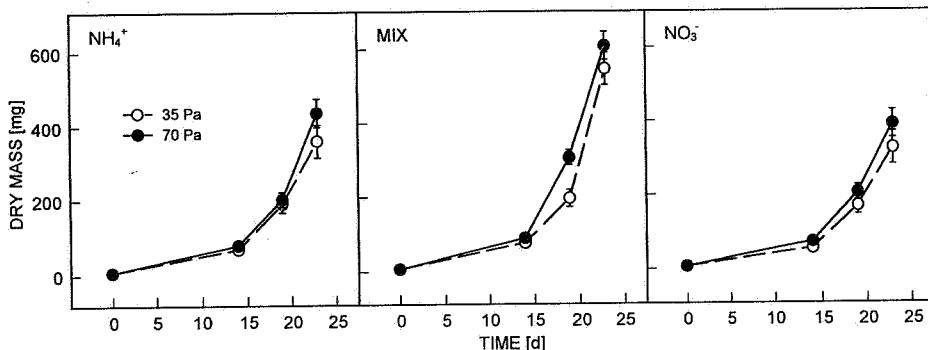


Fig. 1. Time-course of plant dry mass of *Lolium perenne* as affected by atmospheric partial pressure of CO₂ and by the form of nitrogen in the nutrient solution. Vertical bars indicate \pm SE (if greater than the marker size), $n = 8$.

Table 1. Concentrations of total (N_T) and organic (N_O) nitrogen [mg g^{-1} (structural d.m.)] in leaves, stems and roots of *Lolium perenne* after 14 and 23 d of cultivation in nutrient solution with NO_3^- , NH_4^+ or $\text{NH}_4^+ + \text{NO}_3^-$ (MIX) as nitrogen source. Plants were grown at CA (partial pressure of CO_2 35 Pa) or CE (70 Pa). Means \pm SE, $n = 4$. Dissimilar letters denote significant difference ($P < 0.05$) within plant parts between experimental treatments. n.d. - no determination was made because of an insufficient amount of material for analysis.

	[d]	CA - NH_4^+	CA - MIX	CA - NO_3^-	CE - NH_4	CE - MIX	CE - NO_3^-
N_T leaves	14	73.17 \pm 0.66 b	71.33 \pm 0.65 b	61.55 \pm 1.52 a	64.43 \pm 1.29 a	73.76 \pm 1.44 b	62.74 \pm 1.63 a
	23	68.31 \pm 1.80 a	77.78 \pm 1.99 b	66.10 \pm 1.16 a	65.22 \pm 2.13 a	77.37 \pm 1.03 b	63.15 \pm 0.63 a
N_O leaves	14	73.20 \pm 1.30 c	65.30 \pm 1.10 b	53.00 \pm 3.50 a	64.40 \pm 2.60 b	62.80 \pm 4.60 b	54.00 \pm 2.80 a
	23	68.31 \pm 1.80 b	69.70 \pm 1.80 b	56.70 \pm 1.20 a	65.22 \pm 2.13 b	69.70 \pm 1.70 b	55.20 \pm 1.70 a
N_T stems	14	60.20 \pm 0.16 b	62.00 \pm 0.79 bc	56.00 \pm 0.33 a	56.64 \pm 0.91 a	62.54 \pm 1.14 c	n. d.
	23	57.31 \pm 1.85 a	64.40 \pm 1.73 ab	54.92 \pm 1.52 a	57.85 \pm 2.37 a	69.85 \pm 1.10 b	61.52 \pm 5.39 ab
N_O stems	14	60.20 \pm 0.16 c	54.90 \pm 0.80 ab	n.d.	56.64 \pm 0.91 ab	51.60 \pm 3.00 a	n.d.
	23	57.31 \pm 1.85 b	56.04 \pm 2.50 b	45.23 \pm 2.00 a	57.85 \pm 2.37 b	59.51 \pm 1.27 b	47.37 \pm 3.41 a
N_T roots	14	57.57 \pm 1.10 c	49.46 \pm 1.38 b	40.65 \pm 0.52 a	58.01 \pm 1.27 c	50.77 \pm 0.51 b	43.00 \pm 0.72 a
	23	58.46 \pm 2.90 c	50.40 \pm 2.49 b	38.53 \pm 0.89 a	63.02 \pm 1.62 c	53.16 \pm 0.68 b	39.93 \pm 0.54 a
N_O roots	14	57.57 \pm 1.50 c	41.13 \pm 2.30 b	27.70 \pm 1.20 a	58.01 \pm 1.27 c	39.50 \pm 0.60 b	28.30 \pm 0.80 a
	23	58.46 \pm 2.90 c	38.06 \pm 1.94 b	22.49 \pm 0.40 a	63.02 \pm 1.62 c	40.06 \pm 0.96 b	22.70 \pm 0.92 a

