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Leaf nitrogen content as a predictor of photosynthetic capacity in ambient and global change conditions

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Abstract. Leaf assimilation capacity in *Lolium perenne*, grown in elevated CO_2 level (700 μmol mol^{-1}) and/or increased air temperature (ambient + 4°C) could be predicted from leaf N content expressed on an area basis, although the linear relationships between maximum carboxylation rate (Vc_{max}) or maximum electron transport rate (J_{max}) and leaf N depended on treatment. The model, based on Farquhar, Von Caemmerer & Berry (1980) showed negative long-term effects of increased air temperature on Vc_{max} and J_{max}, while long-term exposure to increased CO_2 level affected only Vc_{max}. Acclimation responses to these global changes therefore could not be explained by changes in N-content alone, but also in terms of changes in photosynthetic nitrogen use efficiency. Stimulation of photosynthesis by elevated CO_2 was not affected by reduction of leaf N in leaves developed in ambient air temperature, while part of the CO_2 benefit was lost in leaves developed in increased air temperature. This suggests that N-deficient ecosystems maintain the potential to respond to elevated CO_2 concentration, unless other processes than the primary carbon metabolism become limiting at low N supply. Similar to nitrogen content, changes in photon flux density did not change the CO_2 benefit either, unless a transition occurred from one limiting process to another (electron transport to carboxylation or vice versa). Hypotheses on interaction between CO_2 level, nitrogen status of the leaf and light intensity are formulated to support these findings.

Key words. Carboxylation, electron transport, elevated CO_2 concentration, increased air temperature, photosynthesis, nitrogen, modelling.

INTRODUCTION

While nitrogen represents only a small fraction of plant biomass (usually around 3% of dry matter), its concentration in different plant tissues is a highly limiting factor for growth and productivity. Over the last few years, this essential element is also increasingly recognized as a controlling factor in many plant processes, for example in the distribution of dry matter over different plant parts (e.g. Levin, Mooney & Field, 1989), and in carbon assimilation by photosynthesis (e.g. Field & Mooney, 1986; Gastal & Saugier, 1989). It also has become clear that the use of nitrogen by the plant is subjected to 'economic' principles, and that plants invest N in different processes and compounds to maximize the return in terms of carbon gain and growth. Hirose & Werger (1987), for example, showed that in some species the spatial nitrogen allocation pattern in the stand is optimal for daily canopy photosynthesis. Because of this close coupling between C- and N-metabolism, studies of environmental changes that affect carbon uptake should take into account the link with the nutrient flow in the plant. While it is widely accepted that ongoing and future changes in the global atmosphere (increasing atmospheric carbon dioxide content and likely also increased air temperature) will strongly alter carbon flow in vegetation over the next decades, too little attention has so far been given to the interaction of these changes with nitrogen availability and supply. There are, however, strong indications that N-use is severely affected by these changes, because many species show markedly decreased leaf N concentrations after long-term exposure to elevated CO_2, at

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Abbreviations and symbols: A—leaf CO_2 assimilation rate; Ca—external CO_2 concentration; Ci—CO_2 concentration in the intercellular spaces of the leaf; CO2—treatment with elevated CO_2 concentration; CO2 × TEMP—treatment with both elevated CO_2 concentration and increased air temperature; CONTROL—treatment with ambient CO_2 concentration and air temperature; gs—leaf stomatal conductance for water vapour, J_{max}, maximum electron transport rate; *PFD*—photon flux density of photosynthetically active radiation; RH—relative humidity of the air; TEMP—treatment with elevated air temperature; Vc_{max}—maximum carboxylation rate; α—quantum yield of electron transport.

least when expressed on a dry matter basis (Campbell, Allen & Bowes, 1990; Hocking & Meyer, 1985; Overdieck, Reid & Strain, 1988; Rowland-Bamford *et al.*, 1991). Part of this decrease can be compensated by a coinciding decrease in specific leaf area, but any remaining drop in leaf N generally deteriorates the photosynthetic capacity of the leaf (downward regulation). Our study examines whether, apart from the N concentration itself, the photosynthetic efficiency of this nitrogen will also change through long-term exposure to global changes. This will allow assessment of whether acclimation responses in leaf photosynthesis are merely the result of concentration changes or whether the investment of N in different processes is also altered. Following this question, two further objectives were also put forward.

