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Effects of Elevated CO_2 and Nitrogen on Growth of *Poa pratensis* (L.)

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The growth responses of a grass, *Poa pratensis*, to elevated CO_2 and nitrogen were investigated. Light-saturated photosynthetic rate per unit leaf area increased with exposure to elevated CO_2, while dry weight did not respond to increased CO_2. Patterns of biomass allocation within plants, including leaf area, leaf area ratio, specific leaf area, and root to shoot ratios, were not altered by elevated CO_2, but changed considerably with N treatment. Shoot and whole-plant tissue N concentrations were significantly diluted by elevated CO_2 (Tukey test, $P < 0.05$). Total N content did not differ significantly among CO_2 treatments. The absence of a concomitant increase in N uptake under elevated CO_2 may have caused a dilution in plant tissue [N], probably negating the positive effects of increased photosynthesis on biomass accumulation.

Keywords: Elevated CO_2, Nitrogen, Patterns of biomass allocation, Photosynthesis

Global carbon uptake through photosynthetic activity by green plants and oceanic dissolution may be as much as 120 Gt (gigaton) and 115 Gt of carbon per year, respectively. These rates may approximately balance the amount of CO_2 generated by respiration, decomposition, fires, and ocean release (Bowes, 1991). However, the continuous addition of CO_2 into the atmosphere, mainly from human activities such as fossil-fuel burning, deforestation, and land use, has been a major component of the increasing level of atmospheric CO_2, perhaps even doubling over the next 100 years (Crane, 1985; Houghton et al., 1990; Bowes, 1991).

Such an elevated atmospheric [CO_2] will likely affect photosynthetic (den Hertorg et al., 1998) and respiration rates (Bunce, 1990), as well as dry matter production and biomass partitioning (Farrar and Williams, 1991). This could possibly lead to further alterations in the competitive relationships that exist between neighboring plants, or change species composition in the plant community (Jongen and Jones, 1998). However, plant responses to enriched CO_2 seem to vary with developmental stage, species (Poorter, 1993; Paterson et al., 1996), and environmental factors such as nitrogen levels in the soil (Woodin et al., 1992). In particular, because increased N availability to plants should result from the current increases in anthropogenic N inputs to the ecosystem (Whitehead et al., 1997), it may be worthwhile to study how elevated atmospheric [CO_2] affects plant growth in the presence of additional nitrogen.

The aims of this study were to 1) investigate photosynthetic and nutritional responses of *Poa pratensis* to elevated CO_2, and 2) address whether the extent to which plant growth responds to elevated CO_2 is dependent on N availability.

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MATERIALS AND METHODS

Carbon Dioxide Fumigation System

Two blocks of open-top CO_2 test chambers were constructed according to the specifications of Ashenden et al. (1992). These chambers, located in the Cruickshank Botanic Garden at the University of Aberdeen, United Kingdom, were made of clear corrugated PVC, with removable door panels and adjustable polycarbonate lids. Each block comprised two chambers, with one receiving unaltered ambient CO_2, the other, supplemental enriched CO_2. They were served by a single pump through a split-ducting pipe. Highly concentrated CO_2 from cylinders was mixed with ambient air before entering the enriched chambers, so that actual [CO_2] ranged from 630 - 680 $\mu L\ L^{-1}$. In contrast, the [CO_2] in the ambient chambers was maintained at 340 - 360 $\mu L\ L^{-1}$. Gas was distributed at ground level through a perforated annular polythene tube. [CO_2] in the chambers was measured with an infrared gas analyzer (IRGA, LCA-3; ADC Ltd, Hoddesdon, UK), and was controlled manually by monitoring the concentration and adjusting as necessary.

