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INFLUENCES OF NITROGEN SUPPLY AND ELEVATED CO_2 ON NITROGEN CONSUMPTION, NITROGEN LOSS, TISSUE NITROGEN CONCENTRATION, AND YIELD OF HYDROPONIC WHEAT

by

Karl B. Ritchie

A thesis submitted in partial fulfillment
of the requirements for the degree

of

MASTER OF SCIENCE

in

Plant Science
(Crop Physiology)

Approved:

UTAH STATE UNIVERSITY
Logan, Utah

1994

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Karl B. Ritchie

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ABSTRACT

Influences of Nitrogen Supply and Elevated CO_2 on Nitrogen Consumption, Nitrogen Loss, Tissue Nitrogen Concentration, and Yield of Hydroponic Wheat

by

Karl B. Ritchie, Master of Science

Utah State University, 1994

Major Professor: Dr. Bruce Bugbee
Department: Plants, Soils, and Biometeorology

Wheat was grown hydroponically for 23 days (early boot stage) in a controlled environment at NO_3^- concentrations of 100 and 1000 μM and CO_2 levels of 360 and 1200 μmol mol^{-1}. Nitrogen consumption and transpiration were measured daily. Tissue nitrogen concentration, total biomass, and percent root mass were measured at harvest. Nitrogen recovery and nitrogen use efficiency were calculated. Elevated CO_2 increased nitrogen consumption of the 100 μM NO_3^- treatment by 13.6% and the 1000 μM NO_3^- treatment by 21.3%. These increases were particularly evident during tillering and early grain fill. Whole plant nitrogen, shoot NO_3^-, and root NO_3^- concentrations were increased by elevated CO_2. High CO_2 increased biomass by 15% and increased percent root mass by 11%. Nitrogen recovery and nitrogen use efficiency were similar at both

CO_2 concentrations. Transpiration (L m^{-2}_{ground} d^{-1}) decreased by 40% in elevated CO_2. The 1000 μM NO_3^- treatment consumed more NO_3^- than did the 100 μM NO_3^- treatment (8.1% in ambient CO_2, 15.5% in elevated CO_2); this effect was most pronounced during the last 5 days of the experiment (flag leaf emergence and early grain fill). Percent root mass increased as N concentration decreased from 1000 to 100 μM. Nitrogen levels did not significantly affect tissue N concentration or biomass. Nitrogen losses increased as N supply increased; an average of 16% of the nitrogen added to the 100 μM NO_3^- treatment was lost, while the 1000 μM NO_3^- treatment lost 21%. Nitrogen use efficiency and transpiration were similar in both nitrogen treatments.

(103 pages)

INTRODUCTION

Growing plants at minimal levels of nitrogen is economically and environmentally necessary. Nitrogen management may need to be modified in the future because of rising atmospheric CO_2 levels that are predicted to double from the current concentration of 360 μmol mol^{-1} in the next century (Conroy et al., 1992; Conway et al., 1988). Elevated CO_2 alters nitrogen uptake and tissue concentrations, but these effects are complex and variable among species (Garbutt et al., 1990; Sage et al., 1989). The purpose of this study was to determine effects of low nitrogen supply and elevated CO_2 on nitrogen recovery, yield, daily nitrogen uptake, nitrogen use efficiency, and tissue nitrogen concentrations of wheat during rapid vegetative growth.

Wheat plants were grown hydroponically for 23 days (early boot stage) in a controlled environment at NO_3^- concentrations of 100 and 1000 μM and CO_2 levels of 360 and 1200 μmol mol^{-1}. Continuous monitoring with an ion selective electrode was used to maintain constant solution NO_3^- levels and to determine daily nitrogen removal. Daily transpiration was also measured. Yield components and tissue nitrogen content were determined at harvest. The controlled environment provided conditions that favored rapid growth.

These experiments provide information that should assist in developing nitrogen management strategies as atmospheric CO_2 continues to rise. This information may also be useful for NASA's research to grow wheat in controlled environments.

LITERATURE REVIEW

Minimal nitrogen levels

Nitrogen (N) is the mineral nutrient that most often limits crop production, and N fertilization is usually necessary to achieve maximum yields. Fertilizer N consumption is increasing by 1.3% yr^{-1} in industrialized countries and 4.1% yr^{-1} in developing countries (Burke and Lashof, 1990). While adequate N is essential for maximum yields, excessive N contributes to atmospheric and groundwater pollution. Gaseous nitrous and nitric oxide emissions produced by microbial denitrification and nitrification contribute to global warming processes (Rodhe, 1990). Because fertilizer use is increasing yearly, fertilizer N is predicted to become an increasingly significant source of nitrous oxide emissions (Burke and Lashof, 1990). Additionally, excess nitrate (NO_3^-) in the soil can leach into groundwater and pose health hazards.

Available N levels in soils are difficult to assess because N can undergo many transformations, so fertilizer recommendations often overestimate the amount of N required. Farmers might be able to reduce N applications below soil test recommendations without reducing profits or yields (Hibbard et al., 1992). Finding a minimum N level at which plants can sustain yields without reducing growth may help farmers cut fertilizer costs and reduce the risk of pollution caused by N fertilizers.

Because soils are complex and variable, hydroponic studies are used to study fundamental relationships involving nutrient concentrations, uptake, and plant

growth (Edwards and Asher, 1974). Many hydroponic studies have used N levels greater than 1 mM. However, several different plants can be successfully grown at N levels ≤ 100 μM in hydroponic solutions (Smart and Bloom, 1993). Cox and Reisenauer (1973), for example, achieved maximal yields of wheat seedlings with 100 μM NO_3^- supplied in the bulk hydroponic solution (89 μM in the root zone). These plants were grown for 14 days after emergence with two plants per flow vessel. Nutrient solution flow rates were increased exponentially to minimize N depletion from the root zone with increasing growth rate, as estimated by leaf length measurements.

Flow rates are a critical factor in sustaining minimal N levels. Using published nitrogen uptake data from Cox and Reisenauer (1973), Edwards and Asher (1974) calculated flow rates needed to limit N depletion (the difference between N concentration entering and leaving the root zone) to 5% in wheat root zones with N levels ranging from 5 to 250 μM. Flow rates of 0.40 L min^{-1} pot^{-1} were required to minimize N depletion and keep N solution concentrations at 45 μM when the root dry mass was about 0.65 g. Required flow rates increased proportionately with root mass, so that roots with a dry mass near 2.6 g required 1.6 L min^{-1} pot^{-1}.

Maximum yields in hydroponics are obtained with high growth rates, and consequently, high root densities. These large root densities have a high resistance to solution flow (Bugbee and Salisbury, 1989), and thus solution N levels may need to be increased (Edwards and Asher, 1974). Concentration

gradients of O_2 and other nutrients in the rhizosphere are minimized by rapid, uniform solution flow (Bugbee and Salisbury, 1989). The recirculating hydroponic system described by Bugbee and Salisbury (1989) allows large root densities (> 1000 km m^{-3}) and rapid flow rates ($\approx$ 0.50 L $m^{-2}_{surface}$ min^{-1}).

Chen (1989) grew wheat in a hydroponic system like that described by Bugbee and Salisbury (1989). There were no yield differences between plants grown at 1, 5, and 15 mM NO_3^-. The potential should exist to grow wheat in a hydroponic system like Chen's (1989) at NO_3^- levels near 100 μM.

Nitrogen losses

Bock (1984) cited many studies measuring fertilizer N losses of 30-70% in soils. Both soil and plant processes contribute to N losses from the root zone. When NO_3^- is the sole N source, plants can facilitate gaseous nitrogen losses by supplying denitrifiers with carbon. Plants may contribute to ammonia (NH_3) volatilization during photorespiration, and NH_3 volatilization during leaf senescence. How much N might be lost by processes influenced or caused by plants?

Microbial denitrification

Microbial denitrification is the process by which bacteria reduce NO_3^- to nitric oxide, nitrous oxide, and atmospheric nitrogen. Plants can be an important contributor to denitrification because denitrifying bacteria populations and activity may be much higher in the rhizosphere than in the bulk soil. Plants

influence denitrification by providing soluble carbon root exudates, which are a primary substrate for microorganisms (Christensen et al., 1990; Haider et al., 1985; Newman, 1985). Denitrifying bacteria are present on our hydroponic wheat roots (Smart et al., unpublished data).

Many denitrifying bacteria are facultative anaerobes, although some obligate anaerobes and aerobic bacteria strains have been cultured (Robertson and Kuenen, 1990). Therefore, heterotrophic facultative anaerobes are considered to be the most significant denitrifiers (Haynes and Sherlock, 1986). Although our bulk hydroponic solution is well aerated (> 95% saturated with oxygen), anaerobic microsites might exist in our root zones where roots overlap and plant and microbial respiration consume oxygen at the root surface faster than it can be supplied by the hydroponic solution (Hoberg and Sorensen, 1993). These conditions provide a suitable environment for facultative anaerobic denitrifiers.

Ammonia volatilization

Ammonia (NH_3) is volatilized from senescing plant leaves. NH_3 volatilization increases as tissues age and enter senescence, while NH_3 emissions before anthesis are comparatively small (Parton et al., 1988). Parton et al. (1988) measured loss rates of 60-120 ng NH_3-N $m^{-2} s^{-1}$ during presenescence; during final plant senescence loss rates increased to 200-300 ng NH_3-N $m^{-2} s^{-1}$. NH_3 losses before anthesis were similar in high and low N treatments, but after anthesis, high N plants volatilized more NH_3. NH_3 losses during senescence are usually less than 5% (O'Deen, 1989), so NH_3 volatilization losses may be much smaller

than N loss by denitrification.

Photorespiration

NH_3 is formed during the photorespiration process as oxygen competitively inhibits Rubisco. Most of this NH_3 is probably rapidly reassimilated (Woo et al., 1978). Photorespiratory release of NH_3 accounted for about 20% of total plant NH_3 emission in spring wheat (Morgan and Parton, 1989). Assuming that NH_3 volatilization during senescence accounted for 5% of total N lost (O'Deen, 1989) and that 20% of total volatilization is a result of photorespiration (Morgan and Parton, 1989), then NH_3 losses during photorespiration might account for only about 1% of all plant N losses in ambient CO_2. Plants grown in elevated carbon dioxide (CO_2) have very little photorespiration (Bowes, 1991), so photorespiratory NH_3 loss is probably a minor source of N losses.

Nitrogen losses from hydroponic systems

Hydroponic systems allow the study of N losses due to plant-mediated processes, and significant N losses have occurred in hydroponic studies. Dr. Ray Huffaker has observed N losses from wheat of more than 25% after 30 days (pers. comm.). Chen (1989) measured N losses in a system similar to ours and found losses ranging from 33 to 47% at NO_3^- levels of 1, 5, and 15 mM. He found that N losses decreased as solution N concentrations were decreased.

Nitrogen interactions with elevated CO_2

Increasing atmospheric levels of CO_2, the substrate for photosynthetic carbon

assimilation, may require that current N management practices be modified (Conroy et al., 1992; Hocking and Meyer, 1991). Atmospheric levels of CO_2 will likely double in the next century from the current level near 355 μmol mol^{-1} (Conway et al., 1988). Watson et al. (1990) predicted that ambient CO_2 concentrations will increase to near 550 μmol mol^{-1} by the middle of the next century. Elevated CO_2 alters N uptake and concentration in plant tissues because elevated CO_2 increases aboveground productivity (Acock, 1990). The effects of elevated CO_2 on N nutrition are complex and vary among species (Garbutt et al., 1990; Sage et al., 1989). If elevated CO_2 increases plant productivity without having a positive feedback effect on the N-cycle (Zak et al. 1993), then N could limit beneficial effects of elevated CO_2 on productivity. Understanding the responses of agronomic species in agricultural ecosystems to increased CO_2 may help us to modify current N fertilization practices to sustain yields while minimizing N inputs in the future.

Nitrogen use efficiency

Nitrogen use efficiency (g biomass/g plant N) is typically increased in elevated CO_2 (Cure et al., 1988; Goudriaan and De Ruiter, 1983; Hocking and Meyer, 1991; Larigauderie et al., 1988; Schmitt and Edwards, 1981), although plants grown in elevated CO_2 may require more total N. Nitrogen use efficiency increases because more biomass is produced per unit N assimilated. Nitrogen uptake is usually increased, and tissue N concentrations may be decreased by high CO_2, although this is not always the case.

Nitrogen uptake

Total N uptake per m^2 was increased by elevated CO_2 in wheat (Hocking and Meyer, 1991) and soybean (Allen et al., 1988; Cure et al., 1988). However, Wong (1979) observed no increases in NO_3^- uptake by cotton relative to ambient CO_2 controls. Hocking and Meyer (1985) measured little change in NO_3^- uptake in cocklebur over a range of N concentrations. Acock (1990) reviewed research studying the effects of elevated CO_2 on photosynthesis and plant growth, and concluded that crop plant fertilizer requirements should increase as CO_2 levels increase.

Elevated CO_2 might exacerbate N stress at low N levels. Grain yield of winter wheat was reduced at low N levels, while grain yield at high N increased 15% in elevated CO_2 (Mitchell et al., 1993). This corresponds with other research suggesting that low N supply generally reduces the effect that elevated CO_2 has on increasing growth and yield (Cure et al., 1988; Goudriaan and de Ruiter, 1983). In contrast, Hocking and Meyer (1991) found that N-stressed wheat had a larger proportional increase in dry matter production as a result of CO_2 enrichment than plants receiving ample N. Wong (1979) and Hocking and Meyer (1985) obtained similar results in studies with cotton and cocklebur, respectively.

Tissue nitrogen content

In elevated CO_2, N concentration in biomass usually decreases. Hocking and Meyer (1991) measured a 34% decrease in wheat tissue N concentrations;

NO_3^- and NO_3^- reductase concentrations were greatly reduced. Lower N concentration has been measured in soybeans (Allen et al., 1988) and several other plants (Coleman et al., 1991; Garbutt et al., 1990; Vessey et al., 1990). In wheat, Smart et al. (D. Smart, K. Ritchie, J. Stark, and B. Bugbee) (unpublished data) measured lower N concentration per unit total biomass in CO_2-enriched plants, but found no differences in N concentrations between ambient and enriched CO_2 levels when N concentration was expressed on a structural dry weight basis.

Foliar N concentration is strongly correlated with photosynthetic capacity because over half of the N in C_3 plant leaves is used for photosynthetic machinery construction (Evans, 1989). Ribulose-1,5-bisphosphate carboxylase oxygenase (Rubisco) is the largest sink for N in the photosynthetic apparatus (see Sage et al. 1989). At ambient CO_2, Rubisco in C_3 plants has a low catalytic activity (Bowes, 1991). Photorespiration is inhibited in high CO_2, so Rubisco activity is consistently reduced in high CO_2, and Rubisco concentration may also decrease (Allen et al., 1988; Sage et al., 1989).

Elevated CO_2 may increase denitrification losses

Denitrification activity is generally higher on root surfaces (or in the rhizosphere) than in bulk soil or hydroponic solution away from the root (Newman, 1985; Prade and Trolldenier, 1990). Elevating atmospheric CO_2 to 1000 μmol mol^{-1} CO_2 increased denitrification activity by more than an order of

magnitude greater on hydroponic wheat roots (Smart et al., unpublished data). Labile carbon (C) is an important substrate for denitrification. In studies by Zak et al. (1993), labile C in the rhizosphere of poplar was significantly increased by elevated CO_2, but labile C in the bulk soil was unchanged by elevated CO_2. Microbial biomass was also greater in high CO_2. Consequently, it is possible that the combination of both microbial activity (due to increased C availability) and microbial biomass could increase microbial denitrification.