(1) Can photosynthetic capacity be predicted from leaf N, and is this photosynthesis–nitrogen relationship still valid when plants are grown in a new CO_2 and/or air temperature regime?
(2) Does the magnitude of global change effects depend on the nutrient status of the leaf?

The last question relates to the problem of whether ecosystems with low nitrogen availability will still respond to elevated carbon dioxide (e.g. natural ecosystems with a low N-input), while the former question is interesting for prediction of such responses with mathematical models.

The questions mentioned above were approached in an integrated way based on the original model of Farquhar, Von Caemmerer & Berry (1980), which allows photosynthetic responses to be traced back to the initial photochemistry and biochemistry, and which can at the same time be linked to leaf nitrogen (e.g. Harley *et al.*, 1992). The analysis was performed on measured photosynthetic rates (*A*/Ci curves) of perennial ryegrass plants grown in either elevated carbon dioxide, increased air temperature or a combination of both. A wide range of nitrogen supply was used to produce both optimal and N-deficient conditions.

MATERIALS AND METHODS

Plant material

Seeds of *Lolium perenne* L. cv. Vigor were planted on 7 March 1993 at a density of 6 g m^{-2} in 1.5 l containers (154 10^{-4} m^2 surface area) in steam-sterilized loamy soil, which was fertilized with N, P and K in the following amounts: 10 g m^{-2} N (in NH_4NO_3), 15 g m^{-2} P (in P_2O_5), and 15 g m^{-2} K (in K_2O). The pots were assembled with minimal interspace to form a closed canopy and were irrigated every day with a trickle system (one drip tube per container), which was sufficient to maintain field capacity. Plant material (sixty pots in total) was distributed over four treatments and was allowed to develop undisturbedly. The stands were cut on 10 June, 10 July, 10 August and 10 September at 3 cm cutting height. After cutting, N supply was 9.43 g m^{-2} (optimal treatment) or 55, 30 or 15% of this amount in the suboptimal and deficient treatments, while P and K doses were maintained at 2.17 g m^{-2} and 7.54 g m^{-2}, respectively, regardless of N supply.

Exposure system

Four global change treatments were produced in a system for continuous exposure during growth:

CO2: elevated CO_2 concentration at ± 700 μmol CO_2 mol^{-1} air;
TEMP: increased air temperature at ± 4°C above ambient;
CO2 × TEMP: a simultaneous combination of TEMP and CO2; and
CONTROL: reproduction of field conditions of CO_2 level and air temperature (CO2without subscript is used to denominate the treatment, CO_2 with subscript for the gas).

The system consisted of four independent exposure units, one for every treatment, equipped for tracking field temperature (two units) or field temperature $+4$°C (two units) and for reproducing this at the site of the plants through heating/çooling. Each unit was composed of three elements: a half-open greenhouse, an air-conditioning unit outside and an exposure table inside, on which the plant containers were arranged. The greenhouses were plastic-covered with 180 μm thick transparent polyethylene, containing 14% vinyl acetate for reinforcement, transmitting ± 80% of the UV-B (280–320 nm) in natural sunlight as well as most of the UV-A wavelengths. Intensities in the 400–700 nm range are reduced by ± 15%. To facilitate heat removal through natural convection, the lateral walls of the greenhouses were partially replaced by plastic gauze over a 75-cm high strip from the ground up, over the entire 6.3 m length. The air-conditioning cabinet feeds filtered, temperature-controlled outside air to the exposure table, passing it through it through a dust filter (fibre-glass filled filter, EFC, Herk-de-Stad, Belgium), through SO_2-impregnated active charcoal filters (EFC) to exclude most of the interaction with air pollutants, through a cooling system with separate condenser and an evaporator of 4.28 kW, and through an electrical resistance heating grid (6 kW). The turbine ventilator (2.2 kW) supplied 4000 m^3 h^{-1}, which was reduced to 2000 m^3 h^{-1} through partial obstruction of the air inlet. The containers with the stands were placed on an exposure table inside the greenhouse, which is an aluminium cabinet designed to convert the horizontal air stream, produced by the air-conditioning unit, into a homogeneous upward flow of well-mixed air supplied to the plants. A perforated plate and horizontal and vertical deflectors distribute the vertical air stream uniformly and produce the necessary homogeneity of windspeed over the surface of the exposure table (2.75 m × 1.45 m), minimizing gradients in environmental conditions at the site of the plant. Transparent acrylic shields are placed round the exposure table to minimize influx of 'false' turbulent air into the air column. Elevated CO_2 levels are obtained through injection of a constant flow of pure CO_2 in the air-conditioning cabinet.