Experimental Treatments

Seeds of *P. pratensis* were sown on trays of washed sand on 9th April 1999. They were transferred to individual pots (10×10×10 cm) in the greenhouse on 30th April 1999. Temperature and RH (relative humidity, %) in the greenhouse ranged from 18 to 25°C and 60 and 80%, respectively. All plants were supplied with 50 mL of 1 and 3 mM nitrate-based Long Ashton solution (50% strength) three times a week until the end of the experiment. Ten pots were transferred to each CO_2 chamber on 10th June 1999 and placed randomly. Half of the pots in each chamber received 1 mM N, the other half, 3 mM N. Plants were watered daily to prevent desiccation due to wind generated in the chambers by the air supply. Plants were harvested on 21st August 1999, and shoots and roots were separated.

Measurements

Carbon exchange rates in the plants (at their growth CO_2 concentrations) were measured with an infrared gas analyzer (IRGA), with 350 μL L^{-1} being used for ambient CO_2- and 650 μL L^{-1} for high CO_2-grown plants, respectively. For measurements, saturating irradiance (>1500 μmol m^{-2} s^{-1}) was supplied from a 12 V, 20 W tungsten halide lamp. The temperature within the leaf chamber was maintained at 20 - 23°C. Light-saturated photosynthetic rates (A_{max}) were calculated on a leaf-area basis, using the equations of von Caemmerer and Farquhar (1981). Leaf area was determined with an Area Measurement System (Delta-T Devices LTD, Cambridge, UK). The separated plant materials were oven-dried at 80°C prior to weighing and nutrient analysis. From these primary weight and leaf-area data, the following parameters were calculated: Root/Shoot (R/S) Ratio (DW of root/DW of shoot (mg mg^{-1})), Leaf Area Ratio (LAR; leaf blade area/total plant dry weight (mm^2 mg^{-1})), and Specific Leaf Area (SLA; leaf blade area/leaf blade dry weight (mm^2 mg^{-1})). Nitrogen and carbon concentrations (% DW) were determined with an NCS autoanalyzer (NA1500; Fisons, UK).

Statistical Analysis

All data were analyzed by two-way ANOVA (General Linear Model, Minitab) and Tukey's HSD test (Minitab). To avoid pseudoreplication (Hurlbert, 1984), the main effects of CO_2 were tested for significance against an error term that described overall between-chamber variation, and which was obtained by pooling the block and block X CO_2 interaction terms. The significance of N effectswas tested against residual betweenplant variation because N treatment was nested within chambers. The interaction between CO_2 and N also was tested against this residual.

RESULTS

Photosynthesis and Growth

P. pratensis plants grown and measured at elevated CO_2 exhibited higher photosynthetic rates per unit leaf area (A_{max}) compared with those grown and measured at ambient CO_2 (Fig. 1A; General Linear Model (GLM): $F_{1,2} = 44.1$, $P < 0.05$). However, this increased rate was not reflected in actual plant growth under