Experimental objectives

Chen (1989) found that N loss from hydroponic solutions decreased from 47% to 33% as NO_3^- concentration decreased from 15 to 1 mM. Since yields were the same at all N levels, whereas N loss decreased at the lower N levels, we hypothesized that the amount of N supplied to the plants might be further decreased and N losses reduced while still sustaining high yields.

Our hypotheses presume that microbial denitrification is the primary cause of the N loss measured by Chen (1989). Because NH_3 volatilization is probably a minor source of N loss and hydroponic solution pH is maintained at a level that is not favorable to chemodenitrification (pH = 5.8), this premise seems reasonable. In my short-term experiments, wheat plants were harvested before anthesis, when very few leaves are senescent, so NH_3 volatilization should be minimal.

Smart et al. (unpublished data) previously grew wheat plants to maturity in 100 and 1000 μM NO_3^- solutions. Estimates of weekly N consumption indicated

that most of the N consumption occurred before anthesis. My short-term experiments were designed to examine the effect of elevated CO_2 on daily N use, total biomass, and N recovery on wheat plants harvested before anthesis. This total profile of nitrogen consumption and recovery will provide information for the period where N consumption is greatest.

If denitrification potential in our hydroponic system increases in elevated CO_2, then nitrogen losses might also increase. My experiments were designed to study the effect of high CO_2 on N recovery, daily N consumption, and N use efficiency in our hydroponic system.

My specific objectives were to answer the following questions:

1. *Will reducing NO_3^- concentration from 1000 to 100 μM reduce N losses?*
2. *Will high CO_2 increase N-losses?*
3. *Will 100 μM NO_3^- restrict growth at either ambient or elevated CO_2?*
4. *How will daily N consumption be affected by 100 μM NO_3^-?*
5. *How will daily N consumption be affected by elevated CO_2?*

MATERIALS AND METHODS

Six studies were conducted in a walk-in growth room at the Utah State University Agricultural Experiment Station Research Greenhouse. Trial dates were as follows: trial 1 = June 25-July 23, 1993; trial 2 = Aug. 6-Sept. 3, 1993; trial 3 = Sept. 17-Oct. 15, 1993; trial 4 = Nov. 12-Dec. 10, 1993; trial 5 = Jan. 1-Jan. 28, 1994; trial 6 = Feb. 12-March 12, 1994.

Foliar environment

CO_2 treatments

Three studies were done at ambient CO_2 (360 μmol mol^{-1}) and three studies were done at elevated CO_2 (1200 μmol mol^{-1}). The elevated CO_2 level of 1200 μmol mol^{-1} was chosen because CO_2 saturates the photosynthetic process (no photorespiration occurs) between 1000 to 1200 μmol mol^{-1}, and because several studies of photosynthesis and respiration in our lab have been conducted at this level.

Tub arrangement

The hydroponic design allowed three separate nutrient solutions to be circulated through four tubs within each of three systems. Tubs were set on a platform above the solution reservoirs. The surface of each tub measured 390 mm X 515 mm, which provided a total surface area of 0.2 m^2 per tub. Tubs were arranged in a randomized block design shown in Fig. 1.

A1	C2	B3	C4
B1	A2	C3	A4
C1	B2	A3	B4

Fig. 1. Tub arrangement with 4 replications per N treatment. Each tub appeared once in each block.

Root zone environment

The root zone environment was designed to provide rapid flow rates and ample root zone volume to sustain rapid growth rates.

Nitrogen treatments

Plants were grown for 23 days with three different levels of NO_3^-. Two of the NO_3^- treatments were kept constant at 100 μM and 1000 μM. The third NO_3^- treatment started at 4000 μM NO_3^- and depleted to about 200 μM NO_3^- at harvest. CO_2 treatments were 360 and 1200 μmol mol^{-1}. Daily N use was calculated for the 100 and 1000 μM NO_3^- treatments. The 4000 μM NO_3^- nutrient solution is widely used in hydroponic wheat experiments at Utah State Unviersity and described by Bugbee and Salisbury (1989). Total N use, N recovery, and total biomass were measured for all three N treatments.

The nutrient solution composition for all N treatments is shown in Table 1.

Table 1. Composition of the N treatment hydroponic solutions. The 100 and 1000 μM NO_3^- treatments were alike except for the initial amount of KNO_3. Nitrogen was not added to the refill solution in these treatments because N additions were controlled with syringe pumps.

	4000 μM NO_3^-		100 or 1000 μM NO_3^-	
Salt	Initial Solution	Refill Solution	Initial Solution	Refill Solution
$Ca(NO_3)_2$	1.0mM	1.0mM	0.0mM	0.0mM
$CaSO_4$	0.0mM	0.0mM	1.0mM	1.0mM
KNO_3	2.0mM	2.0mM	0.1/1.0mM	0.0mM
KH_2PO_4	0.6mM	0.5mM	0.6mM	0.6mM
$MgSO_4$	0.5mM	0.25mM	0.5mM	0.5mM
K_2SO_4	0.5mM	0.5mM	1.25mM	1.25mM
$Fe(NO_3)_3$	10μM	2.5μM	0.0μM	0.0μM
$FeCl_3$-HEDTA	25μM	5μM	20μM	20μM
$FeSO_4$-HEDTA	0.0μM	0.0μM	20μM	20μM
$MnCl_2$	3μM	3μM	3μM	3μM
$ZnSO_4$	3μM	1μM	3μM	3μM
H_3BO_3	2μM	1μM	2μM	2μM
$CuSO_4$	0.3μM	0.1μM	0.3μM	0.1μM
Na_2MoO_4	0.1μM	0.1μM	0.1μM	0.1μM
K_2SiO_3	75μM	75μM	75μM	75μM

Nitrogen monitoring and control

Nitrogen in the root zone was continuously monitored with a NO_3^- selective electrode (Bloom, 1989; Smart and Bloom, 1993). A small capacity metering

pump (Fluid Metering Inc., model Q20-2) recirculated calibrating and plant nutrient solutions between their reservoirs and the NO_3^- ion selective electrode. A small volume pump (Little Giant, model 1-EA-42) circulated deionized water through a gland on the Q20-2 pump head to prevent salt buildup on the ceramic parts. A second pump (Fluid Metering Inc., model RHOOCKC) pushed the sampled solutions across the NO_3^- selective electrode. The signal from the electrode was amplified with a high impedence amplifier and sent to a datalogger (Campbell Scientific, model CR-10) where appropriate software converted the signal to a NO_3^- concentration reading.

The N monitor was calibrated daily with 100 and 1000 uM NO_3^- solutions. This calibration procedure minimized the amount of time required for a daily calibration while retaining a high degree of accuracy. Very little drift was detectable in daily calibrations as long as the electrode tips and ground wires were kept clean.

We used two systems simultaneously to control NO_3^- concentrations in each treatment. A syringe pump (Razel Corp., model A-99) supplied 30-50% of daily N by injecting KNO_3 or $Ca(NO_3)_2$ at the same rate that NO_3^- disappeared from the solutions, while nitric acid additions from a pH controller (Omega, model PHCN-36) supplied 50-70% of daily N. The pH controller opened a solenoid (ASCO, model D8260G53V) when pH exceeded 5.8 and allowed acid solution to flow over the tip of a pH electrode in the nutrient solution reservoir. The pH was maintained at 5.8 ± 0.1 in the bulk solution. NO_3^- levels in the 100 and 1000

μM NO_3^- treatments were maintained within ±10% throughout the entire experiment.

Hydroponic system

Three identical hydroponic systems were arranged in a design similar to that of Bugbee and Salisbury (1989). Tub size, flow rate, lid construction, and tub placement were modified for this study.

Flow rates

Hydroponic solution was circulated through each tub at a flow rate of 130 ml s^{-1} tub^{-1} (flow rate measured before planting) and returned to the reservoirs beneath the tubs. By harvest, roots typically plugged some of the inlet manifold holes, and flow rates decreased by about 15% to 108 ml s^{-1} tub^{-1}. Magnetic drive pumps (Little Giant, model 4-MD-SC) recirculated the solution. Solution volume was kept constant by liquid level switches (Omega, model LV91) that opened shielded core solenoids (ASCO, catalog no. D8260G53V), which replenished solution as it was depleted by transpiration.

Oxygen concentration (% of saturation) was measured in preliminary experiments with pumps having flow rates about 20% slower than those used in our experiments. Oxygen concentration in the incoming solution was above 95% of saturation. At peak root mass, oxygen concentration at the bottom of the tub near the inlet manifold ranged between 65 and 95% (average = 80%). Oxygen concentration near the outlet ranged from 10 to 90% (average = 70%).

Root zone and total solution volume

Total root zone depth was 200 mm. For the first six days, solution volumes inside each tub were maintained at 10 mm below the lids, a tub volume of 30 L, to facilitate germination. During the remainder of the trial, 26 L of solution were inside the tubs. Total solution volume (tubs and reservoir) was 286 L during the first six days and 274 L thereafter.

Cultural conditions

The cultural environment provided conditions to maximize growth and yield.

Seeding density

Lids supporting the plants were made from two pieces of plastic grid with fiberglass windowscreen sandwiched in between them. The plastic grid was composed of cells 15 mm by 15 mm by 10 mm deep. Lids were 505 mm long and 383 mm wide. Seeds were placed in every other cell, then covered with inert media (Isolite, size CG-2, Innova Corp.). Seeding density was 1780 seeds per m^2. Seeding densities and yield determinations per unit area are based on the 0.2 m^2 surface area of the tub. Germination sample counts were conducted five days after emergence on rows 3, 9, 13, 16, 22, 27 (rows spanning lid width). Average germination for all trials was 87%. Germination for each trial is shown in table 2.

Table 2. Average germination percentages calculated from sample counts 5 days after emergence. The average is symbolized by $\bar{x}$.

	Germination (%)		
Trial	100 μM NO_3^-	1000 μM NO_3^-	4000 μM NO_3^-
1	92.2	90.7	89.3
2	92.8	92.5	89.8
3	90.8	91.0	94.5
4	88.8	90.5	85.5
5	80.3	81.2	84.3
6	79.0	83.5	80.5
$\bar{x}$	87.3	88.2	87.3

Light intensity and uniformity

Light intensity has a direct effect on photosynthesis and yield, so careful measurements are critical to uniformity. Lighting was provided by 1000-watt high pressure sodium lamps (Energy Technics). On the first day (day 0; 0 to 24 hours after emergence), plants received 4 hours of radiation at a photosynthetic photon flux (PPF) of 600 μmol m^{-2} s^{-1}. On day 1 (24 to 48 hours after emergence), 600 μmol m^{-2} s^{-1} was supplied for 16 hours. Thereafter, the photoperiod was 18 hours at a PPF above 1000 μmol m^{-2} s^{-1}. Reflectors were added on the walls to improve uniformity of light distribution. Light intensity fluctuated about 2.2% during a 30-minute time interval due to temperature fluctuations. PPF declined slowly as the lights aged. At the beginning of the first trial, the PPF at tub level averaged 1140 μmol m^{-2} s^{-1}, at 16 cm above the tub surface, it was 1183 μmol m^{-2} s^{-1}, and at

37 cm above the tub surface (canopy height at harvest), it was 1263 μmol m^{-2} s^{-1}. By the fifth trial, PPF at tub level was 1045 μmol m^{-2} s^{-1}, an 8.3% decrease. PPF levels at tub level for trial 1 are shown in Fig. 2; Fig. 3 shows PPF levels and uniformity for trial 5.

Edges of the tubs not bordering other tubs (guard rows) were shaded by three layers of windowscreen to reduce guard row effects. Table 3 compares light attenuation in a plant canopy to light attenuation through two and three layers of windowscreen.

A1 1109	**C2** 1125	**B3** 1141	**C4** 1138
B1 1142	**A2** 1150	**C3** 1182	**A4** 1177
C1 1118	**B2** 1115	**A3** 1155	**B4** 1162

Fig. 2. Trial 1 PPF intensity and uniformity. All values are expressed as μmol m^{-2} s^{-1}. Average PPF = 1141 μmol m^{-2} s^{-1}.

A1 1033	**C2** 1021	**B3** 1023	**C4** 1035
B1 1069	**A2** 1062	**C3** 1099	**A4** 1086
C1 1012	**B2** 1010	**A3** 1074	**B4** 1017

Fig. 3. Trial 5 PPF intensity and uniformity. All values are expressed as μmol m^{-2} s^{-1}. Average PPF = 1045 μmol m^{-2} s^{-1}.

Table 3. Guard row radiation attenuation. PPF values are μmol m^{-2} s^{-1}.

mm from top of canopy	center of tub	(edge) 3 layers of windowscreen	(edge) 2 layers of windowscreen
0	1100	1100	1100
60	750	750	750
100	265	225	225
170	50	35	100
360	0	0	20

Temperature

Seeds were vernalized for 4 days at 4 °C, then placed in the dark growth chamber for 5 days before receiving light. Germination temperature was 16±1 °C. After the lights were turned on, air temperatures were 22.5±1 °C daytime, and 19.5±1 °C at night. Nutrient solution temperature was consistently 1.5 °C warmer than air temperature. Air temperature was continuously measured with a

Type E thermocouple shielded from radiation and connected to a datalogger (Campbell Scientific, model 21-X). Nutrient solution temperature was monitored continuously throughout trial number three using a Type E thermocouple connected to a datalogger (Campbell Scientific, model CR-10).

Air velocity

Air velocity above the crop canopies was constant at an average speed of 0.65 m s^{-1} (Fig. 4).

A1 0.68	**C2** 0.72	**B3** 0.72	**C4** 0.68
B1 0.69	**A2** 0.56	**C3** 0.65	**A4** 0.67
C1 0.58	**B2** 0.57	**A3** 0.52	**B4** 0.57

Fig. 4. Air velocity measurements (m s^{-1}) made 30 mm above a canopy that was 170 mm high.

Relative humidity

Relative humidity was maintained above 95% while seeds germinated in the dark. Throughout the rest of the trial, relative humidity was 65±3% during the light period and 85±3% during the dark period.

Data analysis

Sample preparation

Roots, shoots, and the stem bases left in the Isolite were separated at harvest. A subsample of shoots was rinsed in DI water to wash away aluminum and sodium deposits on the leaves; the remaining shoots were not rinsed. Roots were separated into three portions. The first sample was not rinsed; N concentrations from this sample were used to calculate total N disappearing from the nutrient solution. The second sample was rinsed with DI water for 60 seconds to wash away occluded NO_3^-; the N levels in this sample were used to calculate N inside the roots. The third sample was rinsed in 100 mM HCl for 60 seconds, then DI water for 60 seconds. The acid rinse washed away occluded iron from the roots.

Plant samples were dried at 50 °C for 72 hours, then at 70 °C for 24 hours. Sample weights were recorded, then samples were ground to pass through a 1-mm screen.

Plant nitrogen measurements

Total N was analyzed by combustion (LECO, model CHN-1000, St. Joseph, MI). Precision of analysis by this instrument compares favorably with the Kjeldahl procedure (see Watson and Isaac, 1990).