Measurement and registration of environmental conditions

Microclimate was monitored with the following sensors

and registered with 10-min intervals: air temperature: copper–constantan thermocouples (Honeywell, Washington, U.S.A.), relative humidity: macro-polymer humidity sensors RH-8 (General Eastern, Watertown, Maine, U.S.A.); photosynthetically active radiation: quantum sensors with gallium arsenide photodiode (Pontailler, 1990); CO_2 concentration: absolute infrared CO_2 analyser SBA-1 OEM (PP-Systems, Herts., U.K.). Air was sampled from each unit alternatively with a datalogger-controlled valve system while tubes which were not being sampled were continuously flushed. Sensor output was registered with a time-controlled datalogger DL 2 (Delta-T, Burwell, Cambridge, U.K.).

Microclimate

During the measurement period, the average difference in air temperature between the ambient treatments and the treatments with increased air temperature was 3.91°C ($n = 17713$), compared to a target value of 4°C. Average air temperatures were 17.3, 17.0, 21.0 and 21.2°C in CONTROL, CO2, TEMP and CO2 × TEMP, respectively. Air temperature in the ambient treatments was on average 0.9°C above the field temperature due to uncoupling of the conditioning control below 10°C. Relative humidity was slightly reduced compared to field values (8% on average). CO_2-increase above ambient was on average 326 μmol CO_2 mol^{-1} ($n = 4341$), while the average background level was 394 μmol CO_2 mol^{-1}.

Photosynthesis measurements

CO_2 exchange rates (A) of fully expanded leaves were determined between 22 June and 9 July 1993, with a combined set-up of a portable LCA3 photosynthesis system (Analytical Development Company, Hoddesdon, U.K.) with a Parkinson Leaf Chamber (2.5 × 2.5 cm measurement area), and a LI-6262 CO_2/H_2O analyser (LI-COR, Lincoln, Neb, U.S.A.). Four leaves were simultaneously inserted into the chamber to obtain sufficiently high fluxes and more reliable estimates compared to single leaf sampling. Rates were measured at different concentrations of external CO_2, Ca (0–2000 μmol CO_2 mol^{-1}), at a leaf temperature of 25°C, a relative humidity (RH) of 80% and a photon flux density (PFD) of photosynthetically active radiation of 1500 μmol m^{-2} s^{-1}. Leaf area of the exposed leaf sections was measured photometrically using a LI-3000 planimeter (LI-COR). Internal CO_2 level in the intercellular space of the leaf (Ci) was calculated from CO_2 and H_2O exchange rates. In total, sixty-four A/Ci curves were obtained: four global change treatments × four nitrogen levels × four replicates. The model of Farquhar *et al.* (1980) was applied according to Harley *et al.* (1992), including the model of Ball, Woodrow & Berry (1987) for simulation of the stomatal response. The latter predicts stomatal conductance as $gs = A \cdot RH/Ca$. Maximum carboxylation rate, Vc_{max}, and maximum electron transport rate, J_{max}, were obtained from points on the A/Ci diagrams below $Ca = 200$ μmol CO_2 mol^{-1} and whole curves, respectively. Initial best fit estimates of dark respiration were replaced by values from sixty-four light–response curves with PFD between 0 and 150 μmol m^{-2} s^{-1} (dark respiration was assumed to remain unaltered in the light). The same light response curves were also used to estimate the quantum yield of electron transport α (mol electrons/mol photons), using the hyperbolic relationship $J = (\alpha \cdot PFD)/((1 + (\alpha^2 \cdot PFD^2/J_{max}^2))^{-2})$. The quantum yield was systematically varied until the least square difference between predicted and measured A was obtained. No allowance was made for possible limitation of CO_2 uptake by inorganic phosphate in triose-phosphate utilization (cf. Sharkey 1985), because limitation by either carboxylation or electron transport provided sufficient explanation of observed rates. The model was not validated with an independent data set, as it is used analytically for determination of the parameters, and not for simulation or prediction purposes.