Table 2. Concentration of non-structural saccharides (TNS) [mg g^{-1} (d.m.)] in leaves, stems and roots of *Lolium perenne* after 14 and 23 d of cultivation in nutrient solution with NH_4^+ , NO_3^- or $\text{NH}_4^+ + \text{NO}_3^-$ (MIX) as nitrogen source. Plants were grown at CA or CE. Means \pm SE, $n = 4$. Dissimilar letters denote significant difference ($P < 0.05$) within plant parts between experimental treatments. In some cases single (s.d.) or no (n.d.) determination was made because of an insufficient amount of material for analysis.

	[d]	CA - NH_4^+	CA - MIX	CA - NO_3^-	CE - NH_4	CE - MIX	CE - NO_3^-
TNC leaves	14	22.00 \pm 3.50 a	19.90 \pm 3.30 a	22.80 \pm 1.20 a	36.30 \pm 1.20 b	37.10 \pm 1.70 b	38.50 \pm 1.80 b
	23	29.30 \pm 3.20 b	24.80 \pm 2.10 a	23.00 \pm 5.00 ab	42.30 \pm 2.90 c	38.60 \pm 1.40 c	43.50 \pm 5.50 c
TNC stems	14	16.50 \pm 0.60 a	17.50 \pm 0.90 a	20.10 (s.d.)	15.80 \pm 1.40 a	17.10 \pm 1.50 a	n.d.
	23	21.30 \pm 1.80 b	20.60 \pm 1.80 b	23.50 \pm 0.60 b	16.90 \pm 1.30 a	20.40 \pm 1.30 b	31.50 \pm 4.10 c
TNC roots	14	4.40 \pm 0.40 abc	4.70 \pm 0.40 bc	4.80 \pm 0.40 c	3.90 \pm 0.40 a	4.70 \pm 0.30 bc	4.30 \pm 0.20 ab
	23	4.70 \pm 0.20 a	4.80 \pm 0.20 a	4.90 \pm 0.30 ac	4.30 \pm 0.40 ab	5.80 \pm 0.50 bc	5.50 \pm 0.70 bc

organic nitrogen content of plant organs on day 23.

The content of total non-structural saccharides (TNS) in leaves of plants exposed to CE increased by 60 to 70 % (Table 2) but the increase in other plant organ was not significant. The increase of TNS in leaves was similar in all types of N nutrition. Compared to plants supplied with nitrate, the presence of ammonium in the nutrient solution increased the content of organic N in all plant parts irrespective of pCO_2 (Table 1). The content of total N in

leaves in NH_4^+ treatment was higher than in NO_3^- treatment only on day 14 in CA.

Specific absorption rate and nitrogen productivity between day 14 and 23 were not affected by pCO_2 (Fig. 3). Plants in MIX showed the highest SAR for nitrogen but plants in nitrate treatment the lowest. Significantly higher nitrogen productivity was found in plants in nitrate treatment (Fig. 3).