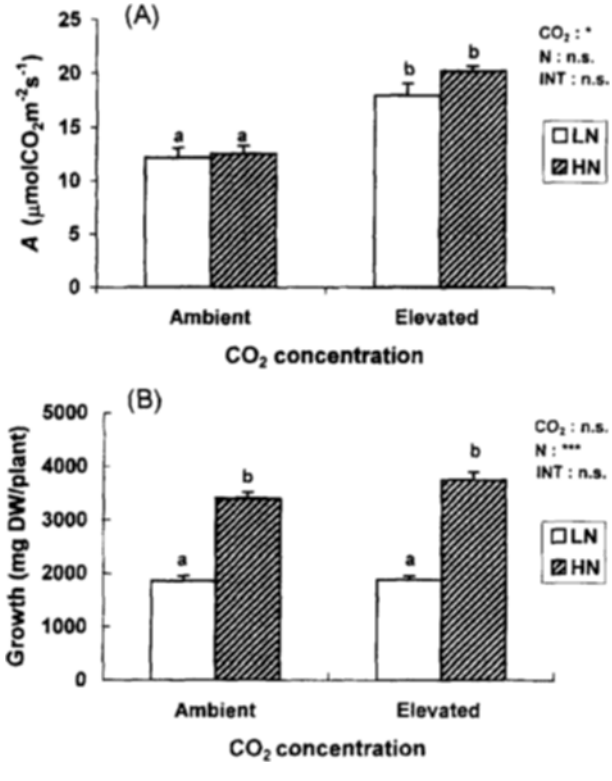

Figure 1. (A) Light-saturated photosynthetic rate per unit leaf area (A_{max}) and (B) dry weight (mg $plant^{-1}$) of *P. pratensis* supplied with 1 mM N (LN) and 3 mM N (HN) under ambient and elevated CO_2. Bars represent means ± 1 s.e. Analyses of two-way ANOVA are shown by symbols (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; n.s., not significant). CO_2, N, and CO_2 X N represent the effects of elevated CO_2, nitrogen, and the interaction between both factors, respectively. Bars with same letters are not significantly different from each other at the 0.05% level (Tukey test).

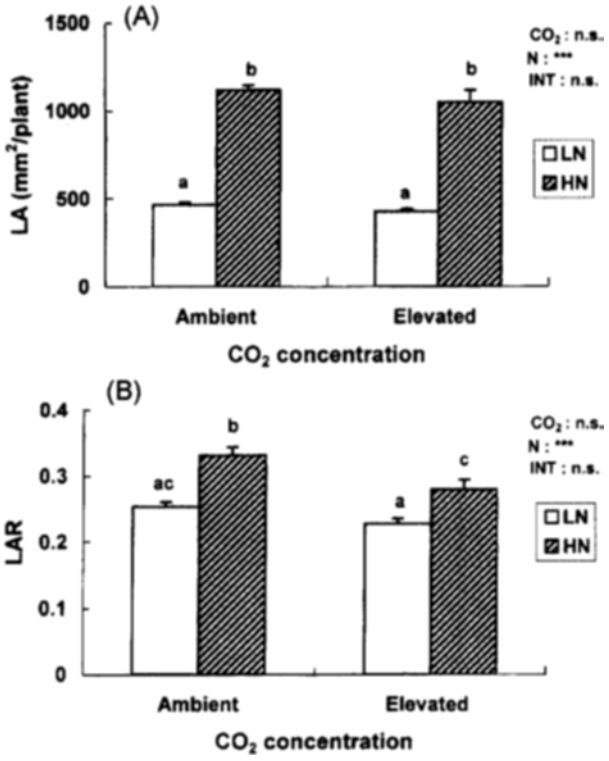

Figure 2. (A) Leaf Area (LA) and (B) leaf Area Ratio (LAR) of *P. pratensis* supplied with 1 mM N (LN) and 3 mM N (HN) under ambient and elevated CO_2. Bars represent means ± 1 s.e. Abbreviations and symbols are described in Figure 1.