Nitrogen content

Total nitrogen in the leaf sections used for photosynthesis measurements was determined with the micro-Kjeldahl technique in a block digestor (Isaac & Johnson, 1976). after drying the fresh matter for 24 h at 80°C.

RESULTS

Maximum carboxylation rates Vc_{max} and maximum electron transport rates J_{max} were regressed versus leaf nitrogen on an area basis (pooled data from all treatments, Fig. 1), yielding linear relationships with coefficients of determination r^2 of 0.70 and 0.71 for Vc_{max} and J_{max}, respectively. In this graph, part of the variation in leaf N originated from the nitrogen treatments while another fraction was caused by the global change treatments. The linear N-dependences of Vc_{max} and J_{max} were, however, not universal but changed slightly with CO_2 and air temperature regime during growth: there was a significant (negative) effect of the factor air temperature on both Vc_{max} and J_{max} (ANCOVA, $p < 0.05$, CO_2 and air temperature level as factors and leaf N as covariate), while the effect of CO_2 level was only significant (negative) on Vc_{max} (same analysis). This means that when leaves of the same N level are compared, the maximum carboxylation rate than can be attained by these leaves is lower for plants grown in 700 μmol CO_2 mol^{-1} air, while both the maximum electron transport rate and the maximum carboxylation rate that can be reached are lower in plants grown in + 4°C above ambient. Photosynthetic nitrogen use efficiency is thus reduced by the global change treatments and simulation of photosynthetic capacity will have to be based on treatment-dependent relationships in this species.

Fig. 1 describes the so-called 'demand' function of photosynthesis (and its dependence on leaf N), referring to the requirement of the photosynthetic apparatus for CO_2 from the intercellular air spaces in the leaf (Ci). Fig. 2 represents the counterpart of this, that is, the 'supply' function, describing the transport of CO_2 over the stomatal barrier into the substomatal cavities. This cannot be related to leaf N, at least not directly, but is modelled from assimilation rate, corrected for RH and Ca. While the linear