Discussion

Plant growth: Total biomass of *Lolium perenne* was significantly higher in CE after 14 d of cultivation but this effect disappeared in the end of cultivation (Fig. 1). A transient stimulation of growth has been explained by an increase in the rate of photosynthesis at CE (Poorter *et al.* 1988, Ryle *et al.* 1992, den Hertog *et al.* 1993). A disappearance of the effect of pCO_2 on total biomass in the end of cultivation was unexpected since plants were young and supply of mineral nutrients as well as irradiance were in all treatments sufficient to prevent

growth limitation. There was an significant effect of CE on SLA, LMR and RMR on day 14, but this effect was missing on day 23. Therefore, to the decrease in the stimulatory effect of CE on dry mass production contributed also acclimatory changes in a leaf morphology and a distribution of plant dry mass. On the other hand, the cultivation of plants was shorter compare to some other short-term experiments where the effect of CE was significant (Baxter *et al.* 1994, Bowler and Press 1996, Schenck *et al.* 1996). Though, we can not exclude

the possibility, that the lack of growth stimulation in our experiment was due to temporal shift in development of plants between CO₂ treatments.

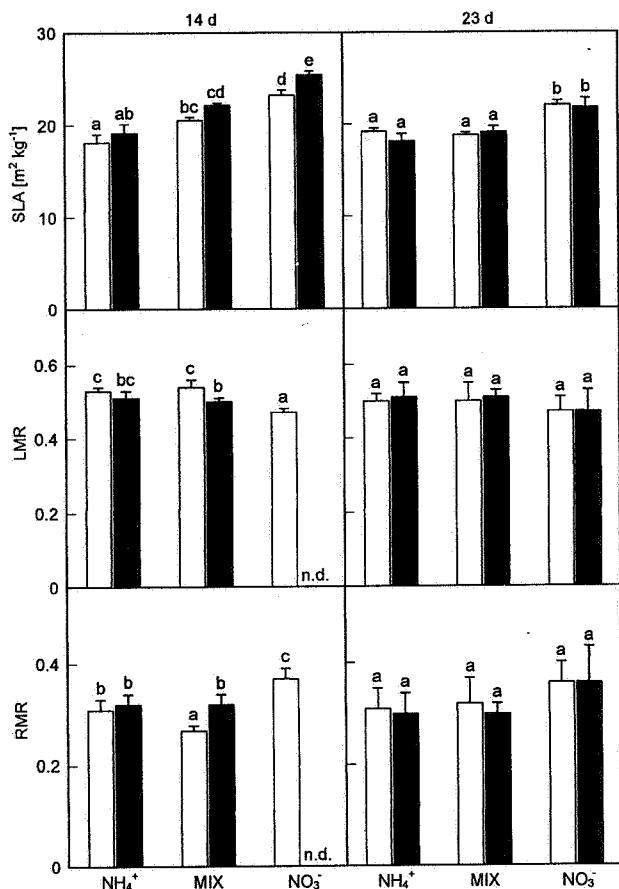


Fig. 2. Specific leaf area (SLA), leaf mass ratio (LMR) and root mass ratio (RMR) of plants after 14 and 23 d of cultivation, as affected by atmospheric partial pressure of CO₂ (35 Pa - open columns, 70 Pa - closed columns) and by the form of nitrogen in the nutrient solution - NH₄⁺, NO₃⁻ or NH₄⁺ + NO₃⁻ (MIX). Vertical bars indicate \pm SE, $n = 8$. Dissimilar letters denote significant difference ($P < 0.05$). n.d. - not determined.

Total dry mass of plants supplied with nitrate or ammonium was the same (Fig. 1), but the mechanisms responsible for the similar growth rates were different. Plants supplied with nitrate had a lower LMR but a much higher SLA compared to plants supplied with ammonium. This supports the hypothesis that SLA is one of the most important traits responsible for environmental plasticity of plants (Poorter and Remkes 1990). The results also suggest that mechanisms governing dry matter production are not the same for plants grown with different forms of nitrogen.

A mixture of ammonium and nitrate was the most beneficial for the growth of *L. perenne* at both pCO₂. The beneficial effects of a combination of NO₃⁻ and NH₄⁺ are well known (Volk *et al.* 1992, Wang and Below 1992)

and have been explained by a faster uptake of N and by lower respiratory costs for the maintaining of the cation/anion balance and the intracellular pH. In contrast, when ammonium or nitrate are supplied alone, a change in the pH of the rhizosphere and changes in the uptake and assimilation of N may severely reduce the rate of N uptake by the plant itself (Volk *et al.* 1992). However, our experiment using hydroponic culture and controlled pH showed that the stimulatory effect of MIX can not be explained only by changes the pH of the rhizosphere. Despite the strong positive effect of MIX on plant growth, the beneficial effect of CE was similar to that in the other two N treatments.