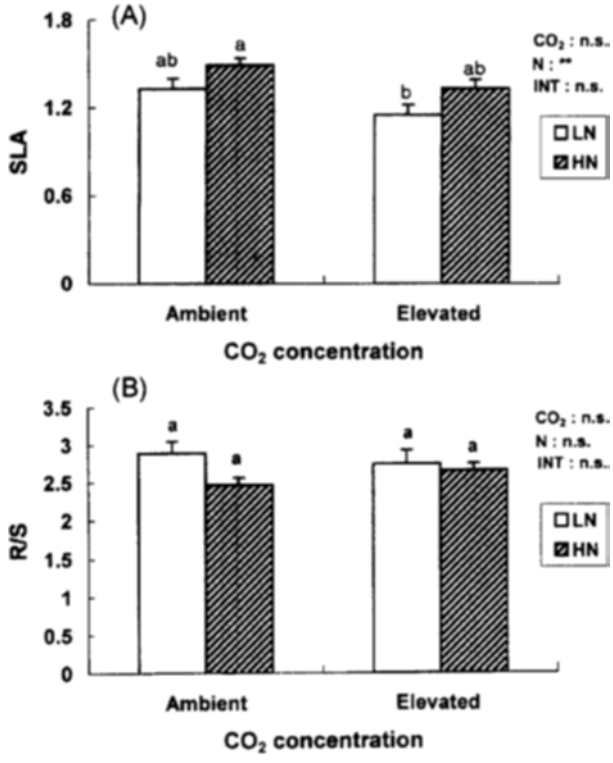

Figure 3. (A) Specific Leaf Area (SLA) and (B) Root to Shoot Ratio (R/S) of *P. pratensis* supplied with 1 mM N (LN) and 3 mM N (HN) under ambient and elevated CO_2. Bars represent means ± 1 s.e. Abbreviations and symbols are described in Figure 1.

elevated CO_2 (Fig. 1B; GLM: $F_{1,2} = 0.75$, not significant (n.s.)). The higher concentration of applied N did not improve A_{max} (Fig. 1A; GLM: $F_{1,29} = 2.06$, n.s.), but it did significantly increase dry weight, irrespective of the [CO_2] supplied (Fig. 1B; GLM: $F_{1,29} = 311.3$, $P < 0.001$). No significant interactions were found for the growth parameters mentioned above.

Biomass Allocation Patterns

Leaf Area (LA; mm^2 $plant^{-1}$) and Leaf Area Ratio (LAR) benefitted from the higher-N treatment (Fig. 2, A and B; GLM: $F_{1,29} = 321.1$, $P < 0.001$ and GLM: $F_{1,29} = 39.9$, $P < 0.001$, respectively). Elevated CO_2, however, did not affect either LA or LAR (Fig. 2, A and B; GLM: $F_{1,2} = 0.36$, n.s. and GLM: $F_{1,2} = 6.76$, n.s., respectively). Although higher nitrogen increased the Specific Leaf area Ratio (SLA), elevated CO_2 did not (Fig. 3A; GLM: $F_{1,29} = 9.09$, $P < 0.01$ and GLM: $F_{1,2} = 1.88$, n.s., respectively). Neither higher N nor elevated CO_2 altered the Root to Shoot Ratio (R/S) (Fig. 3B; GLM: $F_{1,29} = 2.89$, n.s., GLM: $F_{1,2} = 0$, n.s., respectively). No significant interactions were found for the growth parameters mentioned above.

Nutrient Status

Shoot, root, and whole-plant tissue N (% DW) were significantly affected by N treatment, but not by elevated CO_2 (Table 1). Interactive effects were absent. Nevertheless, tissue N concentrations, including the shoot and whole-plant values, tended to be significantly lower under elevated CO_2 (Tukey test, $P < 0.05$), irrespective of the [N] supplied. Total N content (mg $plant^{-1}$) did not increase in response to elevated CO_2, but showed a positive response to the higher N treatment (Table 1).

DISCUSSION

Photosynthetic rates in C_3 plants are limited by the amount of CO_2 currently in the atmosphere, and are further restricted by a high atmospheric [O_2] (Pearcy and Bjorkman, 1983). Therefore, plants should exhibit

Table 1. Tissue nitrogen concentrations (% DW) of root (Root N), shoot (Shoot N) and whole plant (Total N) and total nitrogen content (Total N content (mg plant^{-1})) of *P. pratensis* supplied with low N (1 mM N) and high N (3 mM N) under ambient and elevated CO_2. Values represent means ± 1 s.e. Analyses of two-way ANOVA are shown by symbols (***, $P<0.001$; n.s, not significant). CO_2, N, and CO_2 X N represent the effects of elevated CO_2, nitrogen, and the interaction between both factors, respectively. Values followed by the same letters within each row are not significantly different from each other at the 0.05% level (Tukey test).

