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Nitrogen balance for wheat canopies (*Triticum aestivum* cv. Veery 10) grown under elevated and ambient CO_2 concentrations

D. R. SMART,[1] K. RITCHIE,[2] A. J. BLOOM[1] & B. B. BUGBEE[3]

[1]Department of Vegetable Crops, University of California, One Shields Avenue, Davis, CA 95616-8746, [2]Department of Agronomy, N-305 Turner Hall, University of Illinois, Urbana, IL 61820-0460, and [3]Department of Plants, Soils, and Biometeorology, Utah State University, Logan, UT 84322-4820, USA

ABSTRACT

We examined the hypothesis that elevated CO_2 concentration would increase NO_3^- absorption and assimilation using intact wheat canopies (*Triticum aestivum* cv. Veery 10). Nitrate consumption, the sum of plant absorption and nitrogen loss, was continuously monitored for 23 d following germination under two CO_2 concentrations (360 and 1000 μmol mol^{-1} CO_2) and two root zone NO_3^- concentrations (100 and 1000 mmol m^3 NO_3^-). The plants were grown at high density (1780 m^{-2}) in a 28 m^3 controlled environment chamber using solution culture techniques. Wheat responded to 1000 μmol mol^{-1} CO_2 by increasing carbon allocation to root biomass production. Elevated CO_2 also increased root zone NO_3^- consumption, but most of this increase did not result in higher biomass nitrogen. Rather, nitrogen loss accounted for the greatest part of the difference in NO_3^- consumption between the elevated and ambient [CO_2] treatments. The total amount of NO_3^--N absorbed by roots or the amount of NO_3^--N assimilated per unit area did not significantly differ between elevated and ambient [CO_2] treatments. Instead, specific leaf organic nitrogen content declined, and NO_3^- accumulated in canopies growing under 1000 μmol mol^{-1} CO_2. Our results indicated that 1000 μmol mol^{-1} CO_2 diminished NO_3^- assimilation. If NO_3^- assimilation were impaired by high [CO_2], then this offers an explanation for why organic nitrogen contents are often observed to decline in elevated [CO_2] environments.

Key-words: *Triticum aestivum*; elevated CO_2; nitrogen; NO_3^- absorption; transpiration.

INTRODUCTION

For taxa that respond positively to elevated CO_2 concentrations, higher carbon gain when exposed to high [CO_2] might increase physiological capacity for NH_4^+ or NO_3^- absorption and assimilation because of the high energy costs associated with these processes (Pate & Layzell 1990). For example, absorption and assimilation of NH_4^+ and NO_3^- can consume 14% and 23%, respectively, of the total energy respired by roots (Bloom *et al*. 1992). Because NH_4^+ is almost always assimilated in roots (Raven & Smith 1976), root respiration should reflect the total physiological costs for NH_4^+ absorption and assimilation. Nitrate, on the other hand, can be assimilated in either roots or shoots. If NO_3^- is assimilated in shoots, the energy costs are still high, with as much as 25% of mitochondrial respiration or 26% of photosynthetic electron transport being coupled to NO_3^- assimilation (Bloom *et al*. 1989).

In addition to energetic considerations, the activity of NO_3^- assimilating enzymes is sensitive to both [CO_2] and internal levels of carbon metabolites in a way that would favor higher assimilation under elevated [CO_2]. The absence of CO_2 lowers nitrate reductase activity (Aslam *et al*. 1979; Kaiser & Brendle-Behnisch 1991) and elevated levels of nonstructural carbon metabolites such as glucose and sucrose increase NO_3^- reductase activity (Aslam *et al*. 1976; Aslam & Huffaker 1984). Many taxa exposed to elevated [CO_2] show an increase in both root and shoot concentrations of nonstructural carbohydrates including glucose and sucrose (Havelka *et al*. 1984; Acock *et al*. 1990; Wong 1990; Körner & Arnone 1992; Wullshleger *et al*. 1992). Indeed, such increases are one of the more consistent plant responses to elevated [CO_2] (Körner *et al*. 1995). Wheat, the subject of this investigation, shows a 5–20% increase in nonstructural carbohydrate content when it is exposed to elevated [CO_2] (Lekkerkerk *et al*. 1990; Smart *et al*. 1994).

Although elevated [CO_2] may increase carbon and energy supply for nitrogen acquisition, leaf nitrogen and soluble protein contents decrease in wheat leaves exposed to high [CO_2] (Hocking & Meyer 1991; Mckee & Woodward 1994; Rogers *et al*. 1996b). One generally accepted hypothesis for this decline is that the photosynthetic apparatus acclimates to elevated [CO_2] by reducing enzymatic components (e.g. ribulose bisphosphate carboxylase-oxygenase, Rubisco), while being able to sustain photosynthetic rates comparable to plants exposed to current ambient [CO_2] (Sage *et al*. 1989; Yelle *et al*. 1989;

Correspondence: David R. Smart. Fax: 530 752 9659*; e-mail: DRSMART@VEGMAIL.UCDAVIS.EDU*

Rogers *et al*. 1996a). In spite of a decrease in specific leaf nitrogen contents under elevated [CO_2], which would reduce nitrogen demand, wheat shows an average increase in total biomass production of from 10 to 53%, a response that increases overall demand for nitrogen (Kimball 1983; Hocking & Meyer 1991; Rogers *et al*. 1993; Mitchell *et al*. 1993; McKee & Woodward 1994; Thompson & Woodward 1994; Rogers *et al*. 1996a).

The above considerations indicate that nitrogen demand should be greater for wheat under elevated [CO_2], but that additional carbon skeletons, respiratory ATP, and reductant may compensate for this demand by providing resources that can be used in nitrogen acquisition. As NO_3^- absorption and assimilation can consume a significant fraction of a plant's energy budget, and NO_3^- represents a significant source of nitrogen for cultivated taxa (Lewis 1986; Haynes 1986), we examined the hypothesis that NO_3^- absorption and assimilation by intact wheat canopies would be enhanced by elevated [CO_2]. To conduct these investigations, we used a controlled environment chamber designed to support high-density wheat canopies and a solution culture system that allowed us to monitor NO_3^- consumption, the sum of plant nitrogen absorption and nitrogen loss, over the entire growth cycle. Previous investigators have used cut root segments extracted from soil cores, and have reported that root NO_3^- absorption decreased in response to elevated [CO_2] in one case (Jackson & Reynolds 1996) and greatly increased in response to elevated [CO_2] in another (Bassirirad *et al*. 1996). Ecological considerations do not well explain these discrepancies with ruderal annual taxa showing a negative response to elevated [CO_2] in one instance (Jackson & Reynolds 1996) and a strong positive response in another (Bassirirad *et al*. 1997); and woody perennial taxa showing positive, negative and neutral responses to elevated [CO_2] (Bassirirad *et al*. 1996; Bassirirad *et al*. 1997). Such inconsistencies may reflect differences in internal carbohydrate status in roots at the time of excision, or spatial and temporal heterogeneity in soil NO_3^- pools at the time and locality of excision. For example, elevated [CO_2] can increase microbial NO_3^- assimilation (Mikan *et al*. 1995), and thus might lower NO_3^- availability in some soil microsites. Nitrate absorption systems are highly inducible over very short time periods (< 4 h) and NO_3^- is the primary driving variable in NO_3^- absorption and assimilation induction (Crawford 1995). In the system we describe, [CO_2] effects on ontogenetic processes (e.g. root proliferation), changes in morphology, and physiological responses (e.g. induction of NO_3^- absorption) are integrated, resulting in a more accurate assessment of physiological responses by a community to elevated [CO_2]. Two NO_3^- concentrations were used for the following reasons. A lower concentration of 100 mmol m^{-3} NO_3^- was selected because it is close to the apparent K_m for net NO_3^- uptake by wheat roots (Goyal & Huffaker 1986) as well as for other temperate graminaceous cultivars (Smart & Bloom 1988). This concentration may more accurately reflect NO_3^- levels in agricultural soils and does not generally lead to large leaf NO_3^- accumulation by nitrophilous species (Koch *et al*. 1988). The higher NO_3^- concentration we used was 1000 mmol m^{-3}, a concentration that saturates growth nitrogen demands for similar high-density wheat canopies (Chen 1989), and can lead to high leaf NO_3^- accumulations (Koch *et al*. 1988), thus insuring that leaf NO_3^- reducing enzymes would be fully induced.