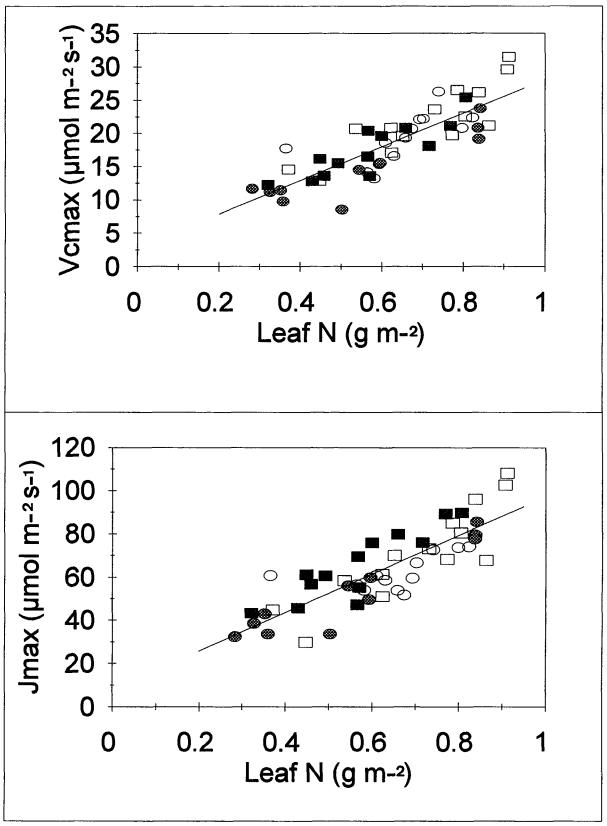

FIG. 1. Effect of leaf nitrogen content on maximum carboxylation rate Vc_{max} and maximum electron transport rate J_{max}. Values were derived from the response between assimilation rate (A) and internal CO_2 level (Ci) measured at a leaf temperature of 25°C, a relative humidity of 80%, and a photon flux density (*PFD*) of photosynthetically active radiation of 1500 μmol m^{-2} s^{-1}. Treatment codes and symbols—(■) (CO_2): plants grown in elevated CO_2 concentration (± 700 μmol mol^{-1}); (○) (TEMP): plants grown in ambient air temperature + 4°C; (●) ($CO_2 \times$ TEMP): plants grown in both elevated CO_2 concentration and increased air temperature; (□) (CONTROL): plants grown in ambient CO_2 concentration and air temperature.

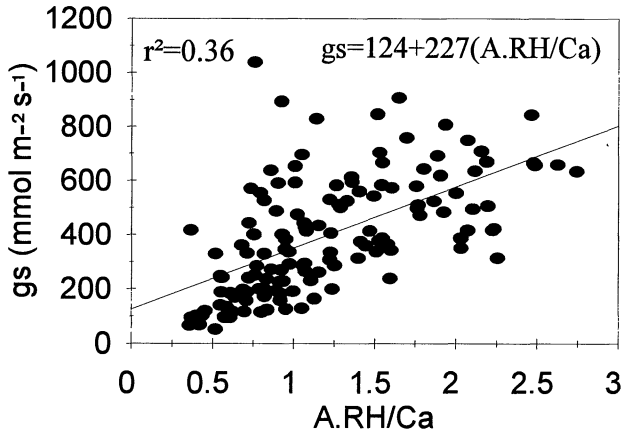

FIG. 2. Dependence of leaf stomatal conductance gs on assimilation rate A, relative humidity RH and external CO_2 concentration Ca (pooled data from all treatments; see Fig. 1 for treatments).

regression of stomatal conductance gs on A·RH/Ca was significant ($p < 0.05$), only 36% of the variation in gs could be explained by this model ($r^2 = 0.36$, pooled data from all treatments). Since the model of Ball *et al.* (1987) used here is the only mechanistic one that we are aware of (stomatal behaviour is generally characterized empirically), it was retained for further processing of the data. Only limited extra variability was added by using this simplification (see Fig. 3).

In the mode of Farquhar *et al.* (1980), the demand and the supply function are connected by the central value of Ci. Because A, gs and Ci are interrelated, the actual assimilation rate in a set of environmental conditions (Ca, RH, *PFD*, air temperature) is calculated in an iterative way, by varying Ci until the calculated A and gs satisfy $A = gs\ (Ca - Ci)$. The result of this is shown in Fig. 3, where simulated A values from all treatments are plotted against measured values. This 'regeneration' of the individual photosynthesis measurements yielded an r^2 value of 0.96, which means that the use of the generalized relationships in Figs 1 and 2 adds only 4% of extra variation to the data. The model also shows a slight tendency to underestimate the measured values (on average 5.7%).

Until now the model was used for saturating *PFD*, because A/Ci curves were made at 1500 μmol m^{-2} s^{-1}. Dependence of A on *PFD* was introduced by using the independent data set of photosynthetic rates measured between 0 and 150 μmol m^{-2} s^{-1}. These assimilation rates were reproduced by the model in a similar way to those in Fig. 3, apart from using J_{max} which was replaced by the light-dependent expression $J = (\alpha \cdot PFD)/((1 + (\alpha^2 \cdot PFD^2/J_{max}^2))^{-2})$. In this analysis, α was systematically varied until the least square difference was obtained between simulated and measured rates, yielding $\alpha = 0.177$ mol electrons/mol photons (see Fig. 4). The same value could be used for all treatments and was adopted as constant in the model.