NO_3^- within plant tissues was measured colorimetrically using a modified Griess-Ilosvay procedure with an autoanalyzer (QuikChem AE, Lachat

Instruments). Methods reviewed by Keeney and Nelson (1982) were modified for our plant tissues. Plant tissue (250 mg) was shaken in 50 ml of 2 M KCl for 15 minutes, refrigerated at 4 °C overnight, then filtered through Whatman 42 paper. The filtrate was diluted 10 fold, then analyzed for NO_3^-. The 2 M KCl solution contained 2 ml of 32 N H_2SO_4 L^{-1} to suppress microbial activity. Reduced N was calculated by subtracting NO_3^--N from total N.

Other nutrient analysis

Inorganic elements in plant tissues from an elevated CO_2 and ambient CO_2 trial including B, Ca, Co, Cu, Fe, K, Mg, Mn, Mo, P, S, Si, and Zn were analyzed using the Inductively Coupled Plasma (ICP) atomic emission spectrophotometer (Watson and Isaac, 1990).

Nitrogen consumption and recovery

Daily nitrogen consumption from the 100 and 1000 μM NO_3^- treatments was calculated by adding N additions from the pH controllers, syringe pumps, and pipette spikes, then measuring the net increase or decrease in solution N concentration at the beginning of each day, and subtracting the solution removed by the NO_3^- monitor (100 ml per hour). Total N disappearing from all nutrient solutions was calculated by subtracting the amount of N added from that remaining in solution at harvest. Nitrogen recovery was determined by dividing the N recovered in the plant by the N removed from the nutrient solution.

Potassium recovery

Potassium recovery was measured as a check of our mass balance approach for measuring N recovery. Potassium was selected because of its high solubility. Other elements such as calcium precipitate in the hydroponic system, thus underreporting the amount remaining in solution at the end of the trial. Potassium recovery was calculated using the same methods as N recovery, except potassium in nutrient solution and plants was measured using ICP.

Potassium analysis by ICP is sensitive to the preanalysis digestion and dilution procedure. Digesting plant tissue with nitric acid/hydrogen peroxide instead of nitric acid/perchloric acid increased potassium recovery by about 2%. Sample dilution had a greater effect on potassium recovery; proper dilution increased recovery about 15%. Measurement of potassium in the hydroponic solution had limitations, too. Potassium values of our nutrient solution were underreported from 8.1 to 22.2% (average = 16.1%).

Statistical analysis

Lid position within each system was changed six days after emergence to minimize possible position effects and allow data to be analyzed as a completely randomized design. Nitrogen treatments were randomized among systems for each trial. Data was analyzed with the assistance of Dr. Donald Sisson. When analysis of variance (ANOVA) procedures indicated statistical significance (α=0.05), least significance difference (LSD) was calculated. Yield and tissue N data were analyzed as a completely randomized design. Daily N uptake data was

analyzed using repeated measures ANOVA.

Twenty-five samples of plant material from each N treatment were analyzed for total N: eight unrinsed shoots, four rinsed shoots, four unrinsed roots, four DI water-rinsed roots, four acid rinsed roots, and one sample of stem bases. Four unrinsed shoots, two acid-rinsed roots, two unrinsed roots, and one stem base sample were analyzed by ICP. NO_3^- was measured in four shoots and four roots from each treatment.

Data analysis was complicated by problems with trials 3-6 of Mn deficiency and *Pythium* fungal infection. Therefore, many of our measurements have less replication than we originally planned.

Extenuating circumstances

The 4000 μM NO_3^- treatment became infected with *Pythium* fungus during trial 2. All N treatments became infected in Trial 3 and Trial 6. Metalaxyl ([N-(2,6-dimethylphenyl)-N-(methoxyacetyl) alanine methyl ester]) CIBA-GEIGY Corporation, Agricultural Division) was used in trials 4 and 5 as a preventative against *Pythium* infection. Metalaxyl was also added in trials 3 and 6 after *Pythium* infection symptoms were seen.

Subsequent analysis of plant tissue from trials 3-6 revealed that all plants were manganese (Mn) deficient. Trial 3 was moderately deficient (Mn levels were 10 $\mu g\ g^{-1}$; about 33% of optimum), while trials 4-6 were severely deficient (manganese levels 3-5 $\mu g\ g^{-1}$; about 10% of optimum). The Mn deficiency was caused by the use of an incorrect salt ($MgCl_2$ instead of $MnCl_2$) in the stock

solution, which had been mixed by an undergraduate student worker.

A summary of metalaxyl additions and *Pythium* symptoms is provided in Table 4.

Table 4. Summary of metalaxyl additions and *Pythium* symptoms in all trials. Visual *Pythium* symptoms were indicated by discoloration of the roots, and in severe cases, some wilting of the shoots. Visual estimates of *Pythium* infection were made as follows: slight = less than 10% of roots discolored; moderate = 10-50% discoloration; severe = more than 50% discoloration. Presence of *Pythium* was confirmed by laboratory analysis.

Trial	CO_2 (ppm)	Metalaxyl (ppm)	Pythium Visual Symptoms
1	360	0	None
2	1200	0	4000 μM N treatment moderate infection
3	360	7.5	1000 and 4000 μM severe, 100 μM moderate
4	1200	5.5	None (metalaxyl added early as preventative)
5	360	5.5	None (metalaxyl added early as preventative)
6	1200	2.25	100 and 1000 moderate, 4000 μM slight

RESULTS

Although three replicate studies were conducted at each CO_2 level, two of each of the three replications (four studies) were confounded by *Pythium* fungus, or by manganese deficiency. Most of the following discussion will compare results obtained from the 100 and 1000 μM NO_3^- treatments grown once at ambient CO_2 and once in elevated CO_2. However, the discussion of N recovery, N use efficiency, and water use efficiency utilizes data from all six trials. Detailed results of yield, nitrogen uptake, and tissue N concentrations from the experiments with the confounding factors (trials 3-6) are presented in Appendix A.

Nitrogen recovery

Nitrogen losses ranged from 5.3 to 40.0%. Nitrogen losses generally decreased as solution N supply decreased (Table 5). This trend was significant at $P = 0.11$ between the 100 and 1000 μM N treatments. There was no consistent effect of CO_2 level on N recovery (Table 6). Table E-1 displays ANOVA results for N recovery from the 100 and 1000 μM N treatments. The ANOVA for all three N treatments is shown in Table E-2.

Table 5. Percentage of N added to hydroponic system that was recovered in the plant tissue and nutrient solution at the end of the trial. The average for all treatments is denoted by $\bar{x}$; s.e. signifies standard error of the mean.

	Nitrogen Recovery (%)							
[N]	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Trial 6	$\bar{x}$	s.e.
100 μM	81.2	89.4	83.9	77.0	88.8	83.7	84.0	1.9
1000 μM	80.9	81.3	83.4	74.2	81.4	73.4	79.1	1.7
4000 μM	81.8	94.7	59.3	60.4	60.0	75.2	71.9	6.0

Table 6. Average N recovery (%) at all N levels in ambient (360 μmol mol^{-1}) and elevated (360 μmol mol^{-1}) CO_2. The average for all treatments is denoted by $\bar{x}$; s.e. signifies standard error of the mean.

	Nitrogen Recovery (%)			
	ambient CO_2		elevated CO_2	
[N]	$\bar{x}$	s.e.	$\bar{x}$	s.e.
100 μM	84.6	2.2	83.4	3.6
1000 μM	81.9	0.8	76.3	2.5
4000 μM	67.0	7.4	76.8	9.9

Growth analysis

Total biomass

Elevating CO_2 from 360 to 1200 μmol mol^{-1} increased total biomass by 16.3% at 100 μM NO_3^- and by 15.2% at 1000 μM NO_3^-. Biomass increased with increasing NO_3^- by 4.1% ($P = 0.11$) (Fig. 5). Table E-3 provides the ANOVA.

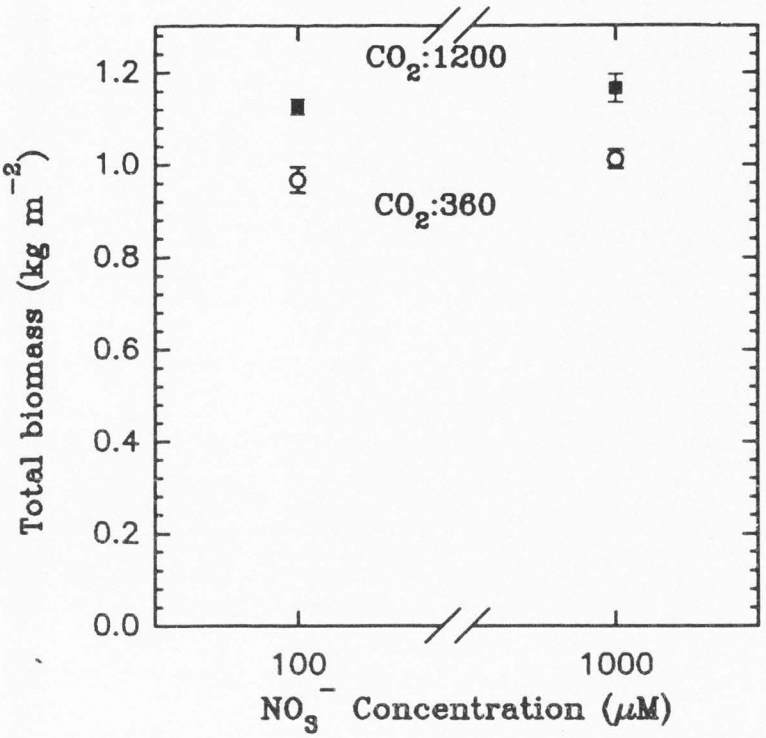

Fig. 5. Total biomass (kg m^{-2}) of wheat grown at CO_2 levels of 360 and 1200 μmol mol^{-1} and NO_3^- concentrations of 100 and 1000 μM. Values are expressed as means with standard errors.

Carbon partitioning to the roots

Percent root mass increased at both NO_3^- levels when plants were grown in elevated CO_2. Percent root mass of the 100 μM N treatment was higher than the 1000 μM N treatment at both CO_2 levels ($P < 0.01$). There was not a significant CO_2 X NO_3^- interaction (Fig. 6, Table E-4).

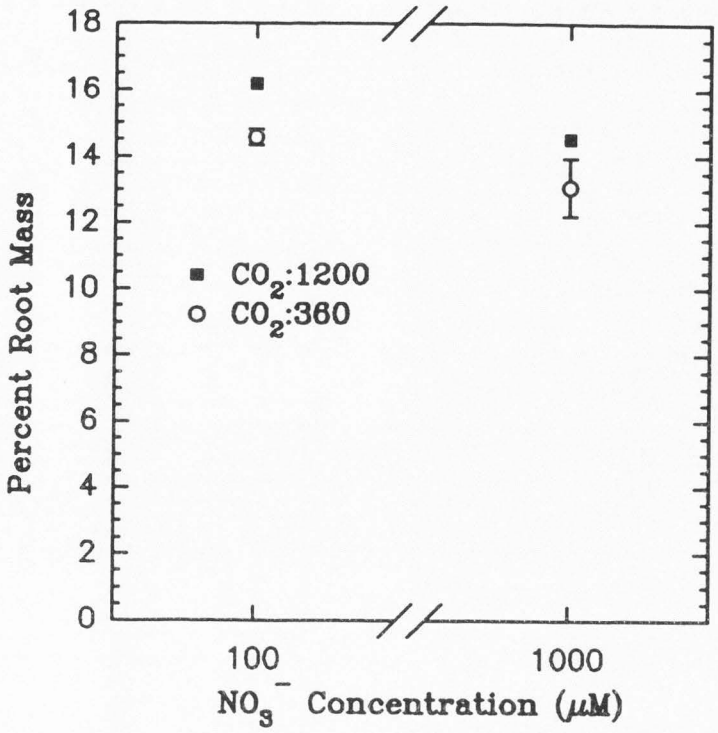

Fig. 6. Percent root mass of wheat grown at CO_2 levels of 360 and 1200 μmol mol^{-1} and NO_3^- concentrations of 100 and 1000 μM. Values are expressed as means with standard errors.

Nitrogen uptake

Ambient CO_2

Rigorous statistical analysis of all 23 days is not possible because confounding factors decreased N uptake after day 10 in some of the trials. However, N uptake during the first 10 days was unaffected by confounding factors, so a repeated measures ANOVA was calculated for days 0-8 (Table E-5). Nitrogen disappearance rates from all experiments are shown for days 0-10, but N disappearance during days 11-22 is not replicated.

There was not a significant difference in average daily uptake between the two N levels through 8 days after emergence (Fig. 7).

Elevated CO_2

Daily N disappearance followed a similar pattern in elevated CO_2 and ambient CO_2, with uptake peaking near 12 days after emergence (Fig. 8).

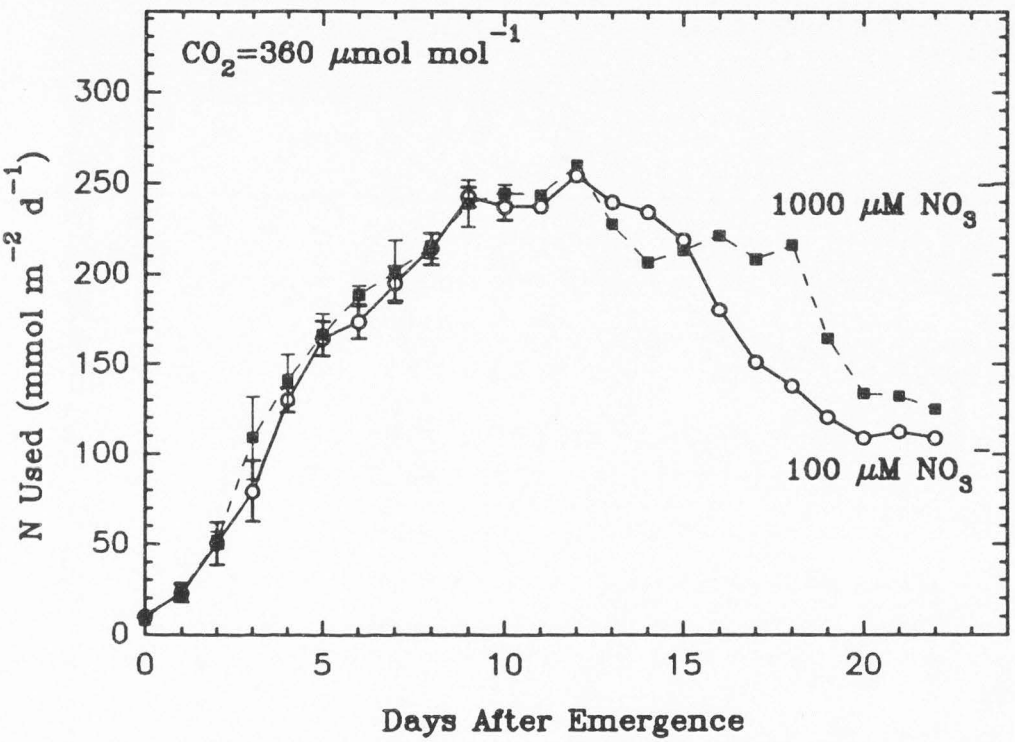

Fig. 7. Daily N disappearance (mmol N m^{-2} d^{-1}) from plants grown in 100 and 1000 μM NO_3^- at a CO_2 concentration of 360 μmol mol^{-1}. Values are expressed as means with standard errors.