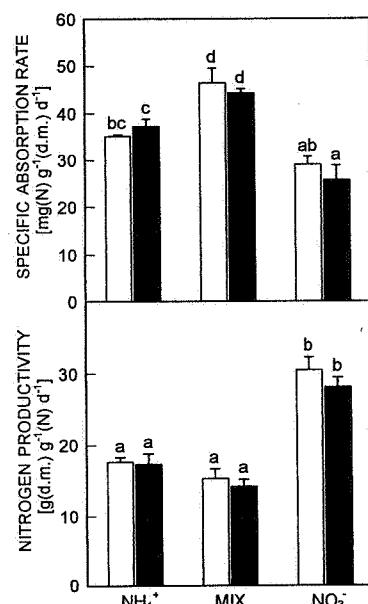


Fig. 3. Specific absorption rates of nitrogen supplied as NH₄⁺, NO₃⁻ or NH₄⁺ + NO₃⁻ (MIX) and nitrogen productivity as affected by atmospheric partial pressure of CO₂ (35 Pa - open columns, 70 Pa - closed columns) between day 14 and 23 of cultivation in nutrient solution. Vertical bars indicate \pm SE, $n = 4$. Dissimilar letters denote significant difference ($P < 0.05$).

Partitioning of biomass: To avoid misinterpretation of growth parameters due to higher accumulation of non-structural saccharides (TNS) under CE (Wong *et al.* 1990), calculations of all parameters were based on biomass without TNS. The distribution of structural dry mass in plants cultivated for 14 d at CE showed a decrease in the LMR and an increased allocation of d.m. to the roots. In many experiments, the allocation of biomass to the roots increased at CE (Bazzaz 1990, McKee and Woodward 1994, Hebeisen *et al.* 1997) but plants in these experiments were grown in solid substrates. Our data indicate increase of RMR under CE after 14 d but no difference between CO₂ treatments on day 23. Stulen and den Hertog (1992) suggested that when the supply of water and nutrients is continuous, no major shifts occur in the root/shoot ratio, thus an

increased root/shoot ratio must be interpreted as an indication for insufficient nutrient supply for respective growth. In agreement with this hypothesis *L. perenne* plants in steady state of exponential growth (day 23 of cultivation) and with sufficient nutrient supply exhibited no change in their RMR.

The effect of the N form on biomass allocation was very significant throughout the experiment even though there was higher variability of the data in the end of cultivation. The reduced rate of root growth in the presence of ammonium alone was repeatedly observed for crops (Findenegg 1987, Cramer and Lewis 1993). Several hypotheses have been proposed to explain this: 1) a direct toxicity of a high intracellular ammonium content plays a key role (Mehrer and Mohr 1989), 2) the negative effect on root growth is due to a decrease in the pH of the rhizosphere, as associated with the uptake and assimilation of NH_4^+ (Findenegg 1987), and 3) root growth is limited by competition for saccharides as related to the assimilation of NH_4^+ by roots (Cramer and Lewis 1993). Direct toxic effects of ammonium ions were usually observed only in external concentrations higher than the concentration used in the present study (1 mM). Since hydroponic cultivation ensured that the pH of the nutrient solution was stable, it was unlikely that low pH would disturb growth of roots. Competition for carbon source used simultaneously for growth and N assimilation can hardly explain our data, because the greater amount of carbon in plants exposed to elevated pCO_2 did not alter the low root mass ratio in plants in the NH_4^+ treatment compared to the NO_3^- treatment. Therefore, we suggest that the demand for N in plants supplied with ammonium can be fully met even at lower amount of root biomass as a result of higher rates of specific uptake of NH_4^+ ions. Higher rates of uptake of ammonium ions as compared to nitrate ions were observed for *L. perenne* (Clarkson *et al.* 1992, Høgh-Jensen *et al.* 1997) which is in line with our calculation of SAR in this experiment (Fig. 4).