Completing the parametrization step in Fig. 4 allows prediction of assimilation rate as a function of global change treatment, nitrogen content, CO_2 level and light intensity. In the following simulations of assimilation rate,

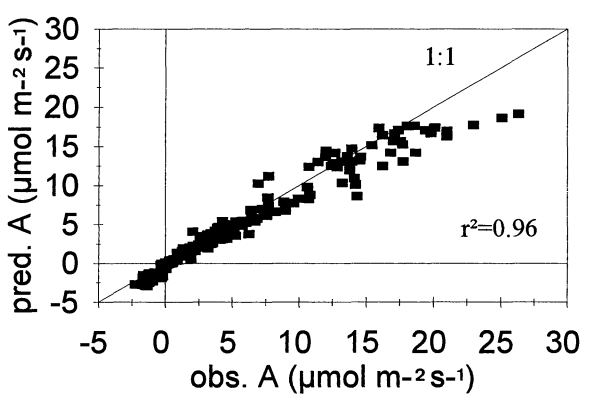

FIG. 3. Simulated (straight line) versus measured leaf assimilation rate A at saturating *PFD* (pooled data from all treatments; see Fig. 1 for treatments).

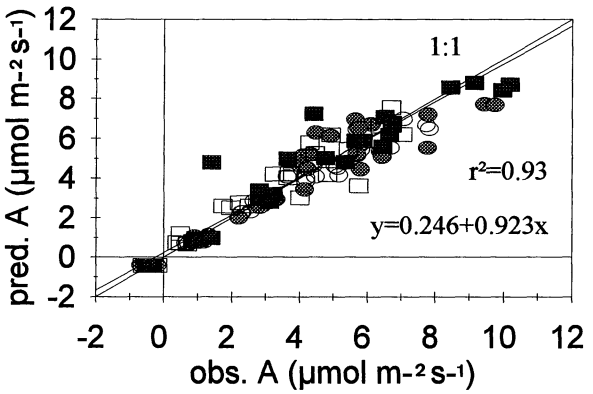

FIG. 4. Simulated (straight line) versus measured leaf assimilation rate *A* (separate data set at low *PFD*: 0–150 μmol m^{-2} s^{-1}). Other measurement conditions and symbols: see Fig. 1. Quantum yield of electron transport α (mol electrons/mol photons) was chosen to obtain the least square difference between simulated and measured values

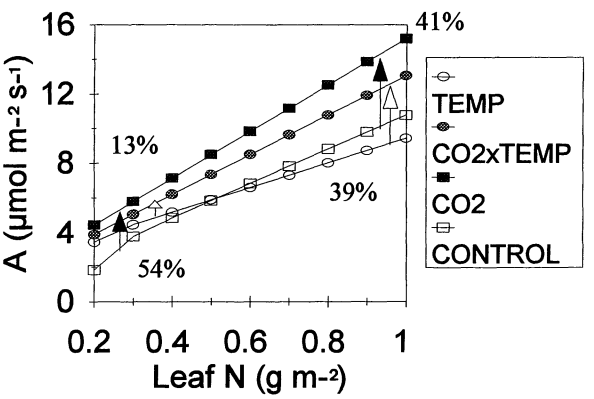

FIG. 5. Simulated leaf assimilation rate *A* at different leaf nitrogen concentrations. Treatment codes and measurement conditions: see Fig. 1. Arrows indicate the relative stimulation by elevated CO_2 (%) in ambient air temperature (closed arrows) or increased air temperature (open arrows) for leaf N = 1.0 g m^{-2} and 0.3 g m^{-2}

TABLE 1. Effect of elevated carbon dioxide level on leaf assimilation rate *A*.