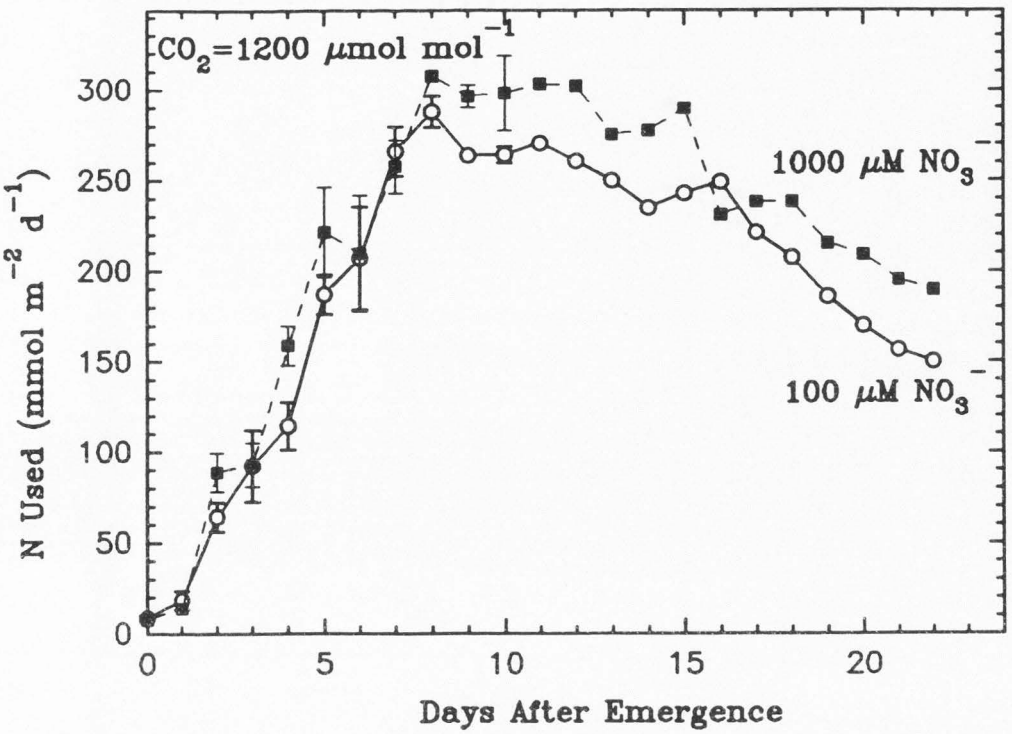

Fig. 8. Daily N disappearance (mmol N m^{-2} d^{-1}) from plants grown in 100 and 1000 μM NO_3 at a CO_2 concentration of 1200 μmol mol^{-1}. Values are expressed as means with standard errors.

Elevated CO_2 vs. ambient CO_2

Elevated CO_2 increased total N consumption in both the 100 and 1000 μM NO_3^- treatments, especially after day 6. Fig. 9 compares daily N consumption from the 100 μM NO_3^- treatment in ambient and elevated CO_2, while Fig. 10 compares the 1000 μM NO_3^- treatments in ambient and elevated CO_2. When both NO_3^- treatments are pooled within each CO_2 level, differences in N uptake caused by CO_2 are evident from days 5-22 (Fig. 11). Fig. 12 shows the ratio of average daily N uptake in elevated CO_2 compared to ambient CO_2 when N levels are pooled.

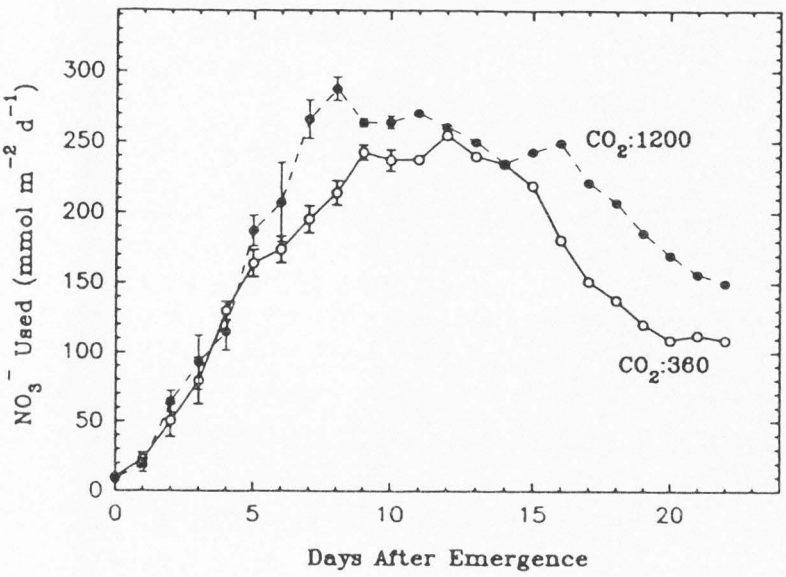

Fig. 9. Daily N disappearance (mmol N m^{-2} d^{-1}) from plants grown in 100 μM NO_3^- at CO_2 concentrations of 360 and 1200 μmol mol^{-1}. Values are expressed as means with standard errors.

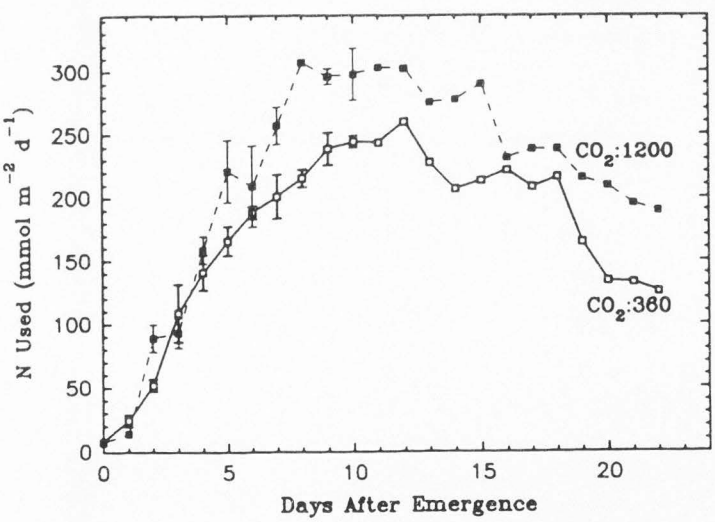

Fig. 10. Daily N disappearance (mmol N m^{-2} d^{-1}) from plants grown in 1000 μM NO_3^- at CO_2 concentrations of 360 and 1200 μmol mol^{-1}. Values are expressed as means with standard errors.

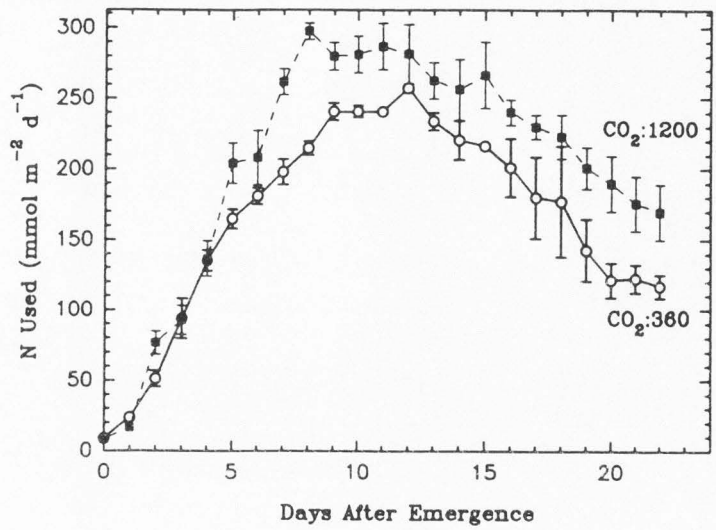

Fig. 11. Daily N disappearance (mmol N m^{-2} d^{-1}) from plants grown in 100 and 1000 μM NO_3^- at CO_2 concentrations of 360 and 1200 μmol mol^{-1}. Values from the N treatments are pooled. Values are expressed as means with standard errors.

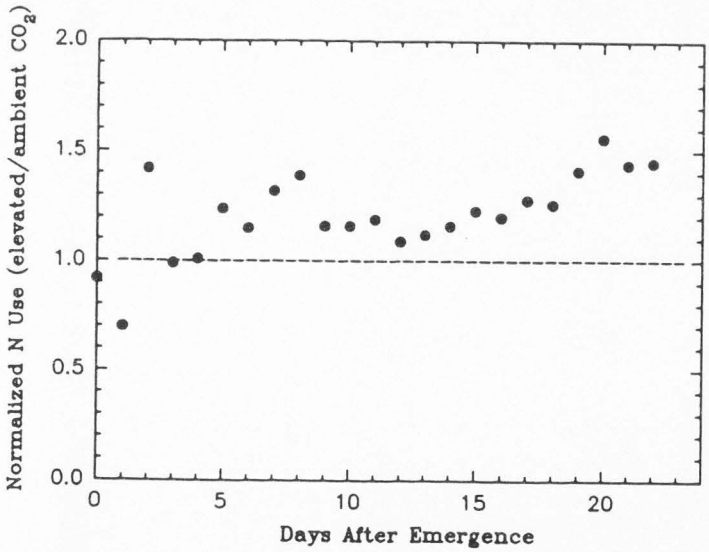

Fig. 12. Ratio of average daily uptake in elevated CO_2 (1200 μmol mol^{-1}) compared to ambient CO_2 (360 μmol mol^{-1}). Values from N treatments are pooled and expressed as a ratio (N uptake elevated CO_2:N uptake ambient CO_2).

Nitrogen use efficiency

Nitrogen use efficiency is often defined as the amount of N in plant biomass per unit of biomass produced (Hocking and Meyer, 1991). Table 7 compares nitrogen use efficiency calculated in this way. There is no effect of N or CO_2 concentration on nitrogen use efficiency.

Because we measured total N applied, N assimilated, and N lost, we may also calculate nitrogen use efficiency as the amount of N disappearing from the hydroponic system per unit of biomass produced. Calculating nitrogen use efficiency in this manner provides different results (Table 8), but there are no significant differences among any treatments (Table E-6).

Table 7. Nitrogen use efficiency ($g_{biomass}\ g_{N\ absorbed}^{-1}$). The average for all treatments is denoted by $\bar{x}$; s.e. signifies standard error of the mean.

	$[CO_2]$ (μmol mol^{-1})			
	360		1200	
$[NO_3^-]$ (μM)	$\bar{x}$	s.e.	$\bar{x}$	s.e.
100	27.4	0.78	27.2	0.38
1000	27.6	0.98	26.6	0.29

Table 8. Nitrogen use efficiency ($g_{biomass}\ g_{N\ removed}^{-1}$). The average for all treatments is denoted by $\bar{x}$; s.e. signifies standard error of the mean.

	$[CO_2]$ (μmol mol^{-1})			
	360		1200	
$[NO_3^-]$ (μM)	$\bar{x}$	s.e.	$\bar{x}$	s.e.
100	23.2	0.11	22.7	0.67
1000	23.1	1.13	20.9	0.56

Tissue nitrogen content

CO_2 effects on shoot nitrogen

Shoot total N increased in elevated CO_2 (5.1%) as compared to ambient CO_2 (4.8%, Fig. 13a). This increase was significant at both N levels (P = 0.04) and was caused by the increased shoot NO_3^- found in plants grown in elevated CO_2. Reduced N (NH_2) concentrations were nearly identical in plants grown at both CO_2 levels (Fig. 13b). ANOVAs for N content in shoots and roots are provided in Appendix E, Tables E-7, E-8, and E-9.

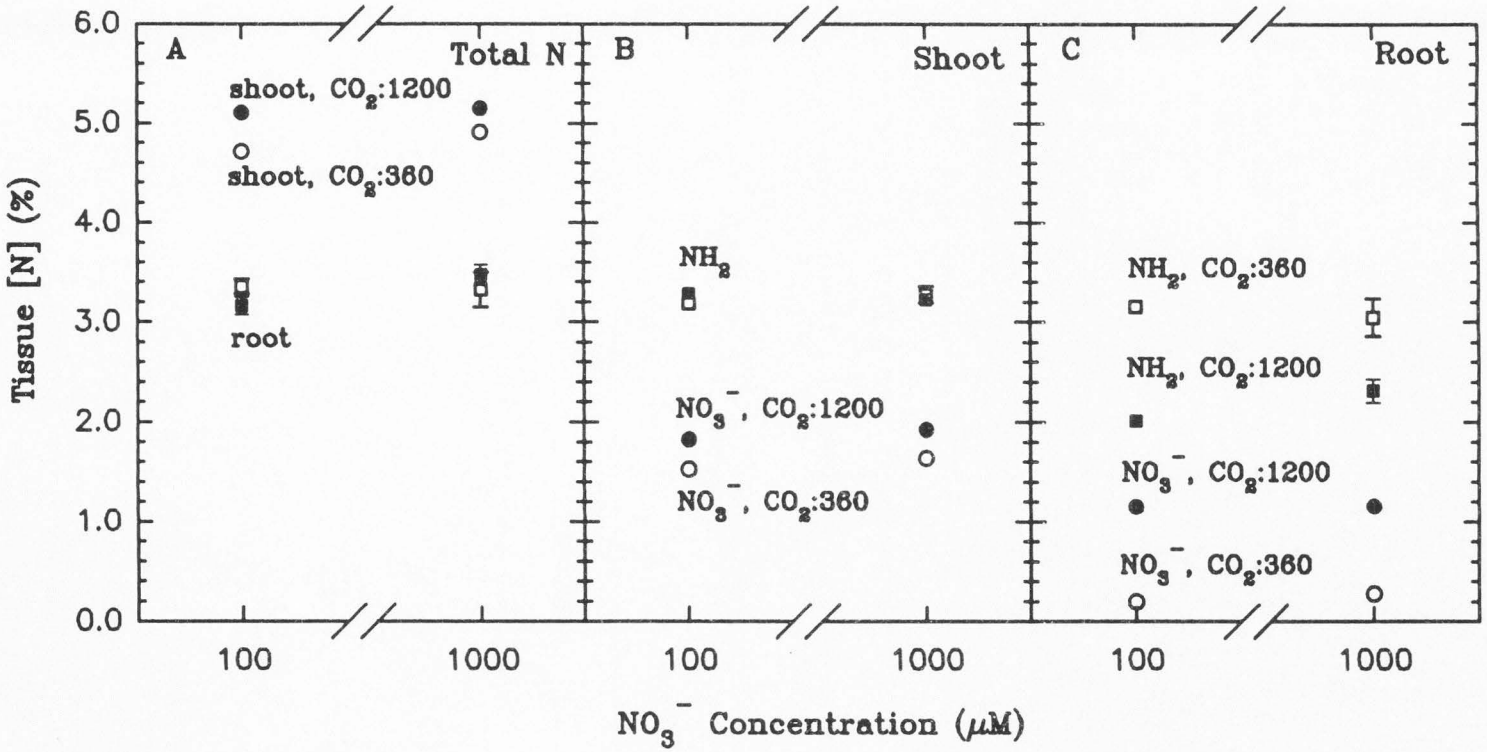

Fig. 13. Total N (%) in shoots and roots (A); N fractions (%) in shoots (B); and N fractions (%) in roots (C) of plants grown in ambient (360 μmol mol^{-1}) or elevated CO_2 (1200 μmol mol^{-1}) at average solution NO_3^- concentrations of 100 and 1000 μM.