MATERIALS AND METHODS

Cultural practices

Wheat canopies were grown in a 2·4 m^2 array (Fig. 1) that was divided into 12 contiguous 0·8 m^2 subcanopies arranged into a randomized block design with three possible nutrient treatments (3 × 4). Two of the nutrient treatments are reported here while the third consisted of an average NO_3^- concentration of 2000 mmol m^3 and was grown in order to have a contiguous canopy and thus eliminate border effects. Seeds were germinated in frames that were made from plastic light diffusers ≈ 50 cm long by 40 cm wide with individual cells of 1·5 cm^2 by 1 cm deep. Two such grids were sandwiched with a 1 mm mesh fibreglass cloth between them. Seeds were placed in individual cells in a uniform pattern that provided a density of 1780 plants per m^2. The seeds were covered with an inert media (Isolite CG-2, Sumitomo Corp., Denver, CO, USA). Each frame supported one individual subcanopy, suspending it over a 27 L root zone that was 48 cm long by 38 cm wide by 15 cm deep.

The seeded grids were first placed in a 4 °C cold room for vernalization. The grids were then thoroughly wetted and moved into the growth chamber where they remained in the dark for five days at 16 °C. On the sixth day, 0–24 h following first emergence, the emerging canopies were provided with four hours of photosynthetic radiation at a photon flux density of just over 1000 μmol m^{-2} s^{-1} (λ = 400–700 nm). At the beginning of the second day, the photoperiod was increased to 16/8 h (light/dark), and on the third day to 18/6 h where it remained for the duration of the experiment.

Physical characteristics of the chamber and treatments

The controlled environment chamber had a total volume of 28 m^3 (Smart *et al*. 1994; see Fig. 1). Light was provided by two rows of five 1000 W high-pressure sodium lamps (Bubenheim *et al*. 1988). The positioning of the lamps ensured that photosynthetic photon flux at any point just above the plant canopies was uniform, the average photon flux being 1094 ± 56 μmol m^{-2} s^{-1} (mean ± SD, λ = 400–700 nm). The integrated daily total was ≈ 74·0 mol m^{-2} d^{-1}, substantially higher than the amount provided by sunlight during a clear summer day.

Three blowers (0·5 m^3 s^{-1}) re-circulated air over a heat exchanger sustaining the temperature within the chamber at 22·5/19·5 °C for the 18/6 h light/dark cycle. The blowers