PFD (μmol m^{-2} s^{-1})	Relative change in assimilation rate *A* ($A_{CO2}/A_{CONTROL}$)	($A_{CO2\times TEMP}/A_{TEMP}$)
0	1.21	1.12
40	1.19	1.24
100	1.20	1.22
180	1.21	1.21
300	1.21	1.22
460	1.22	1.25
650	1.39	1.37
900	1.39	1.37
1300	1.39	1.37
1700	1.39	1.37

Rates were simulated for RH = 80%, leaf temperature = 25°C, leaf N = 1 g m^{-2} and for different photon flux densities *PFD* of photosynthetically active radiation. CO_2 concentration during measurement was the same as during growth. Effects are represented relatively for plants grown in either ambient air temperature ($A_{CO2}/A_{CONTROL}$) or increased air temperature ($A_{CO2\times TEMP}/A_{TEMP}$). Treatment codes: see Fig. 1.

two aspects of long-term elevated CO_2 and increased air temperature are examined: (1) whether the stimulation from elevated CO_2 changes with the N content of the leaf, and (2) whether this stimulation depends on light intensity. In both cases the CO_2 effect is defined as the long-term plus the short-term influence (because the CO_2 level in the simulation is for each treatment the same as during growth). Fig. 5 shows that the relative effect of elevated CO_2 concentration only slightly depends on the nitrogen status of the leaf, and that the stimulation of photosynthesis is not lost in low nitrogen: in the ambient temperature treatment, even a higher relative increase is obtained in leaves with N = 0.3 g m^{-2} (54%) than in leaves with N = 1 g m^{-2} (41%). In the treatments with increased air temperature, however, some of the stimulation from elevated CO_2 level at N = 1 g m^{-2} is lost in leaves with N = 0.3 g m^{-2} (39% vs. 13% stimulation). Low N content is thus not necessarily an impediment for a strong response to elevated CO_2. A similar phenomenon was observed when the CO_2 response was compared at different light intensities (Table 1): the relative effect of elevated CO_2 was maintained in low *PFD*, although it was smaller than in saturating conditions. There was also a distinct transition at approximately 500 μmol m^{-2} s^{-1}, where the higher stimulation associated with higher *PFD* changed to a lower level. This transition was associated with a change in the limiting process for photosynthesis (below this intensity: electron transport rate, above this intensity: carboxylation rate, not shown), which shows that the different processes which can limit photosynthesis have different limitations in their response to elevated CO_2.

DISCUSSION

Leaf N was a good predictor of both the electron transport and carboxylation capacity of the leaf, and the linearity of this relationship indicates that it can be explained by a simple mass effect: more available nitrogen simply yields proportionally higher rates, suggesting that increasing leaf N parallels increasing investment in photosynthetic compounds (thylakoid proteins, enzymes of the Calvin cycle). This is not surprising because about 60% of the leaf soluble proteins consists of Rubisco, which is often regarded as the most limiting factor for biomass production. The relationship between leaf N and assimilatory ability is, however, not fully universal and shifts to lower capacity for the same N levels due to growth in the simulated global changes used here. This means that *Lolium perenne* cannot adapt to losses in photosynthetic potential, resulting from a lower N concentration in the leaf, through changing its photosynthetic N use efficiency. In fact, the reduced efficiencies observed here further aggravate the loss in capacity associated with lower leaf N. According to Ryle, Davidson & McDuff (1992), several causes can be responsible for changes in N use efficiency: changes in relative allocation of N to photosynthetic and non-photosynthetic compounds, shifts in N-investment within the group of photosynthetic compounds itself, changes in the specific activity of N-compounds and, finally, differences in limiting factors

which are not N-related. This also explains why the relationships between photosynthetic rate and leaf N is not narrowly defined when data from different species are pooled (Evans, 1989). We conclude that the loss of photosynthetic potential, generally referred to as acclimation or down-regulation, should not be explained merely in terms of leaf N concentration, but can also be the result of changes in the efficiency of the use of this N. Although the mechanism is not fully elucidated at present, acclimation to elevated CO_2 is a well-documented phenomenon that occurs in many species. Our results indicate that warmer growth temperatures can change the photosynthetic rate of the leaf just as well, so that acclimation to increased air temperature should also be considered in global change research.