Nitrogen effects on shoot nitrogen

In ambient CO_2, the 1000 μM NO_3^- treatment accumulated slightly more total N in the shoots (4.9%) than did the 100 μM treatment (4.7%), but this difference was not statistically significant. In elevated CO_2, tissue N concentrations were nearly identical in both N treatments (5.1%) (Fig. 13a). Shoot NO_3^- and NH_2 levels were not significantly affected by N treatments (Fig. 13b). There was no significant difference in total N concentrations between N treatments at either CO_2 level.

CO_2 effects on root nitrogen

Although root total N concentrations were similar at both CO_2 levels (Fig. 13a), plants grown in ambient CO_2 had increased levels of NH_2 (3.1% vs 2.1%) and decreased levels of NO_3^- (0.2% vs 1.1%) (Fig. 13c).

Nitrogen effects on root nitrogen

Neither root total N (Fig. 13a) nor N fractions were significantly affected by N treatment (Fig. 13c).

Recovery of other inorganic elements

Because of the difficulty of accurately measuring trace amounts of elements with ICP, some micronutrient elements had recoveries greater than 100% (Table 9), which typically occurs from trace contamination in the hydroponics system. The initial data and calculations for the 100 μM N treatment in ambient CO_2 (column one) are shown in Appendix D.

Table 9. Percent recoveries of inorganic elements added to nutrient solution. Cells marked with # indicate that more of this nutrient was measured in the solution remaining at the end of the trial than was added during the trial (see Appendix D).

	Ambient CO_2 Recovery (%)			Elevated CO_2 Recovery (%)		
	Nitrate Treatment (μM)					
Element	100	1000	4000	100	1000	4000
Boron	63.6	50.8	53.2	87.5	21.3	#
Calcium	40.5	48.6	56.1	38.5	30.2	72.2
Copper	1000.0	862.3	739.8	100.8	57.3	20.9
Iron	43.6	38.1	53.5	64.9	38.1	70.5
Magnesium	60.3	56.2	81.6	71.0	52.0	96.7
Manganese	288.1	290.6	48.8	11.8	3.7	0.5
Molybdenum	#	#	#	87.8	#	4.2
Potassium	76.3	89.5	86.3	65.5	90.7	87.9
Phosphorus	75.1	74.3	69.9	78.9	83.1	77.0
Silicon	30.9	47.6	24.4	17.2	24.6	22.6
Sulfur	54.0	35.6	97.0	40.7	36.3	67.3
Zinc	#	#	97.7	73.1	53.0	66.7

Transpiration

Transpiration rates were similar between NO_3^- levels at each CO_2 level, but transpiration decreased in elevated CO_2. Fig. 14 shows daily transpiration per m^2 ground area. Note that plants grown in elevated CO_2 weighed about 15% more than ambient-grown plants; if transpiration were expressed per kg biomass or per m^2 leaf area, the CO_2-induced reduction in transpiration rate would be even

larger. As expected, plants grown in elevated CO_2 had greater water use efficiency (g_{plant} $kg_{water\ transpired}^{-1}$) (Table E-10).

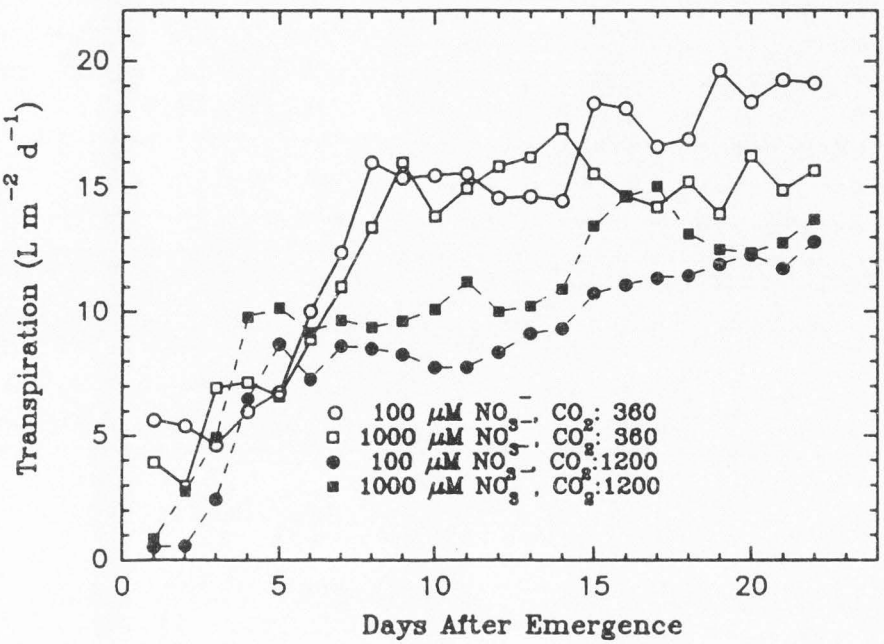

Fig. 14. Daily transpiration rate (L m^{-2}_{ground} d^{-1}) of plants grown in 100 and 1000 μM NO_3^- at CO_2 levels of 360 and 1200 μmol mol^{-1}.

DISCUSSION

Nitrogen recovery

Decreasing N levels from 1000 to 100 μM NO_3^- reduced N losses by 6.2% ($P = 0.11$). However, elevated CO_2 did not alter N recovery. These findings may be important to NASA's efforts to produce wheat in contained environments in space for two reasons. First, accumulation of gaseous products from volatile N losses such as ammonia and nitrous oxides would be undesirable in an enclosed environment. Secondly, resources such as N are expensive to supply in space, so minimal N supply levels need to be found.

Nitrogen losses measured by mass balance ranged from 5.3 to 40.0%. These results could be affected by Mn deficiencies and *Pythium* infections, as well as possible shortcomings in the mass balance N recovery approach. Because root health did not significantly affect N recovery when it was blocked in the ANOVA comparing the 100 and 1000 μM N treatments (Table E-1), it is unlikely that the Mn deficiencies and *Pythium* infections strongly affected N recovery. There are several possible sources of experimental error in the mass balance N recovery measurements, such as inexact N concentrations in the stock hydroponic solutions, solution spilling from the N monitoring system, errors in reading solution level in the syringes or pH control buckets, or improper calibration of the ion selective electrode. Great effort was taken to mimimize these errors, and they should have occurred at similar magnitudes in all trials. These errors could equally overestimate, as well as underestimate, N recovery. Therefore, although the trend

of increasing N recovery with decreasing N content was not statistically significant ($P = 0.11$), it appears to be real. The exact amount of N lost from our hydroponic systems may need to be established by more replications using mass balance measurements or by alternative methods of measuring N recovery, such as using ^{15}N tracers.

Potassium recovery is probably not an accurate reference ion for mass balance N recovery in our hydroponic systems. We had difficulty recovering more than 90% of the potassium added using the mass balance approach. Potassium recovery was often less than N recovery. Nitrogen recovery should be more accurate than potassium recovery because the instruments used to measure both solution and plant N are more accurate than those used for potassium. Potassium measurement by ICP was highly influenced by dilution and digestion procedures.

My studies did not indicate a CO_2 effect on N recovery ($P > 0.25$), although N losses due to microbial denitrification may increase in elevated CO_2. Results by Smart et al. (unpublished data) show that denitrifying enzyme activity is substantially increased in elevated CO_2. Zak et al. (1993) found that labile C (an important denitrifying bacteria substrate) in the rhizosphere significantly increases in elevated CO_2. Microbial denitrification, however, may not be a significant contributor to N losses in this hydroponic system. If denitrification occurred in my experiments at the potential rate measured by Smart et al. (unpublished data), only 0.5% of the added N would have been lost by denitrification processes. However, the denitrification rates measured by Smart et al. may not indicate the

full denitrification potential because diffusion limitations or energy source (glucose) in the reaction flasks differ from typical root zone conditions.

Ammonia volatilization may be a more important mechanism for N losses than suggested by my literature review. While these experiments were being conducted, measurements of ammonia losses from hydroponic wheat (grown in a similar environment) were made in our lab and estimated to be about 7% of the added N (Monje and Bugbee, unpublished data).

Photorespiration may have accounted for some N loss as ammonia. However, if losses from photorespiration were a major factor, N recovery in elevated CO_2 should increase (all other things being equal) because photorespiration should be minimal in high CO_2 (Bowes, 1991). Nitrogen losses did not increase in elevated CO_2 in our studies, suggesting that photorespiration was not an important source of N loss.

Determination of the importance of the mechanisms by which N is lost from our hydroponic system was beyond the scope of this study. Research conducted in this area would need to measure ammonia and nitrous oxide production. Although ammonia production is relatively easy to measure, nitrous oxide is very difficult to measure because it is quickly reduced to atmospheric nitrogen. Nitrogen isotopes or an in-line gas chromatograph would probably have to be used to measure nitrous oxide production.

Growth analysis

Total biomass

Elevated CO_2 increased total biomass by about 15%. Mitchell et al. (1993) found that elevated CO_2 increased grain yield of winter wheat by 15%. Hocking and Meyer (1991) reported a 112% increase in dry matter production due to elevated CO_2, but conceded that this response may have been much smaller if their plants had been grown as communities at commercial densities instead of one plant per pot. Several studies have found increased biomass production in elevated CO_2 in a variety of plant species (Acock, 1990; Lawlor and Mitchell, 1991).

There was no significant difference in total biomass produced between the 100 and 1000 μM NO_3^- treatments at either ambient or elevated CO_2 (Fig. 5). Previous experiments conducted in our lab suggested that plants supplied with 100 μM NO_3^- would yield less than plants supplied with 1000 μM NO_3^- in elevated CO_2 (Smart et al., unpublished data). However, these preliminary experiments differed from my current experiments in that they were conducted in a hydroponic system with a smaller root zone volume and slower flow rates (about 20%). My current studies were less likely to be N limited because a larger root zone volume and faster flow rates provide more room for root growth and allow plants to grow at lower N concentrations (Edwards and Asher, 1974). My results show that vegetative wheat can be successfully grown in hydroponic systems at NO_3^- concentrations of 100 μM. Further research should be conducted to

determine if grain yields would also be equivalent in both NO_3^- treatments.

Percent root mass

Plants grown in elevated CO_2 had a higher percent root mass than at ambient CO_2 in both N treatments (Fig. 6). Vessey et al. (1990) measured lower root mass in elevated CO_2 in soybeans. Hocking and Meyer (1991) found that wheat grown in elevated CO_2 had a lower percent root mass than in ambient CO_2. They also found that wheat grown with deficient N supply had about 25% higher root mass in elevated CO_2, and over 50% higher root mass in ambient CO_2. In our studies, percent root mass increased in elevated CO_2 by 4.7% at 100 μM NO_3^- and by 3.0% at 1000 μM NO_3^-. Although this interaction between CO_2 and NO_3^- was not significant (Table E-4), this may suggest that 100 μM NO_3^- stressed the plants for N enough to increase carbon partitioning to the roots, even though biomass was more similar ($P = 0.11$) between the N treatments than was percent root mass ($P < 0.01$).

High CO_2 might exacerbate N stress by increasing biomass production. Hocking and Meyer (1991) reported that N stress developed at a faster rate in CO_2-enriched wheat that was N deficient. It might also limit the ability of plants to take advantage of increased CO_2 availability. For example, Cure et al. (1988) cited research finding that increases in dry mass due to elevated CO_2 were reduced in low N treatments.

Nitrogen uptake

Elevated CO_2 should increase crop plant fertilizer requirements (Acock, 1990; Allen et al., 1988; Hocking and Meyer, 1991), but there is little information on the magnitude of this projected increase or the life cycle stages at which this might occur. Knowledge of the life cycle stages at which N uptake is increased by elevated CO_2 might be useful in developing fertilizer management programs as global CO_2 levels increase.

Nitrogen concentration

In ambient CO_2, daily N disappearance rates were similar in both N treatments until 16 days after emergence, at which point plants grown in 100 μM NO_3^- consumed less N. This extra uptake during the last 7 days probably accounted for the slightly higher total N concentration in the 1000 μM NO_3^- treatment, since yields and N recovery from the two treatments were similar. Since the 1000 μM NO_3^- yielded 4.6% more than the 100 μM NO_3^- treatment, this extra growth in the higher N treatment may have occurred in the last week before harvest, thus increasing N uptake.

In elevated CO_2, plants grown in 1000 μM NO_3^- began taking up more N on day 9 than the 100 μM NO_3^- treatment (Fig. 8). This extra N uptake did not result in significantly higher tissue N concentrations (Fig. 13) or biomass (Fig. 5). The 100 μM NO_3^- treatment had a higher N recovery in this experiment (89.4 vs 81.3%); therefore, some of the extra N disappearance from the 1000 μM NO_3^- treatment during days 9-22 may be N losses from the system.

CO_2 concentration

At NO_3^- concentrations of 100 μM, wheat grown in elevated CO_2 consumed more N from days 7-11 and 16-22 than in ambient CO_2. The 7- to 11-day period features tiller formation and rapid leaf expansion. The first tiller began forming at day 6, and the second tiller emerged on day 7. Leaf 3 expanded on day 7 to about 85% of full size, and then leaf 4 emerged on day 8. By day 9, leaf 4 was about 50% of full size. Therefore, high N demand during this period probably reflects high plant growth demand. During the 16- to 22-day period, the flag leaf was expanding, and the grain head was beginning to form. Plant N uptake declined during this period and may indicate that flag leaf expansion and grain fill N demands may come more from reallocation of tissue N than from root uptake. Plants were harvested in the early boot stage, when the grain head was just beginning to emerge.

At NO_3^- concentrations of 1000 μM, N consumption was increased by elevated CO_2 during days 7-15 and 19-22 (Fig. 10). Plants grown in 1000 μM NO_3^- followed similar ontogenetic development patterns as those grown in 100 μM NO_3^-. Because days 11-22 are not replicated at each CO_2 level, it is difficult to form conclusions regarding the importance of NO_3^- level (100 vs 1000) and the days later in the life cycle when CO_2 caused increased N disappearance (16-22 at ambient CO_2, 19-22 at elevated CO_2).

Pooling the values from the 100 and 1000 μM NO_3^- treatments (Fig. 11) showed that N uptake was consistently higher from day 5 to harvest in elevated

CO_2, although this difference was not always statistically significant. These increases were greatest on days 7 and 8, when the plants grown in elevated CO_2 consumed over 30% more N than ambient-grown plants, and during days 19-22, when N consumption in elevated CO_2 was 45% higher than in ambient CO_2 (Fig. 12).

This research should assist the development of N management programs in elevated CO_2 by providing useful information regarding the magnitude and timing at which fertilizer N requirements for wheat will differ from current practices. If fertilizer is applied only once, then 15-20% more fertilizer may need to be applied. Based on this research, split N applications should be increased at tillering and early boot stages by about 40% over current rates. More research studying N uptake needs to be conducted in soils with lighting and temperature conditions similar to agricultural environments to better estimate the effects that elevated CO_2 will have on current N fertilizer management practices.