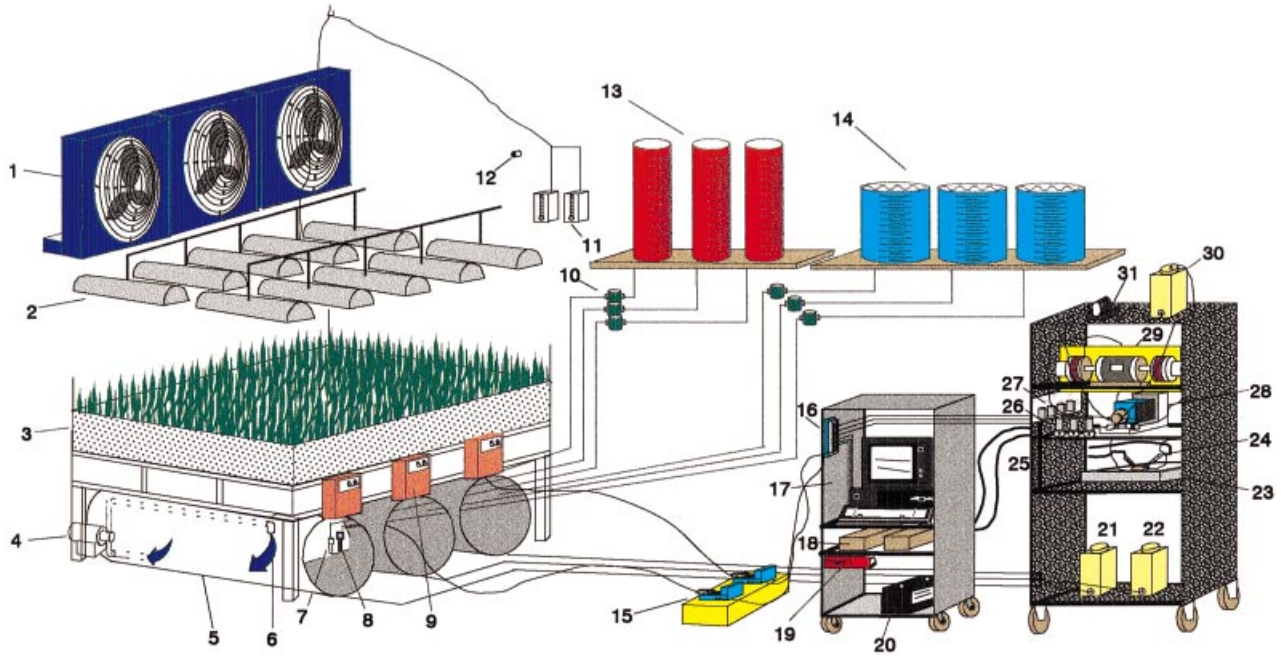

Figure 1. System used to develop nitrogen balances for each of three 0·8 m^2 intact wheat canopies. 1. Heat exchange system consisting of a bank of three blowers (Colmac Coil, Colville, WA, USA) that recirculated air in the chamber at 0·5 m^3 s^{-1}. 2. Ten 1000 W high-pressure sodium lamps (Energy Technics, York, PA, USA). 3. Shade screen border to eliminate border effects. 4. Nutrient solution recirculating pump (Little Giant, model 4-MD-SC, Oklahoma City, OK, USA). 5. 280 L capacity nutrient solution reservoir. 6. Aeration cascade. 7. pH electrode (Orion Research Inc., model 915600, Boston, MA, USA). 8. Water level activated switches (Omega Engineering, model LV-90, Stamford, CN, USA). 9. pH control relay (Omega Engineering, model PHCN-36). 10. 24 VDC normally closed solenoid valves used for H^+ and water addition by the pH and water level control systems, respectively (ASCO, model D8260G53 V, Florham Park, NJ, USA). 11. 50 and 150 cm^3 min^{-1} rotameters for adding pure CO_2 to the system. 12. Sample port for monitoring and controlling CO_2 concentration in the chamber. 13. 10 L capacity reservoir for solution containing 50 mol m^{-3} H_2SO_4 plus 50 mol m^{-3} HNO_3 for pH control. 14. 25 L capacity reservoirs for purified water to control solution volume. 15. Syringe pumps for adding 1 and 2 kmol m^{-3} KNO_3 and $Ca(NO_3)_2$ (Razel Scientific Instruments Inc., model A-99, Stamford, CN, USA). 16. Data acquisition and control system (Campbell's Scientific Inc., model CR10, Logan, UT, USA). 17. PC-XT. 18. Electrode amplifier (Campbell's Scientific Inc., model HiZ). 19. 12 V DC power supply. 20. 12 V battery that serves as back-up power supply. 21. 8 L capacity 100 mmol m^{-3} NO_3^- calibration standard. 22. 8 L capacity 1000 mmol m^{-3} NO_3^- calibration standard. 23. Controlled temperature water bath. 24. NO_3^- electrode (Orion Research Inc., model 92–07) and Ag/AgCl double junction reference electrode (Fisher Scientific Inc., model 13-623-270, Pittsburg, PA, USA). 25. 12 V DC isolatching solenoid valves for switching between hydroponic system and NO_3^- electrode calibrating solutions (General Valve Corp., series 1, East Hanover, NJ, USA). 26. Solid state relays (CRYDOM, model 3611, San Diego, CA, USA). 27. Metering pump (Fluid Metering Inc., model Q20–2, Oyster Bay, NY, USA). 28. Pulse dampening micrometering pump (Fluid Metering Inc., model RH00CKC). 29. Recirculating pump used to wipe ionic strength solutions from metering pump heads (Little Giant, model 1-EA-42). 30. 8 L water supply reservoir for recirculating pump.

were positioned so that wind speed at the top of the canopy was ≈ 1 m s^{-1}. The relative humidity was 90–95% during the dark germination period, and 65 ± 3%/85 ± 3% during the light/dark cycle for the remainder of each experiment. Relative humidity was lowered by circulating dry air into the chamber with a small wall mounted blower.

Carbon dioxide was enriched in the chamber using pure CO_2 gas filtered through a potassium permanganate column to remove contaminating hydrocarbons (Air Repair, Cleveland, OH, USA). The CO_2 concentration in the chamber was continuously monitored using an infrared gas analyser (LI-COR, model 6251, Lincoln, NE, USA). Two metering valves were used to add the CO_2 and to control its concentration in the chamber during the light period. Two CO_2 concentrations were used in these experiments: 360 μmol mol^{-1} CO_2, a concentration close to the current ambient and 1000 μmol mol^{-1} CO_2, a concentration that saturates net CO_2 assimilation rates for these communities (Wheeler *et al*. 1993; Monje & Bugbee 1998). A CO_2 concentration of 1000 μmol mol^{-1} also maximizes yields under the described conditions (Bugbee & Salisbury 1988). The CO_2 concentration was not controlled during the dark period, and climbed to about 10% above the set-point concentrations in a manner that would be similar to night-time increases under natural conditions.

The concentrations of NO_3^- and H^+ were held constant by two simultaneously operating control systems. The first system used the output signal from a NO_3^- selective electrode to control metered additions (Razel Corp., model A-99, Stamford, CN, USA) of equal amounts of 1–2 kmol m^{-3} KNO_3 and 1–2 kmol m^{-3} $Ca(NO_3)_2$. The concentration of the NO_3^- sources was increased from 1 kmol m^{-3} to 2 kmol m^{-3} KNO_3 and $Ca(NO_3)_2$ on about the seventh day as the canopies grew and nitrogen demand increased. An

electrometer instrumentation amplifier with active shielding (Campbell's Scientific, model HiZ, Logan, UT, USA), with the NO_3^- selective electrode connected to one input, a silver/silver chloride reference electrode connected to the other input, and the water in the electrode water bath set to a floating ground, was used for signal conditioning (Bloom 1989). The daily zero drift of this instrument when used in this manner was less than 1%. The NO_3^- electrode was calibrated each 24 h against standard solutions consisting of 100 or 1000 mmol m^{-3} NO_3^- to correct for this slight error. The calibration solutions contained the same cationic and anionic backgrounds as the plant nutrient solutions. This system added between 30 and 50% of the total NO_3^- demanded by root zones. The second control system used the output signal from pH electrodes to control additions of a mixture of 50 mol m^{-3} HNO_3 plus 50 mol m^{-3} $H_2(SO_4)$ that sustained pH at 5·8 ± 0·1 (Omega Engineering, model PHCN-36, Stamford, CN, USA). Preliminary experiments indicated that the above mixtures and use of the dual control modules resulted in accurate simultaneous control of NO_3^- and H^+ concentrations throughout the growth period. The activity of the pH and NO_3^- control systems generally maintained NO_3^- concentration within 5–20% of the set-point levels of 100 and 1000 mmol m^{-3}. The quantity of NO_3^- added to the root zones to maintain the set-point concentrations was determined by summing the amount of NO_3^- added by both the NO_3^- and pH control systems on a daily basis. We call this quantity NO_3^- consumption in that several processes in addition to root absorption contribute to nitrogen consumption by the root zone (see 'Discussion').

The amount of water added to the individual hydroponic systems was monitored over the 23 d growth period. There were two water sources. To sustain the nutrient solution at a constant level, float-activated leveling switches added a dilute refill solution back to the system each time the nutrient solution surface dropped to 1 cm below its initial level (Omega Engineering, model LV-91, Fig. 1). The water added by this system, plus that introduced by the NO_3^- and pH control systems, yielded an evapotranspiration budget. Evaporative losses from exposed water surfaces in the nutrient supply reservoirs were estimated by monitoring the system without plants on two separate occasions. The apparent evaporative loss was subtracted from the total water consumption to yield an apparent transpiration loss.