To assess the influence of elevated CO_2 level and increased air temperature on a global scale, it is important whether low-nutrient ecosystems will respond similarly to well-fertilized vegetation, since large carbon pools of the global carbon cycle are located precisely in these low-productive systems. At present, however, the literature on $CO_2 \times$ nitrogen interaction is contradictory, and a fundamental explanation why the response to elevated CO_2 should change or remain the same when the nitrogen supply is changed is lacking. In C3-species a constant relative effect of elevated CO_2 is sometimes found with varying N-supply (e.g. Coleman *et al.*, 1991; Hocking & Meyer, 1985; Wong, 1979 for biomass; Zangerl & Bazzaz, 1984 in a multi-species ecosystem). Others have found a reduced effect in N-deficient ecosystems or plants (Coleman *et al.*, 1991; Conroy, Milham & Barlow, 1992; Goudriaan & de Ruiter, 1983; Patterson & Flint, 1982 for biomass; Wong, 1979 for photosynthesis), but an increased CO_2 effect at suboptimal N-levels has been observed as well (Marks & Clay, 1990 for *Lolium perenne* biomass). In *Populus tremuloides* and *Picea glauca* the influence of N-supply on CO_2 stimulation was, furthermore, a function of growth stage (Brown & Higginbotham, 1986), and these authors suggest a gradually developing nutrient deficiency and a coinciding change in the effect of elevated CO_2. A review by Kimbal (1986) on species grown in nutrient solution showed that reduced mineral supply generally negatively affects CO_2 stimulation, but the degree is species-dependent. The mechanistic approach in *Lolium perenne* used here shows that plants can maintain the CO_2 benefit even when the leaf nitrogen concentration is low. We suggest that the primary mechanism of CO_2 stimulation potentially allows this, because the shift from oxygenation to carboxylation that is caused by elevated CO_2 does not, in principle, depend on the magnitude of the pool of Rubisco molecules (but only on CO_2 and O_2 levels). When a large Rubisco pool is present due to abundant N supply, the same fraction of these molecules will switch from oxygenation to carboxylation as in a small Rubisco pool in N-deficient conditions. If this hypothesis is true, it implies that all species potentially maintain the CO_2 benefit at least in the primary mechanism of action of this environmental change, and further that (partial) loss of this benefit is due to limitations further down the assimilatory pathway.

An analogous explanation can support the findings in Table 1: similar to changes in leaf N, changes in light intensity do not affect the stimulation by elevated CO_2, unless a transition occurs from one limiting process to another (carboxylation to electron transport or vice versa). We assume that within the same limiting process, the CO_2 benefit is relatively constant because changes in *PFD* only affect electron transport rate, and thus the amounts of ATP and NADPH available for regeneration of RuBP. The ratio in which CO_2 and O_2 molecules bind on these available RuBP molecules mediated by the Rubisco enzyme, however, again only depends on CO_2 level (since O_2 level and leaf temperature were constant in all measurements). As a result, changes in *PFD* do not affect the ratio between photosynthesis in elevated and in ambient CO_2 level. The consequences of this in generally dense grass canopies is that the poorly illuminated leaves in the lower leaf layers still add to the overall CO_2 benefit of the stand, although to a lesser degree than the sunlit upper leaves. The experimental literature confirms this partial conservation of the CO_2-effect (e.g. Dietz, 1986 for leaf photosynthesis; Campbell, Allen & Bowes, 1990 for canopy photosynthesis; Zangerl & Bazzaz, 1984 for production).

To conclude, the results in *Lolium perenne* demonstrate that nutritional aspects cannot be neglected in global change studies. While they indicate that the potential for photosynthesis to respond to elevated of CO_2 is not *per se* affected by low N-supply, they do not explain why many species lose at least some of this potential. At the moment, it is not clear which processes become limiting in this case.

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