Nitrogen use efficiency

Nitrogen use efficiency, whether defined as plant growth per unit N taken up or per unit N removed from the hydroponic system, was not affected by either N level or CO_2 level. Many other researchers have measured increased N use efficiency in elevated CO_2 (Cure et al., 1988; Goudriaan and De Ruiter, 1983; Hocking and Meyer, 1991; Larigauderie et al., 1988; Schmitt and Edwards, 1981), primarily because plants grown in elevated CO_2 increased in biomass while tissue N concentrations decreased. However, in my experiments, plants grown in

elevated CO_2 accumulated more tissue N than those grown in ambient CO_2. This increase in tissue N offset the expected improvement in N use efficiency due to increased growth. My tissue N results should be replicated, though, because in my experiments that were Mn deficient, total tissue N increased in ambient CO_2.

Nitrogen content

CO_2 effects on shoot nitrogen

Elevated CO_2 increased shoot total N (5.1%) as compared with ambient CO_2 (4.8%) (Fig. 13a). This finding is not consistent with many published results which have found that tissue N concentrations either remained constant or decreased in elevated CO_2 (Allen et al., 1988; Hocking and Meyer, 1991; Garbutt et al., 1990; Vessey et al., 1990). However, most of the increase in N concentration in elevated CO_2 was attributed to increased shoot NO_3^- found in plants grown in elevated CO_2, while reduced N concentrations were similar in plants grown at both CO_2 concentrations (Fig. 13b). Hocking and Meyer (1991) measured a decrease in nitrate reductase activity in wheat leaves. If nitrate reductase activity decreases but NO_3^- transport to the leaves does not decrease at the same magnitude, then this might account for the shoots accumulating more NO_3^- in elevated CO_2.

CO_2 effects on root nitrogen

Although root total N concentrations were similar at both CO_2 levels (Fig. 13a), N partitioning was altered by CO_2. Roots grown in ambient CO_2 had

substantially more reduced N (3.1% vs. 2.1%) (Fig. 13c). Hocking and Meyer (1991) also measured lower concentrations of reduced N in elevated CO_2. However, in our experiments, levels of NO_3^--N were much lower in ambient CO_2 (0.2% vs. 1.1%). This finding differs sharply from that of Hocking and Meyer (1991), who measured lower concentrations of root NO_3^--N in elevated CO_2. Their studies were conducted in soils that may have experienced limitations to N supply by mass flow to the root surface due to reduced transpiration in elevated CO_2. The rapid flow rates of our hydroponic system should minimize mass flow limitations.

More replication is necessary to establish whether my results are significant or due to experimental error, especially since my later experiments (although Mn deficient) followed trends similar to those observed by Hocking and Meyer (1991). Many published studies of elevated CO_2 effects are limited by the lack of replication, or pseudoreplication of the CO_2 treatment. CO_2 enrichment is expensive and replication using single growth chambers is time consuming. In my experiments, I attempted to specifically avoid the problem of pseudoreplication by repeating each experimental treatment three times in the same growth chamber (CO_2 concentration). Unfortunately, only one trial at each CO_2 level had adequate Mn and absence of *Pythium* by harvest. Since N concentrations were determined only at harvest, the experimental results with the confounding factors were ignored because they varied sharply from the healthy plants. Some of the differences between my investigation and others that have been reported without

replication (Hocking and Meyer, 1991) may be caused by inadequate replication.

There are also other cultural differences in addition to root zone environment (hydroponic vs. soil) between our studies and those of Hocking and Meyer (1991) that could contribute to discrepancies in results. Photoperiod was dramatically different. Their glasshouse study used natural light which averaged 25 $mol_{photons}$ d^{-1}. Our wheat was supplied 71 $mol_{photons}$ d^{-1}. Hocking and Meyer (1991) used temperatures ranging from 12 °C (min.) to 32.5 °C (max.); our temperatures were 22.5 °C/19.5 °C. Two of our experiments which were manganese deficient resulted in tissue N fractions following a trend similar to that found by Hocking and Meyer (1991), but root NH_2 and NO_3^- were lower in plants supplied elevated CO_2 (Appendix A). Our root N fraction results may deviate from those of Hocking and Meyer (1991) due to experimental error, or it may be that our conditions of fast growth actually influenced roots to accumulate more NO_3^- in elevated CO_2. Nitrogen uptake rates (Fig. 15) show that plants grown in elevated CO_2 take up more N daily, particularly during the last half of the experiments (11-22 days after emergence). It might be that this extra N is accumulated in the roots as NO_3^- while awaiting transport to the leaves.

Root zone effects on plant nitrogen

Shoot NO_3^- and NH_2 levels were not significantly affected by N treatments (Fig. 13b), suggesting that the 100 μM NO_3^- treatment supplied adequate N. Similarly, N concentration did not significantly affect root total N or N fractions within CO_2 levels, which also suggests that 100 and 1000 μM NO_3^- provided

equally adequate N supply.

Transpiration

Transpiration rates were similar between NO_3^- levels at each CO_2 level (Fig. 14), but plants grown in CO_2 had a higher water use efficiency. This finding is consistent with those of other researchers (Acock, 1990; Lawlor and Mitchell, 1991). Plants growing in elevated CO_2 open their stomates less than ambient-grown plants, thus increasing resistance to water diffusion from the leaf and reducing transpiration. Since transpiration within each CO_2 level is dependent on absorbed radiation, the 100 and 1000 μM NO_3^- treatments should have similar transpiration rates.

CONCLUSIONS

Future research should replicate minimal N supply, N recovery, daily N uptake, and N use efficiency in ambient and elevated CO_2. In addition to 23-day studies, these areas should be addressed throughout the full life cycle of wheat.

This research suggests that 100 μM NO_3^- was adequate N to sustain total biomass production in our hydroponic system. Nitrogen losses decreased as solution N decreased. Therefore, N supply less than 100 μM might further reduce N losses without reducing yields.

These studies clearly show that N is lost from our hydroponic systems. Although we measured N losses between 5 and 40%, we cannot account for the importance of the mechanisms that cause N losses. Future research should explore these N loss mechanisms, as well as their relative importance. An ammonia trap, N isotopes, and an in-line gas chromatograph might be used for this research.

Because our research in a rigorously controlled environment shows that elevated CO_2 increases N uptake by 17.6%, particularly during tillering and early grain fill, daily N uptake as influenced by elevated CO_2 should be studied in field conditions.

Nitrogen use efficiency (plant biomass/unit N absorbed by the plant) was similar at all CO_2 and N levels in our experiments. This contradicts the results of Hocking and Meyer (1991). Our results should be further replicated to determine their possible significance.

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APPENDICES

APPENDIX A: EXPERIMENTS WITH CONFOUNDING FACTORS

This appendix compares yield, N uptake, and tissue N results from experiments affected by *Pythium* infection, fungicide additions, and manganese deficiency. These results from the treatment that started with 4000 μM NO_3- are also presented.

Total biomass

Ambient CO_2

Because later experiments were infected with *Pythium* fungal species, metalaxyl [N-(2,6-dimethylphenyl)-N-(methoxyacetyl)-DL-alanine, methyl ester] was added to one ambient and one elevated CO_2 trial in small doses throughout the trial as a preventative. Metalaxyl prevented *Pythium* growth, but appeared to decrease yield. However, experiments in which metalaxyl was added were also manganese deficient, so it is unknown whether the results observed are due to metalaxyl, manganese deficiency, or a combination of both. Cumulative metalaxyl additions were about half of the recommended rate for a one-time soil drench, so it seems probable that manganese deficiency was the cause of the observed results.

Plants treated with metalaxyl (and manganese deficient) had total biomass reduced by about 20% as compared to healthy plants at all N levels. Within the metalaxyl experiment, the 4000 μM NO_3^- treatment yielded less than the 100 and 1000 μM NO_3^- treatments (Fig. A-1).

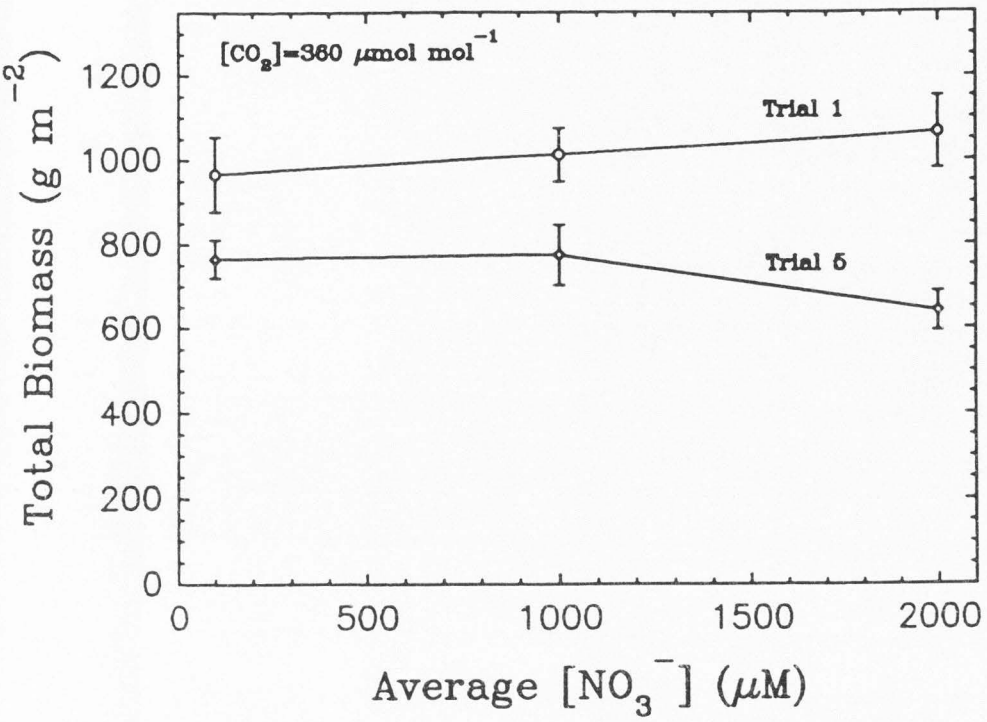

Fig. A-1. Total biomass (g m^{-2}) of trials 1 and 5. The 4000 μM NO_3^- treatment is represented by it's average concentration throughout the trial, 2000 μM NO_3^-. Values are expressed as means with standard errors. Trial 1 represents ambient CO_2 with no metalaxyl added. Trial 5 was ambient CO_2 with metalaxyl.

Elevated CO_2

Additions of metalaxyl to plants grown in elevated CO_2 reduced yields by about 25% compared to healthy plants with no metalaxyl added. There was no significant yield difference between the 100 and 1000 μM NO_3^- treatment within the metalaxyl treatment, but the 4000 μM NO_3^- treatment had a significantly lower yield than the 1000 μM NO_3^- treatment (Fig. A-2).

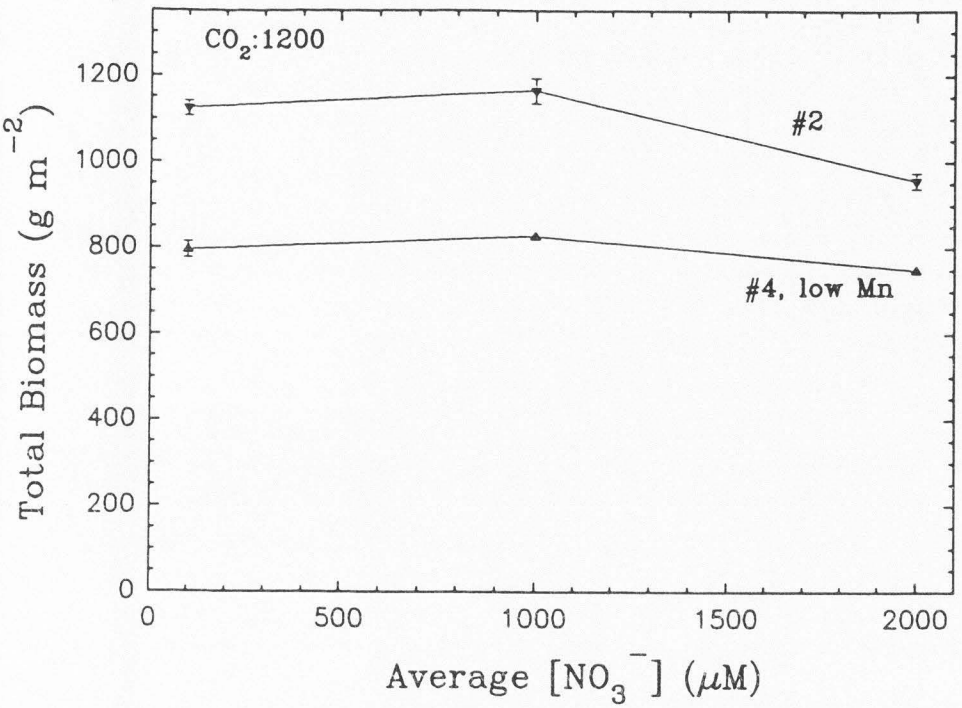

Fig. A-2. Total biomass (g m^{-2}) of plants grown in elevated CO_2. The 4000 μM NO_3^- treatment is represented by it's average concentration throughout the trial, 2000 μM NO_3^-. Values are expressed as means with standard errors. Trial 2 represents elevated CO_2 with healthy plants. Trial 4 was elevated CO_2 with metalaxyl added and Mn deficiency.

Yield Summary

As previously mentioned, *Pythium* infected some of the experiments and reduced yields. Fig. A-3 is a summary of the yield from all experiments, including those infected with *Pythium*.

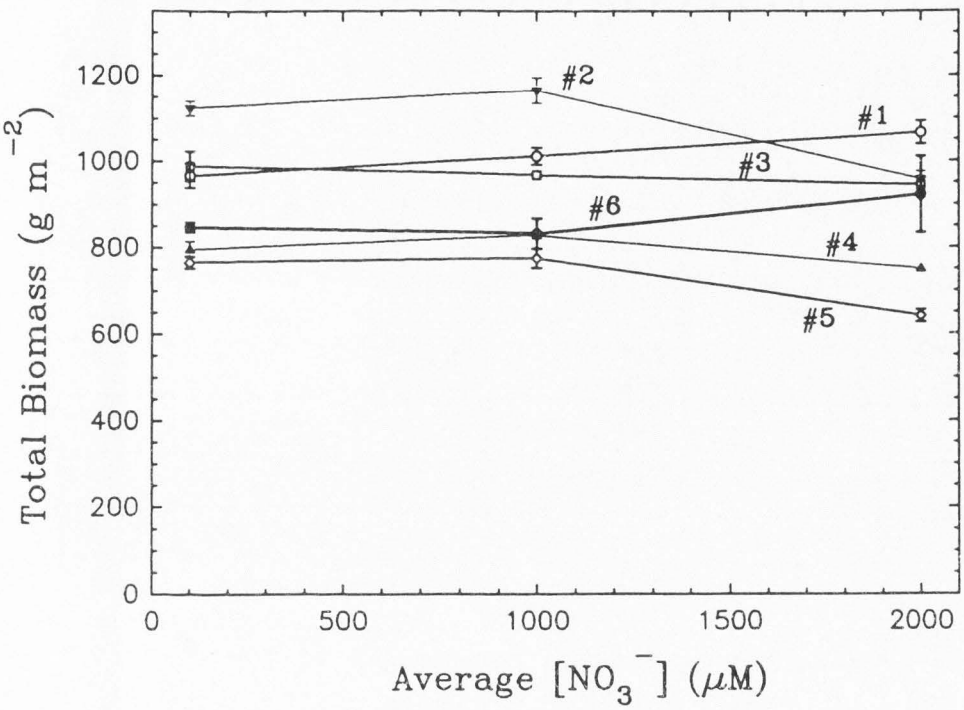

Fig. A-3. Yield summary (g m^{-2}) of all six trials. Trials 1, 3, and 5 were grown in ambient CO_2; trials 2, 4, and 6 were elevated CO_2. Trials 1 and 2 had healthy roots (except for the 4000 μM NO_3^- treatment in trial 2), trials 4 and 5 were treated with metalaxyl to prevent fungal infections, and trials 3 and 6 were infected with *Pythium*. Trials 3-6 were Mn deficient. Values are expressed as means with standard errors.