Tissue analyses

At the end of each 23 d experiment, each subcanopy was cut at the surface of the Isolite planting medium. The plastic frames were taken apart and any stem or root material within the cells was removed and separated at the stem-root intersection. Shoots and stems were placed in a forced air drying oven and dried at 80 °C. Roots were rinsed in deionized water and then dried in the same manner as the shoots. The total amount of biomass nitrogen was determined by combusting two finely milled root and shoot subsamples of 150 mg each in a C,H,N analyser (LECO Corp., model CHN-1000, St. Joseph, MI, USA). The term biomass nitrogen includes absorbed unassimilated NO_3^--N, NO_3^--N that was absorbed and assimilated into plant biomass, and a very small amount of microbial biomass attached to root surfaces. The microbial biomass on root surfaces generally amounts to less than 1% of the total biomass (Smart *et al*. 1995), so we heretofore refer to it together with plant biomass as biomass nitrogen. One shoot and one root sample were combusted for each subcanopy of the $[CO_2] \times [NO_3^-]$ treatments, and nitrogen concentration in roots and shoots used to calculate the total canopy nitrogen contents. Nitrate in these samples was analysed by two independent procedures. In the first procedure, a subsample of 250 mg of plant tissue was shaken for 15 min in 50 cm^3 of 2 kmol m^{-3} KCl and 6·4 mol m^{-3} H_2SO_4 to suppress microbial activity. The extraction solution was allowed to stand at 4°C overnight and then filtered through Whatman 42 filter paper. The filter paper was pre-soaked in 2 kmol m^{-3} KCl to remove ClO_3^- which can interfere with NO_3^- analyses. The concentration of NO_3^- in the solution was analysed by autoanalysis using a modified Griess-Ilsovay procedure (Lachat Instruments Inc., model QuickChem AE, Milwaukee, WI, USA). In the second procedure, a subsample of ≈ 2 mg of very finely ground tissue was weighed directly into a 3 cm^{-3} conical centrifuge tube. Three ml of 1 mol m^{-3} $CaSO_4$ solution was added and the samples were agitated vigorously and allowed to stand at 4°C for 48 h. They were centrifuged at 14000 r.p.m. for 8 min. Then 1·5 cm^{-3} of the supernatant was analysed for NO_3^- concentration spectrophotometrically at 210 nm following HPLC separation using a Whatman Partisil-10-SAX anion exchange column in a 35 mol m^{-3} KH_2PO_4 solution at pH = 3·0 (Aslam & Huffaker 1984). The two methods gave very similar results, so the data sets were combined for analysis.

Nitrogen loss was calculated as the difference between the amount of NO_3^-–N added by the control systems to support total NO_3^- consumption minus that remaining in nutrient solutions at the end of each experiment, minus biomass nitrogen. Such nitrogen balances have been used previously for estimating denitrification loss from the plant soil system (Paul & Clark 1996).

Statistical analyses

We conducted eight experiments, each over a 23 d interval corresponding to exponential growth of the plants. The data for yield, nitrogen mass balances and tissue N contents were analysed using a split-plot design (General Linear Models Procedure, SAS 1986), where $[CO_2]$ represented the main plot treatments and NO_3^- concentration in the root zone the subplot treatments. The NO_3^- consumption and transpiration trajectories (Figs 4 & 5) were analysed according to a split-plot design with repeated measures, where daily NO_3^- consumption or apparent transpiration rates were the repeated measure. Again, CO_2 concentration represented the main plot treatment and NO_3^- concentration in the root zone the subplot treatments.

RESULTS

As expected, aerial CO_2 concentration had a significant effect on biomass production ($P = 0{\cdot}015$, Fig. 2). Root zone NO_3^- concentration, on the other hand, did not significantly influence total biomass ($P = 0{\cdot}26$, Fig. 2). Canopies grown under an elevated CO_2 concentration of 1000 μmol mol^{-1} yielded $\approx$ 16·3% more biomass than ambient [CO_2] treatments when the root zone NO_3^- concentration was 100 mmol m^{-3}. When the NO_3^- concentration was sustained at 1000 mmol m^{-3}, canopies grown under 1000 μmol mol^{-1} CO_2 yielded 15·2% more biomass (Fig. 2). Root biomass production increased slightly more than shoot biomass under elevated [CO_2]. Root biomass represented 14·5% of the total when the NO_3^- concentration was 100 mmol m^{-3}, and this value increased to 16·1% when the CO_2 concentration was raised to 1000 μmol mol^{-1} (Fig. 3). When the NO_3^- concentration was 1000 mmol m^{-3}, the percentage of root biomass increased from 13·1% under ambient [CO_2] to 14·5% under elevated [CO_2] (Fig. 3).

Nitrate consumption

Data describing NO_3^- consumption over the entire 23 d growing period showed highly significant fits to second order polynomial curves (Table 1). For the first 10 d following first illumination, NO_3^- consumption rates increased exponentially (Table 1). This observation would be expected because plant growth rates increase exponentially over the first 10 d growing in these systems, hence nitrogen absorption would increase to keep pace with demand. The rates then levelled off (Fig. 4a,b) because the canopies fill and light interception reaches its maximum, causing growth rates to decline (see Smart *et al.* 1994). The consistency of these responses and observed differences between various treatments indicated that the systems controlling NO_3^- concentration accurately tracked NO_3^- consumption by plants and microbial organisms in the system.

Daily NO_3^- consumption rates under 1000 μmol mol^{-1} CO_2 were significantly greater over time than the rates observed under 360 μmol mol^{-1} CO_2 ($P < 0{\cdot}01$, Fig. 4a,b). Root zone NO_3^- concentration, on the other hand, had no statistically significant effect upon NO_3^- consumption (Fig. 4, compare a with b). The total amount of NO_3^--N consumed, derived from the nitrogen mass balances assembled at the conclusion of each experiment, reflected the differences we observed in daily NO_3^- consumption rates (Table 2). For example, higher NO_3^- consumption rates by the canopies growing under 1000 μmol mol^{-1} CO_2 directly corresponded to an increase in the amount of nitrogen in standing biomass at the end of each experiment (NO_3^--N plus organic-N contained in the total standing biomass). But the total NO_3^--N consumed was much greater than the amounts contained in standing biomass (Table 2) because nitrogen loss from these systems, the difference between biomass nitrogen and NO_3^--N consumed, were large. Overall, when the root zone NO_3^- concentration was 1000 mmol m^{-3}, nitrogen loss averaged 8·41 ± 1·12 g

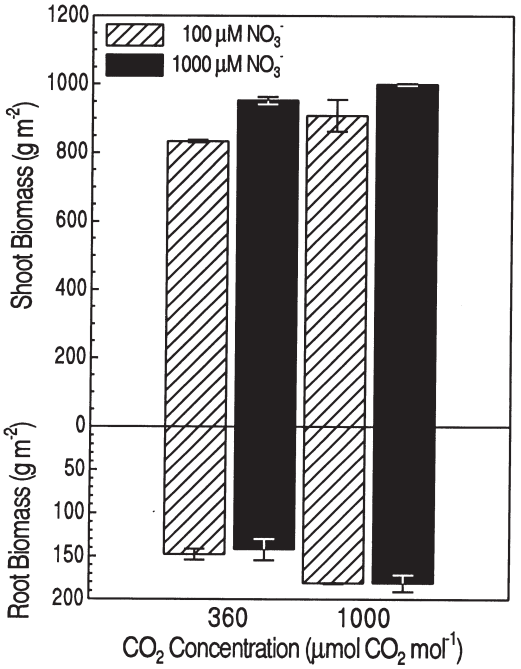

Figure 3. Shoot and root biomass production (g m^{-2}) by wheat (*Triticum aestivum* cv. Veery 10) grown as 0·8 m^2 canopies in a 28 m^3 controlled environment chamber (Fig. 1). Shown are the means and standard errors of the means for four 23 d grow-outs that were conducted under two CO_2 concentrations and two root zone NO_3^- concentrations as indicated.