	Ambient CO_2		Elevated CO_2		CO_2	N	CO_2 x N
	Low N	High N	Low N	High N			
Shoot N (% DW)	1.47 ± 0.05^a	1.83 ± 0.06^b	1.19 ± 0.06^c	1.51 ± 0.05^a	n.s.	***	n.s.
Root N (% DW)	0.63 ± 0.02^a	0.67 ± 0.04^a	0.55 ± 0.05^a	0.69 ± 0.04^a	n.s.	***	n.s.
Total N (% DW)	0.85 ± 0.02^a	1.01 ± 0.03^b	0.72 ± 0.04^c	0.91 ± 0.04^{ab}	n.s.	***	n.s.
Total N content (mg plant^{-1})	15.80 ± 0.94^a	34.00 ± 0.65^b	13.60 ± 0.98^a	34.10 ± 1.41^b	n.s.	***	n.s.

enhanced photosynthetic rates, on a leaf-area basis, under increased atmospheric CO_2 (Cure and Acock, 1986; den Hertorg et al., 1998). CO_2 entering into photosynthetic metabolism is catalyzed by RUBISCO (Ribulose Bisphosphate Carboxylase/Oxygenase), which is present at high concentrations in the leaf. Because this enzyme can catalyze O_2 as well, competition between CO_2 and O_2 is inevitable. Therefore, during reactions, more available CO_2 may increase the possibility for CO_2 to be catalyzed (carboxylation) rather than O_2 (oxygenation). Thus, such photosynthetic enhancement may be due partly to a stimulatory effect of elevated CO_2 on carboxylation activity of RUBISCO, as well as to the inhibitory effects on oxygenation and photorespiration (Bowes, 1991). That was true in this study, where the increased availability of CO_2 improved photosynthesis per unit leaf area for *P. pratensis*.

However, despite the increased photosynthetic rate under elevated CO_2, plant growth was not significantly stimulated. The absence of a significant CO_2 growth response may be attributed to increased biomass allocation toward heterotrophic rather than autotrophic tissue, thereby leading to a higher R/S Ratio and lower LAR (Norby et al., 1992; Callaway et al., 1994). For instance, the accumulation of nonstructural carbohydrates in plant roots, which causes increases in either the respiration rate or the R/S under elevated CO_2 (Schappi and Korner, 1997), may offset the positive effects of improved carbon assimilation on growth. In the current study, however, there was no indication that the proportion of heterotrophic tissue increased under elevated CO_2. This was supported by the absence of alterations in either R/S or LAR for those particular plants.

Alternatively, nitrogen may limit plant growth (Woodin et al., 1992; Bowler and Press, 1996). This results from the absence of an increase in N uptake that corresponds to an improved photosynthetic rate under elevated CO_2 (O'Neill et al., 1987). Indeed, the N content per plant was independent of the CO_2 concentrations supplied in the current study, indicating that N uptake was not stimulated by elevated CO_2. Moreover, irrespective of the [N] applied, both the shoot and the whole-plant tissue N were diluted under elevated CO_2 (Tukey test, $P < 0.05$). This was also seen by Cotrufo et al. (1998). Likewise, Hattenschwiler and Schafellner (1999) showed that increasing the N supply to elevated CO_2-grown plants could not completely prevent CO_2 enrichment from diluting plant-tissue [N]. If this is the case, the elevated CO_2-induced reduction in tissue [N] may have been partly responsible for the absence of a growth response to increased photosynthesis at both nitrogen concentrations in the current study.

Furthermore, whether plant growth benefits from an increased photosynthetic rate under elevated CO_2 seems to depend on the type of nutrients supplied (Bowler and Press, 1996). For instance, Whitehead et al., (1997) observed that growth was not improved when only N was supplied compared with that found when N was applied along with additional phosphorus under elevated CO_2. In the current study, the concentrations of other components (including P and K) in the Long Ashton solution were constant for both N treatments. Although this study may not have been conducted long enough to allow elevated CO_2 to make a difference in plant growth, it is more likely that the increased photosynthesis under elevated CO_2 may not have been reflected in growth without a concomitant increase in N uptake from the soil.

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