Chemical composition: The reduction of the tissue nitrogen content is often found at CE especially in leaves (see Poorter *et al.* 1997 for review). In contrast to leaves, the nitrogen content is usually not affected in other plant organs (Hocking and Mayer 1991, Yelle *et al.* 1987, Hartwig *et al.* 2000) as was also the case in our experiment. Decreased nitrogen content, however, in some cases may result from the CO_2 -dependent increase in pool of non-structural saccharides. Therefore, it is better to express a nitrogen content per unit of structural dry mass as an appropriate measure of the amount of nitrogen available for growth.

The nitrogen content in the leaves at CE calculated on structural dry mass basis was either lower (Wong 1990, Rogers *et al.* 1993, den Hertog *et al.* 1996) or the same as in CA (Chu *et al.* 1992). In the present study, we found a

significant decrease in the content of organic nitrogen only in leaves of plants supplied with ammonium at CE.

Coleman *et al.* (1993) argued that the decrease in the N content observed at CE may be due to an accelerated growth rate and is, thus, the result of an ontogenetic shift. In this study, the ontogenetic effects probably did not play any significant role, because we investigated only very young plants at the exponential growth stage. The regression analysis of all nitrogen treatments between \ln -transformed, TNS-corrected content of organic nitrogen in leaves, and the structural dry mass *vs.* the \ln of structural plant dry mass did not show a significant relationship (data not shown).

The decrease in the N content in the leaves is usually ascribed to substantial decrease in content of Rubisco (Long 1991). More N can then be devoted to other components of a plant. Nakano *et al.* (1997) showed that a decrease in N in leaves of rice (*Oryza sativa*) under CE is not only due to a reduction of Rubisco content, but also due to decreased amount of some other nitrogenous compounds in the leaves.

Irrespective of pCO_2 , plants supplied with ammonium showed higher rates of N uptake and higher contents of total N in all organs compared to their counterparts in nitrate treatment. The TNS-corrected content of organic nitrogen in the leaves decreased under CE only in plants supplied with ammonium. This suggests a possible negative effect of CE on the rate of uptake of ammonium ions. Our calculation of the specific adsorption rate (SAR) of NH_4^+ , however, did not confirm such a negative effect of CE on ammonium uptake. Conversely, in experiments with six wild herbaceous species grown in soil, Jackson and Reynolds (1996) found an average increase of 22 % in the NH_4^+ uptake after doubling pCO_2 .

There was no shift in nitrogen productivity in *L. perenne* exposed to CE in our experiment. However, an increase in nitrogen productivity at CE is usually observed along with a decrease of plant nitrogen content (Luo *et al.* 1994, Roumet *et al.* 1996). Experiments with three grass species (Baxter *et al.* 1994) after more than 50 d of cultivation did not confirm an increase in nitrogen productivity under doubled atmospheric pCO_2 indicating that the change in nitrogen productivity is probably not a general response to CE.

Plants in nitrate treatment showed much higher nitrogen productivity than plants in ammonium or mixed treatments. Plants supplied with ammonium were able to take up greater amounts of N but their growth was not stimulated compare to nitrate-fed plants in spite of increased availability of carbon under CE. Limitation of the rate of photosynthesis due to excessive accumulation of saccharides in the leaves (Stitt 1991, Drake *et al.* 1997) is not likely because the content of TNS in leaves was similar in all nitrogen treatments. This suggests that the growth of plants at ammonium and mixed treatments was primarily regulated by other factors than the amount of carbon and nitrogen available for growth. A possible

explanation offers finding of Ryle *et al.* (1992) that C sink limitation is a possible factor responsible for the weak growth response to CE, because CE did not have a positive effect on tiller formation.

It is possible to conclude that significant effect of different N source namely on plant morphology and

biomass distribution was quite obvious in both pCO₂. Surprisingly similar overall growth response to CE had different mechanisms in different N treatments. A decrease of leaf N content in NH₄⁺-fed plants under CE indicates that available form of N contributes to changes of leaf N content under CE.

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