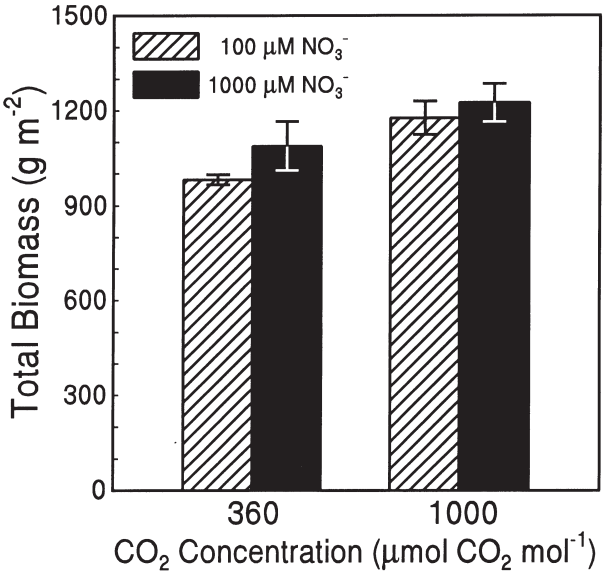

Figure 2. Total biomass production (g m^{-2}) by wheat (*Triticum aestivum* cv Veery 10) grown as 0·8 m^2 canopies in a 28 m^3 controlled environment chamber (Fig. 1). Shown are the means and standard errors of the means for four 23d grow-outs that were conducted under two CO_2 concentrations and two root zone NO_3^- concentrations as indicated.

Table 1. Equations describing NO_3^- consumption by root zones of 0·8 m^2 wheat canopies grown under two CO_2 concentrations and two NO_3^- concentrations as indicated. The exponential equations were fitted using a least squares estimator (SAS Institute 1986) where the dependent variable was NO_3^- consumption (mmol NO_3^--N m^{-2} d^{-1}) and time (t) was the independent variable. The quadratic equations were determined using stepwise polynomial regression (Cohort Software)

CO_2 (μmol mol^{-1})	NO_3^- (mmol m^{-3})	Exponential (10 d)	R^2	$P > F$	Quadratic (23 d)	R^2	$P > F$
360	100	$y = 15{\cdot}04e^{0{\cdot}299t}$	0·789	0·0004	$y = -27{\cdot}13 + 33{\cdot}49x - 1{\cdot}31x^2$	0·919	0·001
360	100	$y = 14{\cdot}82e^{0{\cdot}301t}$	0·851	0·0001	$y = -36{\cdot}38 + 34{\cdot}34x - 1{\cdot}21x^2$	0·966	0·001
360	100	$y = 6{\cdot}22e^{0{\cdot}402t}$	0·876	0·0001	$y = -29{\cdot}26 + 21{\cdot}36x - 0{\cdot}15x^2$	0·973	0·001
360	100	$y = 11{\cdot}82e^{0{\cdot}275t}$	0·962	< 0·0001	$y = -40{\cdot}66 + 26{\cdot}58x - 0{\cdot}84x^2$	0·891	0·002
360	1000	$y = 12{\cdot}62e^{0{\cdot}322t}$	0·759	0·0006	$y = -29{\cdot}89 + 33{\cdot}15x - 1{\cdot}23x^2$	0·939	0·001
360	1000	$y = 14{\cdot}19e^{0{\cdot}319t}$	0·777	0·0007	$y = -45{\cdot}16 + 42{\cdot}12x - 1{\cdot}81x^2$	0·942	0·001
360	1000	$y = 7{\cdot}77e^{0{\cdot}381t}$	0·755	0·0011	$y = -37{\cdot}19 + 31{\cdot}42x - 0{\cdot}94x^2$	0·988	0·001
360	1000	$y = 18{\cdot}82e^{0{\cdot}277t}$	0·910	< 0·0001	$y = -55{\cdot}79 + 48{\cdot}43x - 3{\cdot}10x^2$	0·869	0·004
1000	100	$y = 10{\cdot}69e^{0{\cdot}355t}$	0·834	0·0002	$y = -41{\cdot}44 + 37{\cdot}30x - 1{\cdot}36x^2$	0·916	0·001
1000	100	$y = 6{\cdot}94e^{0{\cdot}413t}$	0·839	0·0002	$y = -52{\cdot}25 + 36{\cdot}37x - 0{\cdot}95x^2$	0·941	0·001
1000	100	$y = 11{\cdot}31e^{0{\cdot}397t}$	0·869	0·0002	$y = -39{\cdot}86 + 34{\cdot}86x - 0{\cdot}29x^2$	0·969	0·001
1000	100	$y = 11{\cdot}61e^{0{\cdot}366t}$	0·856	0·0001	$y = -33{\cdot}99 + 37{\cdot}04x - 1{\cdot}10x^2$	0·918	0·001
1000	1000	$y = 8{\cdot}41e^{0{\cdot}409t}$	0·731	0·0012	$y = -33{\cdot}91 + 40{\cdot}08x - 1{\cdot}46x^2$	0·822	0·006
1000	1000	$y = 9{\cdot}98e^{0{\cdot}371t}$	0·834	0·0002	$y = -42{\cdot}95 + 34{\cdot}06x - 0{\cdot}69x^2$	0·949	0·001
1000	1000	$y = 8{\cdot}99e^{0{\cdot}439t}$	0·785	0·0015	$y = -46{\cdot}79 + 41{\cdot}37x - 0{\cdot}89x^2$	0·975	0·001
1000	1000	$y = 13{\cdot}42e^{0{\cdot}331t}$	0·917	< 0·0001	$y = -36{\cdot}63 + 34{\cdot}26x - 1{\cdot}01x^2$	0·932	0·001

m^{-2} (mean ± SE, n = 8) as compared with 6·60 ± 1·30 g m^{-2} when the root zone NO_3^- concentration was 100 mmol m^{-3}. For aerial CO_2 concentration, nitrogen loss was 9·53 ± 1·21 g m^{-2} (mean ± SE, n = 8) when the CO_2 level was 1000 μmol mol^{-1} as compared with 5·49 ± 0·75 g m^{-2} when it was 360 μmol mol^{-1}.

Specific leaf and root contents of total-N, organic-N and NO_3^--N

Wheat grown in a CO_2 concentration of 1000 μmol mol^{-1} did not accumulate more total nitrogen (NO_3^--N plus organic-N) per unit of shoot or root biomass than wheat grown in ambient [CO_2] (Table 3). However, both roots and shoots grown in 1000 μmol mol^{-1} CO_2 contained less organic-N and higher amounts of NO_3^--N than roots and shoots of canopies grown under 360 μmol mol^{-1} (Table 3). These differences were consistent for both of the NO_3^- treatments. The NO_3^- treatments, on the other hand, had no statistically significant effect upon total-N, organic-N or NO_3^- accumulation in root or shoot tissues (Table 3). Although wheat grown in 1000 μmol mol^{-1} CO_2 had diminished leaf organic-N contents, it did not result in lower organic-N per unit area (Table 2). All of the canopies had the same quantities of organic-N per square meter of surface area (Table 2). However, canopies grown under elevated [CO_2] did have greater quantities of NO_3^--N even when it was expressed on a unit area basis (Table 2), directly reflecting that NO_3^- accumulated in roots and leaves (Table 3).