Percent root mass

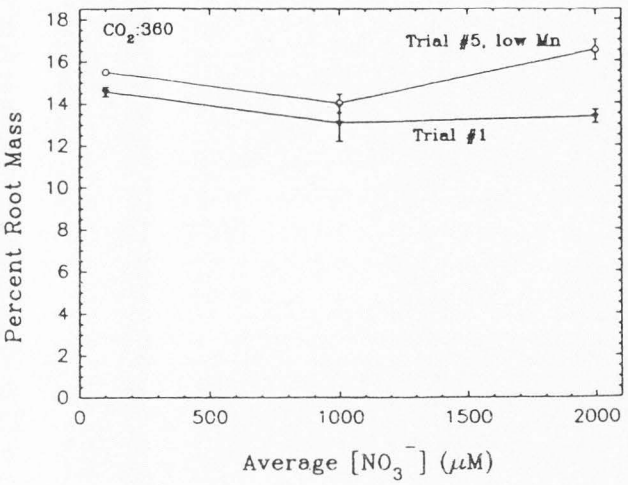

Fig. A-4. Percent root mass at ambient CO_2. Trial 5 had metalaxyl added and was Mn deficient. Values are expressed as means with standard errors.

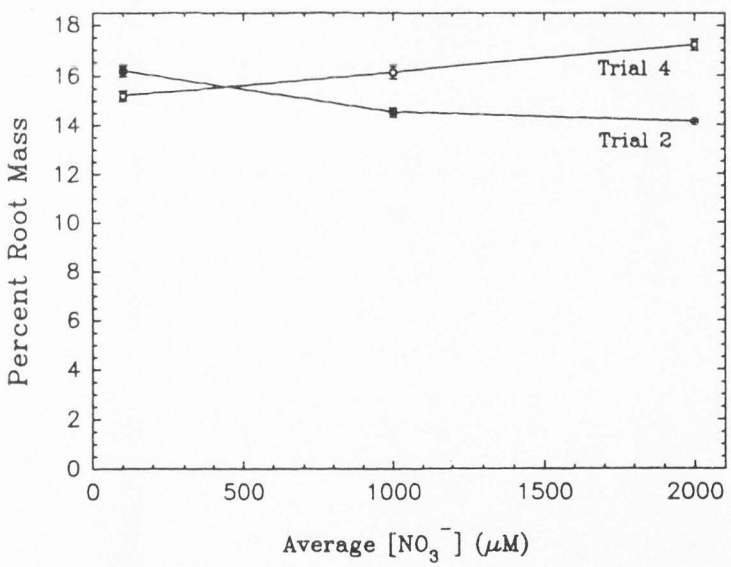

Fig. A-5. Percent root mass at elevated CO_2. Trial 4 had metalaxyl added and was Mn deficient. Values are expressed as means with standard errors.

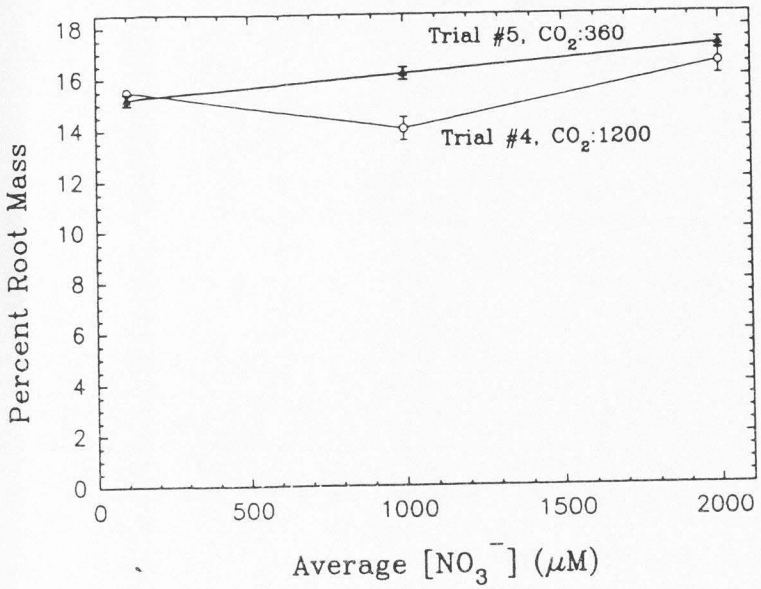

Fig. A-6. Percentage root mass as affected by elevated CO_2. Trial 5 was at ambient CO_2, while trial 4 was conducted at elevated CO_2. Both trials were Mn deficient. Values are expressed as means with standard errors.

Nitrogen consumption

Nitrogen consumption dropped off near day 9 in both ambient and elevated CO_2. This was probably due to plants exhausting their Mn reserves and becoming N stressed (Fig.s A-7 and A-8).

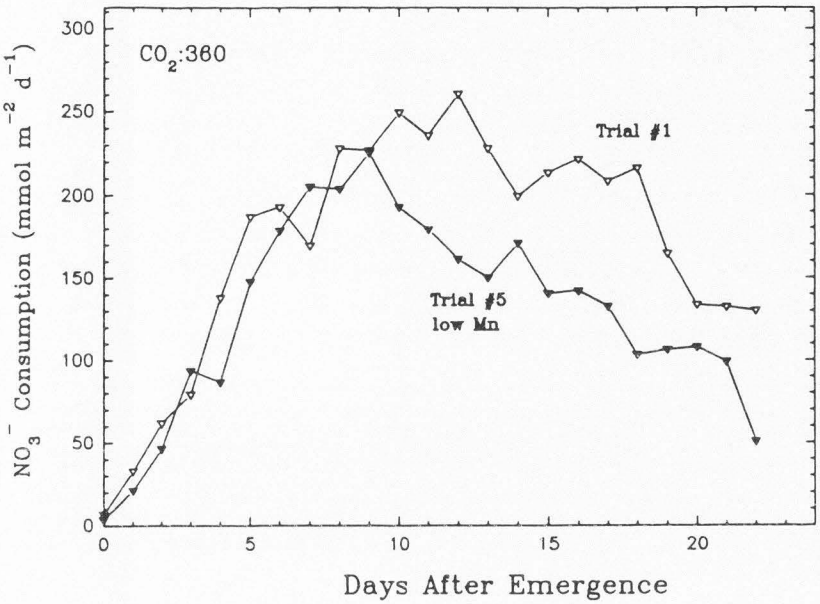

Fig. A-7. Daily N consumption (mmol m^{-2} d^{-1}) from plants grown in ambient CO_2 (360 μmol mol^{-1}). Trial 1 had healthy plants, while trial 5 plants were Mn deficient.

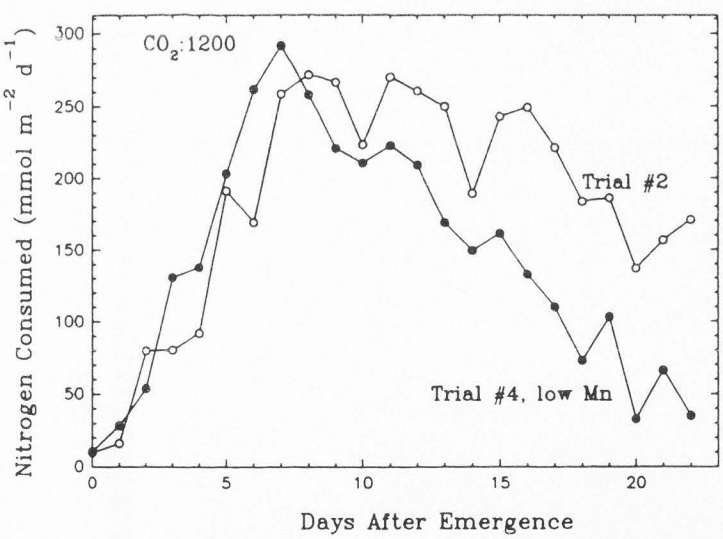

Fig. A-8. Daily N consumption (mmol m^{-2} d^{-1}) from plants grown in elevated CO_2 (1200 μmol mol^{-1}). Trial 2 had healthy plants, while trial 4 plants were Mn deficient.

Nitrogen content

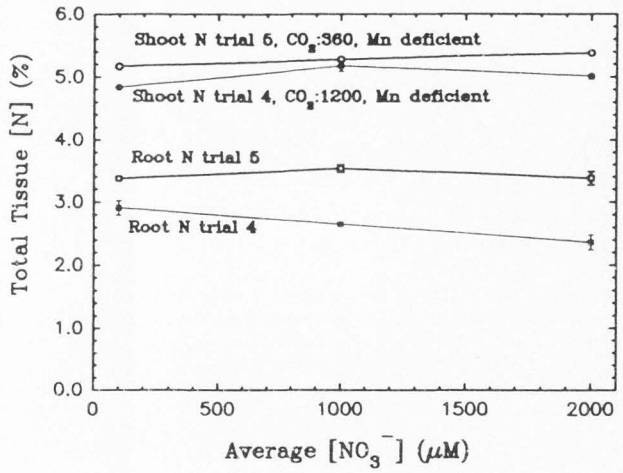

Fig. A-9. Concentration of total N (%) in shoots and roots of plants grown in ambient CO_2 (trial 5) and elevated CO_2 (trial 4) with 5.5 ppm of metalaxyl added to the root zone and Mn deficient. Values are expressed as means with standard errors.

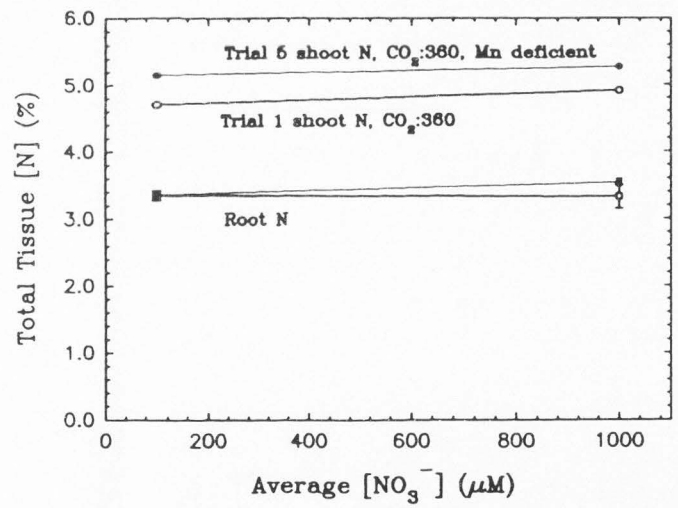

Fig. A-10. Concentration of total N (%) in shoots and roots of plants grown in ambient CO_2 without metalaxyl (trial 1) and with 5.5 ppm of metalaxyl and Mn deficient (trial 5) added to the root zone. Values are expressed as means with standard errors.

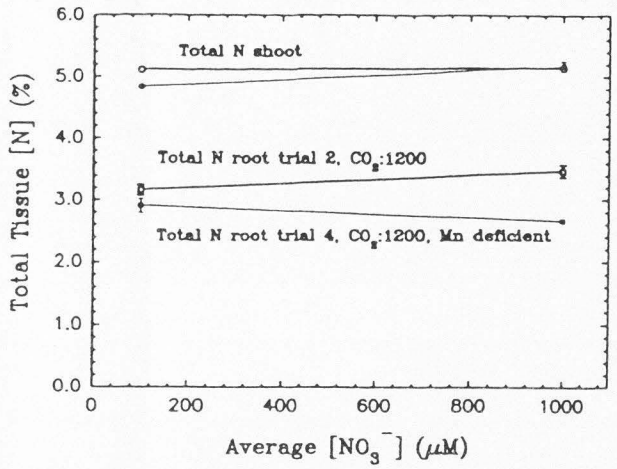

Fig. A-11. Concentration of total N (%) in shoots and roots of plants grown in elevated CO_2 without metalaxyl (trial 2) and with 5.5 ppm of metalaxyl and Mn deficient (trial 4). Values are expressed as means with standard errors.

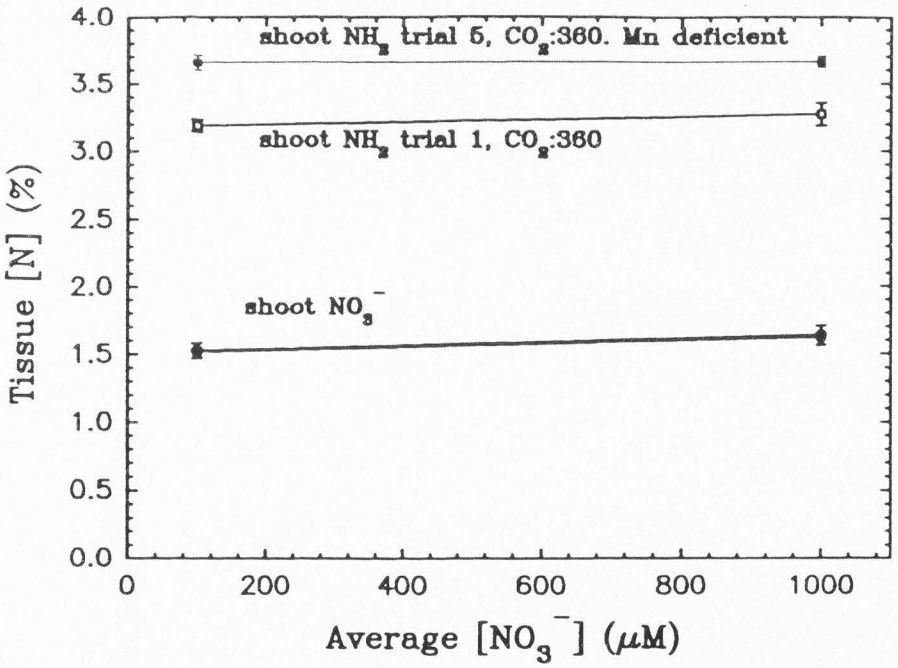

Fig. A-12. Concentration (%) of reduced N (NH_2) and NO_3^--N in shoots of plants grown in ambient CO_2 at average solution NO_3^- concentrations of 100, 1000, and 2000 μM. Trial 5 had 5.5 ppm of metalaxyl added and was Mn deficient. Values are expressed as means with standard errors.