We calculated a nitrogen use efficiency term (NUE) based on the amount of biomass produced per unit of nitrogen consumed by the root zone. This NUE term (g biomass per mg N consumed × 100) accounts for both nitrogen assimilation and losses. Hence, it will be smaller than more traditional terms that are based only on the amount of nitrogen assimilated (e.g. Hocking & Meyer 1991). NUE averaged 2·31 ± 0·35% for canopies grown under 360 μmol mol^{-1} CO_2 (mean ± range of means, n = 8), and dropped to 2·18 ± 0·39% under 1000 μmol mol^{-1}. The differences between ambient and elevated [CO_2] were not statistically significant.

Transpiration

Six of the eight experiments were closely monitored for transpiration losses. As expected, elevated [CO_2] lowered transpiration water loss, in this case by more than 30% over the course of the growing period (Fig. 5a,b). Root zone NO_3^- concentration, on the other hand, had no statistically significant effect upon transpiration (Fig. 5, compare a with b). When the data were pooled for the NO_3^- treatments, a total of 180·2 ± 12·1 kg water (mean ± one standard error, n = 6) was transpired over 23 d from canopies grown under elevated [CO_2], versus 266·3 ± 13·3 kg water for canopies grown under ambient [CO_2].

DISCUSSION

Biomass production

Wheat responded to elevated CO_2 concentration through an increase in root biomass production (Fig. 2). These

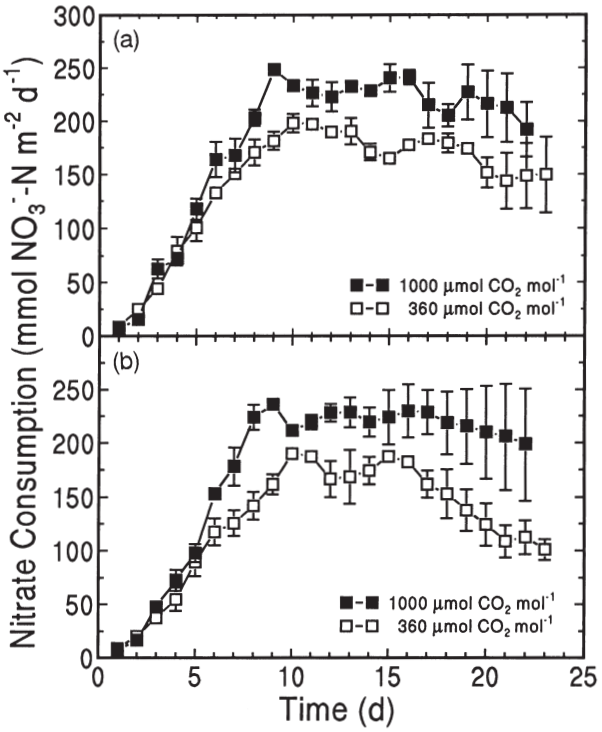

Figure 4. Nitrogen consumption rates (mmol NO_3^--N consumed m^{-2} d^{-1}) for 0·8 m^2 high-density wheat canopies (*Triticum aestivum* cv. Veery 10) growing in a controlled environment chamber. Day/night temperatures were 22·5 /19·5 °C for an 18/6 h light/dark cycle with a photosynthetic photon flux density at canopy height of ≈ 1100 μmol m^{-2} s^{-1}. Shown are the means and standard errors of the means for observations from four 23d experiments (n = 4) that began 5d following emergence, in solutions containing (a) 1000 mmol m^{-3} NO_3^-, or (b) 100 mmol m^{-3} NO_3^- and under either 360 μmol mol^{-1} CO_2 (□) or 1000 μmol mol^{-1} CO_2 (■).

results support evidence from microcosm studies conducted in soils showing that elevated [CO_2] increases carbon partitioning to roots (Hungate *et al*. 1997). Elevated [CO_2] increased root:shoot ratio in this investigation by 11·0% when the root zone NO_3^- concentration was 100 mmol m^{-3} and 11·1% when it was 1000 mmol m^{-3} NO_3^- (Fig. 2). This slight shift in allocation is accompanied by a 24% increase in root zone respiration by canopies maintained under elevated [CO_2] (Monje & Bugbee 1998). Our observations do not support that an increase in root biomass production was a direct compensatory response for higher nitrogen demand under high [CO_2] because elevated [CO_2] did not significantly increase total nitrogen absorbed or assimilated per unit area of biomass (Table 2). Our results do suggest that root growth was probably more carbon limited than shoot growth at 360 μmol mol^{-1} CO_2.

NO_3^- absorption

Our hypothesis that elevating CO_2 concentration would enhance NO_3^- absorption was not strongly supported by the results of this investigation. Although elevated [CO_2] appeared to raise the amount of nitrogen absorbed by plants (total-N in plant biomass; Table 2) the apparent increase of 9·7% when the root zone NO_3^- concentration was 100 mmol m^{-3} and 15·1% when it was 1000 mmol m^{-3} (Table 2) was not statistically significant (P = 0·16). The total amount of nitrogen in plant biomass at the conclusion of such experiments is the net of several processes that include not only root absorption, but also microbial assimilation onto root surfaces and leaf nitrogen trace gas emissions. As pointed out previously, the microbial component on root surfaces is extremely small (< 1%, Smart *et al*. 1995). Leaf trace gas losses include that of NH_3 (Farquhar *et al*. 1980), nitrogen dioxide (Thoene *et al*. 1996) and nitrous oxide (D.R. Smart, unpublished results). Ammonia is the nitrogen trace gas that is emitted in largest quantities by plants (Farquhar *et al*. 1983). Our preliminary measures of NH_3 emissions indicated that during the same 23 d growth period only ≈ 1·4 mg NH_3–N m^{-2} d^{-1} were being emitted from the 360 μmol mol^{-1} CO_2 treatments and 1·2 mg NH_3–N m^{-2} d^{-1} from canopies grown in the 1000 μmol mol^{-1} CO_2 treatments (O.J. Monje and D.R. Smart, unpublished results). These emission rates would account for less than 1% of the quantity of biomass-N that accumulated in ambient and elevated [CO_2] treatments. Thus, N-loss

Table 2. Nitrogen balances for wheat canopies grown in a contained environment under two CO_2 concentrations and with two root zone NO_3^- concentrations as indicated. Shown are the means ± the standard error of each mean, in g m^{-2} of surface area, for four 23 d experiments that were conducted under 360 μmol mol^{-1} CO_2 (n = 4), and four 23 d experiments conducted under 1000 μmol mol^{-1} CO_2 (n = 4). Nitrogen consumed was measured directly as described in the text. Biomass nitrogen, organic nitrogen and nitrate nitrogen were derived from tissue analyses that are summarized in Table 3. Nitrogen loss represents the difference between the total nitrogen consumed over 23 days and that absorbed and assimilated into plant and microbial biomass. Means within the same column followed by the same lower case letter do not differ significantly (LSD, α = 0.1)

NO_3^- (mmol m^{-3})	CO_2 (μmol mol^{-1})	Nitrogen consumed	Biomass nitrogen	Organic nitrogen	Nitrate nitrogen	Nitrogen loss
100	360	39·5 ± 2·1^{a}	34·6 ± 2·0^{a}	23·8 ± 1·3^{a}	10·8 ± 0·5^{a}	4·9 ± 1·5^{a}
100	1000	45·7 ± 5·8^{ab}	36·4 ± 3·9^{a}	23·1 ± 2·4^{a}	13·3 ± 1·0^{ab}	9·3 ± 2·4^{ab}
1000	360	43·1 ± 3·7^{ab}	37·1 ± 3·0^{a}	25·4 ± 2·4^{a}	11·5 ± 1·1^{a}	6·0 ± 0·5^{a}
1000	1000	49·0 ± 5·3^{b}	38·2 ± 4·3^{a}	21·9 ± 2·2^{a}	16·4 ± 2·7^{b}	10·8 ± 1·4^{b}

Nitrate concentration (mmol m^{-3})	Ambient CO_2 concentration (μmol mol^{-1})	Total nitrogen (g kg^{-1})	Organic nitrogen (g kg^{-1})	Nitrate nitrogen (g kg^{-1})
		Shoots		
100	360	49·4 ± 0·6[a]	33·0 ± 0·7[a]	16·4 ± 0·5[a]
100	1000	48·8 ± 0·8[a]	29·4 ± 1·1[b]	19·4 ± 1·2[b]
1000	360	49·9 ± 0·8[a]	33·2 ± 0·9[a]	16·8 ± 0·4[a]
1000	1000	50·9 ± 0·3[a]	28·8 ± 1·2[b]	22·1 ± 1·0[b]
		Roots		
100	360	33·5 ± 0·9[a]	24·4 ± 1·4[a]	9·1 ± 1·8[a]
100	1000	31·6 ± 1·3[a]	18·1 ± 1·3[b]	13·2 ± 1·5[b]
1000	360	35·0 ± 1·0[a]	26·7 ± 1·8[a]	7·2 ± 1·3[a]
1000	1000	32·1 ± 1·1[a]	18·5 ± 1·0[b]	12·3 ± 1·9[b]

Table 3. Total nitrogen, organic nitrogen and nitrate nitrogen, g kg^{-1}, for wheat canopies grown in contained environments under two CO_2 concentrations and with two root zone NO_3^- concentrations as indicated. Shown are the means ± the standard error of each mean for four 23 d experiments conducted under 360 μmol mol^{-1} CO_2 (n = 4), and four 23 d experiments conducted under 1000 μmol mol^{-1} CO_2 (n = 4). Means within the same column followed by the same lower case letter do not differ significantly (LSD, α = 0·1)

from leaves also played a minor role in determining the final amount of nitrogen contained in standing biomass, and CO_2 concentration did not influence this loss.

Next to root absorption, nitrogen loss comprised the largest fraction of root zone NO_3^- consumption. From 11·2% (360 μmol mol^{-1} CO_2, 100 mmol m^{-3} NO_3^-) to 27·6% (1000 μmol mol^{-1} CO_2, 1000 mmol m^{-3} NO_3^-) of the NO_3^--N consumed in the root zone was not recovered in the final biomass-N (lowest and highest percentage for the eight individual experiments). Both elevated [CO_2] and NO_3^- concentration in the rooting zone enhanced such nitrogen loss (Table 2). There are several nitrogen loss pathways in this system including denitrification losses from the root zone, leaf NH_3 emissions, and leaf emissions of other oxidized nitrogen trace gases (see Farquhar *et al*. 1983). Leaf nitrogen trace gas emissions have been discussed and cannot account for the nitrogen loss quantities we observed. Denitrification is the dissimilatory reduction of nitrate (NO_3^-) or nitrite (NO_2^-) to gaseous nitrous oxide (N_2O) and dinitrogen (N_2) by heterotrophic facultatively anaerobic microorganisms. Denitrification accounted for the largest percentage of nitrogen loss from these systems (Smart *et al*. 1997). Denitrifying bacteria have high carbon demands and this may explain why they have a propensity for colonization of the rhizosphere, where carbon availability is high, and why elevated [CO_2] increases their activity (Smart *et al*. 1997).

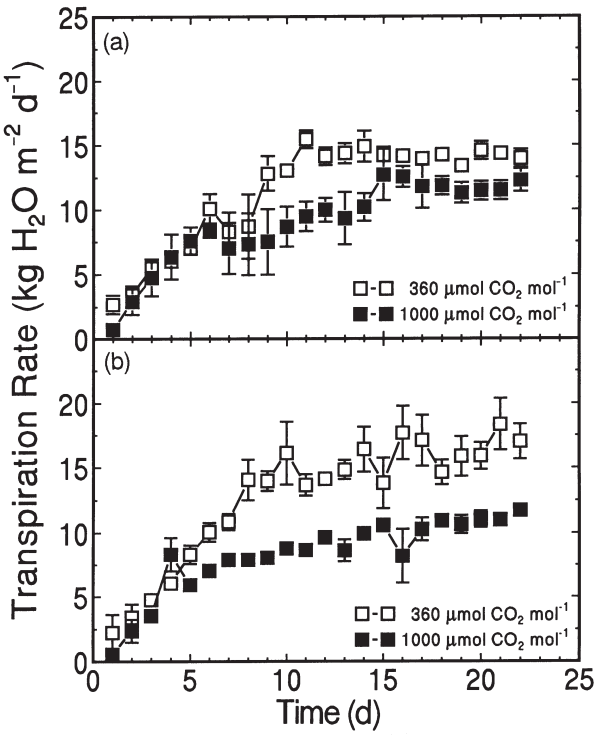

Figure 5. Apparent transpiration rates (kg H_2O consumed m^{-2} d^{-1}) for 0·8 m^2 intact wheat canopies (*Triticum aestivum* cv. Veery 10) growing in a controlled environment chamber in nutrient solutions containing either (a) 1000 mmol m^{-3} NO_3^- or (b) 100 mmol m^{-3} NO_3^-. Shown are the means and standard errors of the means (n = 3) from canopies grown under 360 μmol mol^{-1} CO_2 (□) and 1000 μmol mol^{-1} CO_2 (■) as indicated.

NO_3^- assimilation

Our hypothesis that elevated [CO_2] would increase NO_3^- assimilation also was not supported by the results of this investigation. The total amount of NO_3^- assimilated into organic-N per unit area did not change under elevated [CO_2] (Table 2). What changed was the relative proportions of organic-N and NO_3^--N. Significantly larger quantities of NO_3^- accumulated in both roots and shoots of plants grown under 1000 μmol mol^{-1} CO_2 (Table 3) and the amount that accumulated could easily account for the decline in organic-N content (Table 3).

Why does NO_3^- accumulate under elevated [CO_2]?

There are a number of possible explanations for why elevated [CO_2] caused NO_3^- to accumulate and specific organic nitrogen contents to decline in these canopies. (1) Lowered transpiration rates in 1000 μmol mol^{-1} CO_2 may have increased tissue NO_3^- concentrations because NO_3^- concentration in xylem sap is a function of absorption rate

and sap flow. Although elevated [CO_2] did not significantly change total NO_3^- absorption, it slowed transpiration losses and therefore sap flow rates (Fig. 5a,b). (2) Down-regulation of the photosynthetic apparatus may have resulted in lowered leaf organic-N concentration and exercised negative feedback control on NO_3^- assimilation. (3) Elevated intercellular CO_2 concentrations (C_i) may have resulted in competition between CO_2 and NO_3^- for photoreductant generated by the light reactions of photosynthesis, resulting in a decline in NO_3^- assimilation. We discuss each of these possibilities in greater detail below.

Our data may reflect that xylem NO_3^- concentrations may have been higher under elevated [CO_2] because 1000 μmol mol^{-1} CO_2 decreased transpiration rates by more than 30% (Fig. 5a,b) and NO_3^- concentrations were increased by ≈ 25% in shoots and more than 30% in roots (Table 3). But the possibility that lowered transpiration was the sole cause of higher tissue NO_3^- contents seems unlikely. Nitrate absorption is independent of transpiration rate (Schulze & Bloom 1984), provided that root temperature remains constant (Ali *et al*. 1996). Therefore, the rates of NO_3^- delivery to shoots, a function of xylem sap concentration × transpiration rate, would also be independent of transpiration rate because slowly transpiring plants have proportionally higher xylem NO_3^- concentrations than rapidly transpiring plants (Shaner & Boyer 1976). Xylem NO_3^- concentrations are generally too low (Shaner & Boyer 1976) to account for the quantities of NO_3^- that accumulated in root and leaf tissues in this investigation (Table 3); and NO_3^- is stored in vacuoles when the concentrations approach those reported here (Granstedt & Huffaker 1982). If the rates of NO_3^- delivery were the same for elevated and ambient [CO_2] grown canopies, then the organic nitrogen contents would be similar if no other process were interfering with NO_3^- assimilation. This was not observed (Table 3).

There are no current reports to our knowledge that directly support that down-regulation of enzyme systems associated with photosynthesis has a negative feedback effect on NO_3^- assimilation. NO_3^- is the primary signal for induction of nitrate reductase. Nevertheless, processes directly or indirectly related to carbon metabolism can also serve as signals for induction of nitrate reductase enzyme (Crawford 1995). Such factors can also determine assimilation capacity. For example, light quantity or quality (Aslam *et al*. 1979; Becker *et al*. 1992), carbon metabolites such as sucrose and glucose (Aslam *et al*. 1976; Aslam & Huffaker 1984) and CO_2 availability (Aslam *et al*. 1979; Kaiser & Brendle-Behnisch 1991) can play a regulatory role in nitrate reductase activity in a manner similar to that of NO_3^- availability. In the investigations reported here, wheat canopies were grown in high densities and exhibit high growth rates (Bugbee & Salisbury 1988). They achieve canopy closure (> 95% light attenuation at the base of the canopy) within 15 d for the elevated [CO_2] treatments and within 16 d for the ambient [CO_2] treatments (Smart *et al*. 1994). Canopies grown under elevated [CO_2] have higher leaf area indices, but the same proportion of illuminated leaf area to nonilluminated leaf area (Smart *et al*. 1994). Hence, differences in light interception or attenuation and its effect upon NO_3^- assimilating enzyme systems probably did not cause the change in NO_3^- accumulation observed here. Elevated [CO_2], like high light intensities, raises carbon metabolite concentrations in wheat leaves (Smart *et al*. 1994). For this reason one would expect NO_3^- assimilation to increase rather than decrease in response to elevated [CO_2]. But this was not observed because organic-N content declined and NO_3^- accumulated in canopies exposed to 1000 μmol mol^{-1} CO_2 (Table 3). Finally, it is known that diminished leaf nitrogen content (McKee & Woodward 1994; Hocking & Meyer 1991), soluble protein content (Rogers *et al*. 1996b) and Rubisco content (Rogers *et al*. 1996b) occurs in wheat leaves exposed to elevated [CO_2], although it should be noted that Delgado *et al*. (1994) did not observe such a decrease. If Rubisco down-regulates in response to elevated [CO_2], it is possible that decreased protein content might exercise negative feedback regulation on NO_3^- assimilation. We are not aware of any direct reports to this effect, but some investigators have noted a reduction of *in vivo* nitrate reductase activity for wheat (Hocking & Meyer 1991) and tobacco (Ferrario-Méry *et al*. 1997) growing under elevated [CO_2]. Ferrario-Méry *et al*. (1997) concluded from their investigation that high CO_2 concentration had the direct effect of accelerating nitrate reductase mRNA turnover (Ferrario-Méry *et al*. 1997).

Nitrate assimilation can be coupled to photosynthetic electron transport either directly (Robinson 1988) or indirectly by shuttling malate between the chloroplast and cytosol (Kow *et al*. 1982). Photosynthetic CO_2 assimilation is dependent on C_i and photosynthesis is generally CO_2-limited at ambient [CO_2]. When photosynthesis is CO_2-limited, photosynthetic electron transport generates sufficient reductant that adequate quantities can be diverted to the reduction of NO_3^- or NO_2^- (Robinson 1988; Bloom *et al*. 1989). For example, under light-limited conditions (restricted electron transport), NO_3^- or NO_2^- is unable to compete with CO_2 for reductant (Bloom *et al*. 1989). Elevating [CO_2] around leaves raises C_i and increases photosynthesis rates. Consequently, elevated [CO_2] may curtail NO_2^- photoassimilation because C_i would be saturating and electron transport would limit photosynthesis under these conditions. If NO_2^-, which is toxic, starts to accumulate under elevated [CO_2], then perhaps it is an appropriate candidate for a metabolic signal that leads to the acceleration of nitrate reductase mRNA message and protein turnover (Ferrario-Méry *et al*. 1997). Nitrate accumulation would then be independent of any regulation that might invoke negative feedback from organic nitrogen availability or protein synthesis.

Although the mechanisms underlying NO_3^- accumulation are not presently clear, our observations offer an alternative hypothesis for the commonly observed phenomenon that organic nitrogen contents decline under elevated [CO_2]. Our canopies were planted at very high densities in

which light, more than CO_2 concentration, more strongly limits productivity (Bugbee & Salisbury 1988). Only the uppermost 10% of the canopy was illuminated (Smart *et al*. 1994). But such conditions may better reflect natural canopies than individual plants that are more uniformly illuminated. When wheat is grown so that leaves are more uniformly illuminated, and in sand culture, both organic-N and NO_3^--N concentrations have been observed to decline in elevated [CO_2] (Hocking & Meyer 1991). When wheat is grown to maturity under elevated [CO_2], overall nitrogen contents decline independent of growth conditions (Hocking & Meyer 1991; Monje & Bugbee 1998). Nitrate is an important source of nitrogen in agricultural ecosystems (Haynes 1986) and recent evidence suggests that internal cycling of NO_3^- may be equally important in undisturbed natural ecosystems (Stark & Hart 1997). Consequently, impairment of NO_3^- assimilation could represent an important plant response to elevated [CO_2]. For example, species that rely on NO_3^- as a nitrogen source may be at a competitive disadvantage when exposed to high [CO_2], unless taxa that rely on NO_3^- photoassimilation can compensate by diverting resources to root NO_3^- assimilation or NH_4^+ acquisition.

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