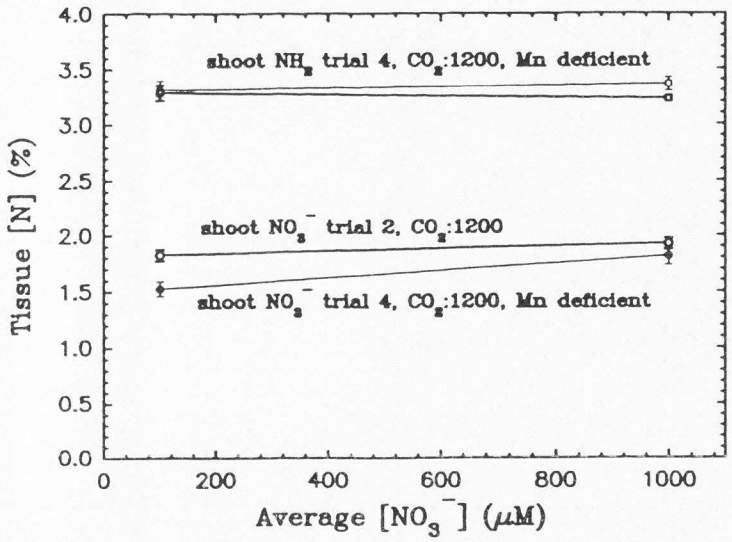

Fig. A-13. Concentration (%) of reduced N (NH_2) and NO_3^--N in shoots of plants grown in elevated CO_2 at average solution NO_3^- concentrations of 100, 1000, and 2000 μM. Trial 4 had 5.5 ppm of metalaxyl added and was Mn deficient. Values are expressed as means with standard errors.

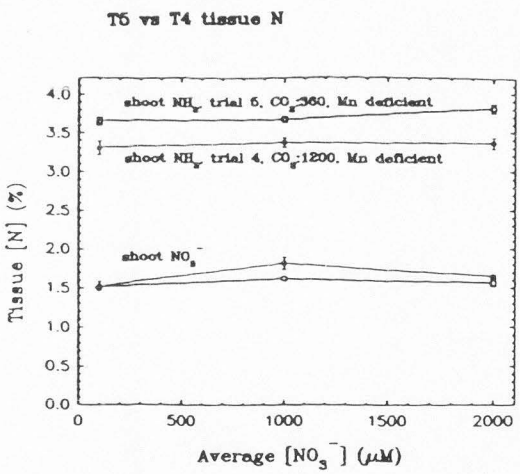

Fig. A-14. Concentration (%) of reduced N (NH_2) and NO_3^--N in shoots of plants grown in elevated CO_2 at average solution NO_3^- concentrations of 100, 1000, and 2000 μM. Trial 4 had 5.5 ppm of metalaxyl added and was Mn deficient. Values are expressed as means with standard errors.

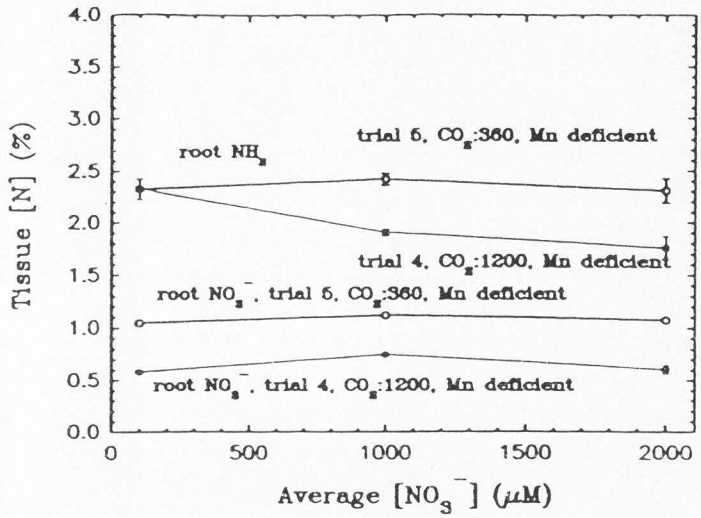

Fig. A-15. Concentration (%) of reduced N (NH_2) and NO_3^--N in roots of plants grown in ambient (trial 5) and elevated CO_2 (trial 4) at average solution NO_3^- concentrations of 100 and 1000 μM with 5.5 ppm of metalaxyl and Mn deficient. Values are expressed as means with standard errors.

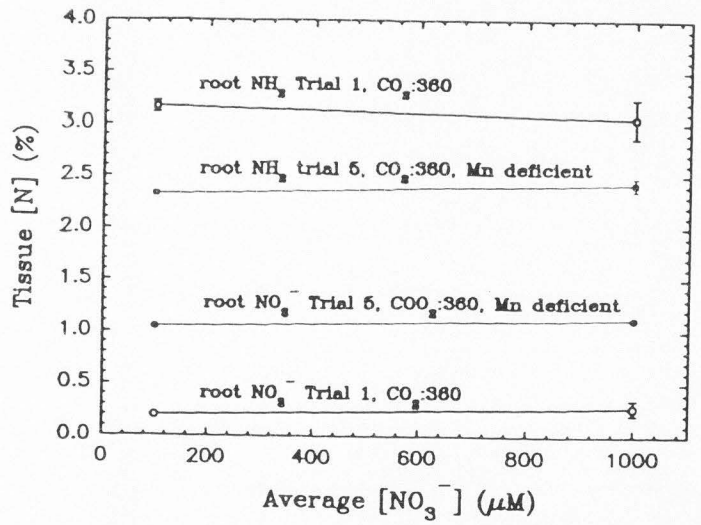

Fig. A-16. Concentration (%) of reduced N (NH_2) and NO_3^--N in roots of plants grown in ambient CO_2 at average solution NO_3^- concentrations of 100 and 1000 μM. Trial 5 was treated with 5.5 ppm of metalaxyl and was Mn deficient. Values are expressed as means with standard errors.

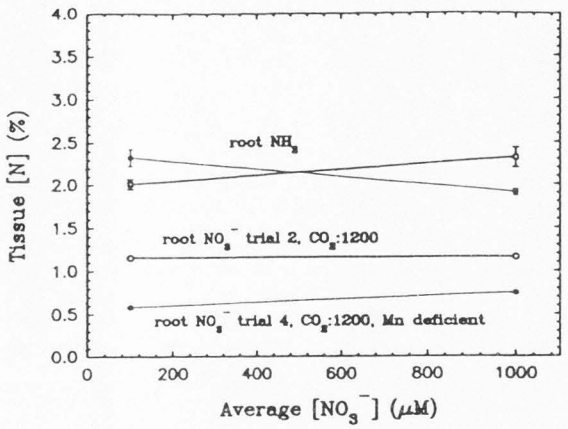

Fig. A-17. Concentration (%) of reduced N (NH_2) and NO_3^--N in roots of plants grown in elevated CO_2 at average solution NO_3^- concentrations of 100 and 1000 μM. Trial 4 was treated with 5.5 ppm of metalaxyl and was Mn deficient. Values are expressed as means with standard errors.

APPENDIX B: 4000 μM N DEPLETION AND YIELD

The treatment that started at 4000 μM NO_3^- has been used to grow hydroponic wheat at the Utah State University Research Greenhouses for several years (Bugbee and Salisbury, 1989), but no thorough characterization of solution N depletion has been made during this time. Fig. B-1 shows N depletion from this treatment during trials 2-5. One trial was conducted at ambient CO_2 in which the results from the 4000 μM N treatment were not confounded by Mn deficiency or fungus. An ANOVA of these results is shown in Table B-1.

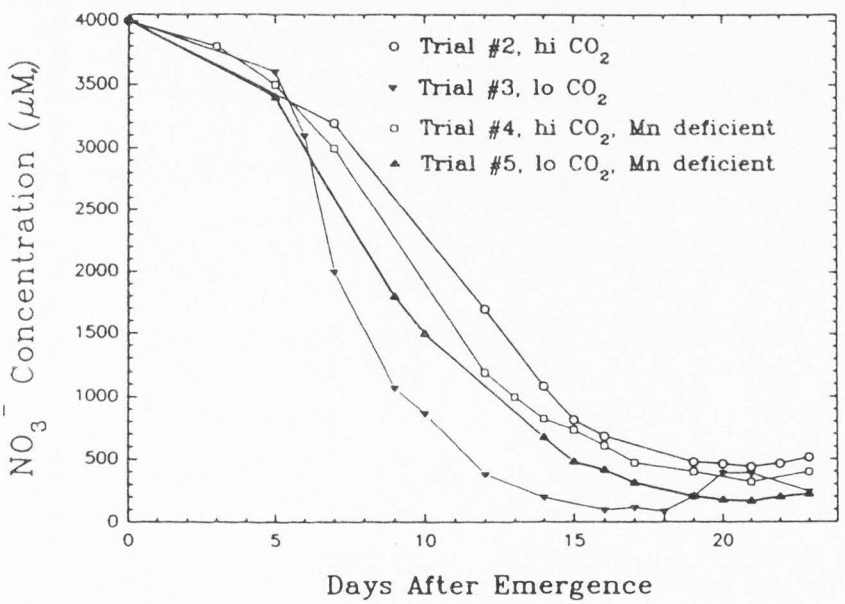

Fig. B-1. Depletion of solution NO_3^- (μM) of the treatment that started with 4000 μM NO_3^-.

Table B-1. ANOVA comparing total biomass (g m^{-2}) of the three N treatments at ambient CO_2. Data were analyzed as a completely randomized design.

Source	DF	MS	F	P
NO_3^-	2	288	3.88	0.061
Error	9	100		
Total	11			

APPENDIX C: EFFECTS OF ROOT RINSES ON NITROGEN CONTENT

Table C-1. Comparison of three types of root rinses on N content. Nonrinsed roots (NR), roots rinsed with deionized water (DI), and roots rinsed with 100 mM HCl (AC) are compared. Values are normalized, with the nonrinsed roots representing 100% nitrogen content.

			Nitrogen Content (% of NR)		
CO_2	Metalaxyl (ppm)	$[NO_3^-]$	NR	DI	AC
360	0	100	100.0	91.3	74.3
		1000	100.0	90.1	81.3
		4000	100.0	90.9	73.3
1200	0	100	100.0	93.4	74.4
		1000	100.0	91.0	75.8
		4000	100.0	92.4	83.5
360	5.5	100	100.0	88.1	
		1000	100.0	86.0	
		4000	100.0	89.3	
1200	5.5	100	100.0	91.7	
		1000	100.0	88.8	
		4000	100.0	91.7	

APPENDIX D: ICP ANALYSIS

Calculation of nutrient percent recovery

Calculation for percent recoveries of nutrients ([nutrient] denotes nutrient concentration):

$$\frac{\text{[nutrient in plant tissue]}}{\text{[nutrient added to solution] - [nutrient in solution at trial's end]}}$$

Table D-1. Concentration (mmoles) of nutrients added to and removed from the nutrient solution and recovered in the biomass from the 100 μM N treatment grown in ambient CO_2 (360 μmol mol^{-1}).

Element	Initial Solution	Final Solution	Total Added	Amount in Biomass	% Recovery
Boron	0.60	0.00	0.34	0.60	63.6
Calcium	300.00	710.70	586.60	71.10	40.5
Copper	0.01	0.00	0.01	0.20	1000.0
Iron	12.00	11.40	8.11	3.80	43.6
Magnesium	150.00	177.10	102.90	45.70	60.3
Manganese	0.09	0.00	0.06	0.60	387.6
Molybdenum	0.03	0.30	0.03	0.10	-41.4
Potassium	975.00	763.40	1212.90	1086.90	76.3
Phosphorus	180.00	80.00	123.00	167.50	75.1
Silicon	22.50	4.50	16.00	10.50	30.9
Sulfur	825.10	1232.10	566.60	86.20	54.0
Zinc	0.10	0.80	0.01	0.50	-72.5

Table D-2. List of tank that N treatment was in for each trial. This is needed to read ICP analysis results.

	TANK		
Trial #	100 μM NO_3^-	1000 μM NO_3^-	4000 μM NO_3^-
1	C	B	A
2	B	A	C
3	A	C	B
4	C	B	A
5	A	C	B
6	C	B	A

Many analyses were conducted for trials 1 and 2. A typical identification entry from trial 1 is "A1 S R-16." This is read as follows: tub A1 (4000 μM N, Table D-2), shoots, rinsed with DI water, 16 row portion of the lid from tub A1. Shoots and roots were sampled, samples were rinsed with DI water or not rinsed (NR), and the shoots were split into 15 or 16 row portions of the lid.

For trials 3-6, one shoot sample was taken from each N treatment.

Trial 1

Plant Samples

USU #	Ident.	Al	B	Ca	Cd	Co	Cr	Cu	Fe	K	Mg	Mn	Mo	Na	Ni	P	Pb	S	Se	Sr	Zn
		mg/kg		%	mg/kg					%		mg/kg				%	mg/kg	%	mg/kg		
5936	A1 S R·16	40	7.3	0.54	<	<	<	9.9	142.3	7.27	0.20	43.5	7.3	74	<	0.65	<	0.33	<	4.1	42.2
5937	S+B A1 SB	194	6.5	0.30	<	<	<	66.1	290.7	5.15	0.10	45.7	5.9	208	<	0.52	<	0.31	<	5.2	69.3
5938	A1 R R	30	<	0.06	<	<	<	10.0	242.7	1.00	0.03	7.2	2.6	90	<	0.35	<	0.19	<	1.5	19.2
5939	A1 R NR	141	<	0.19	<	<	3.4	18.6	724.8	4.26	0.11	10.5	<	216	<	0.51	<	0.30	<	3.6	58.0
5940	A2 R R	47	<	0.11	<	<	1.4	13.0	310.6	1.51	0.05	8.0	2.3	96	<	0.42	<	0.21	<	2.6	29.8
5941	A2 R NR	220	7.5	0.23	<	<	4.0	21.6	885.8	3.69	0.11	15.0	2.3	483	<	0.46	<	0.29	<	4.4	56.5
5942	A2 S NR	27	<	0.46	<	<	<	10.9	46.7	6.76	0.18	34.4	7.4	58	<	0.65	<	0.29	<	3.8	38.0
5943	A4 S R·15	24	6.2	0.47	<	<	<	10.1	47.1	6.77	0.19	47.0	6.6	77	<	0.61	<	0.29	<	3.6	40.8
5944	A3 S R·16	18	6.5	0.54	<	<	<	10.8	45.4	7.09	0.19	37.8	7.2	71	<	0.66	<	0.31	<	4.1	38.2
5945	A2 S R·16	19	<	0.58	<	<	<	10.9	47.4	6.92	0.21	46.8	7.6	78	<	0.70	<	0.33	<	4.4	39.3
5946	S+B B2	208	5.8	0.29	<	<	<	17.0	337.4	4.96	0.09	51.4	9.0	189	<	0.53	<	0.34	<	4.3	121.9
5947	B1 S R·15	15	6.6	0.51	<	<	<	10.5	60.8	7.67	0.16	47.2	9.1	79	<	0.70	<	0.33	<	3.0	52.7
5948	C2 S R·16	43	10.5	0.39	<	<	<	9.0	113.0	6.56	0.16	45.2	9.3	109	<	0.70	<	0.31	<	2.7	27.7
5949	B2 S R·16	31	8.1	0.45	<	<	<	10.0	158.8	7.38	0.15	44.3	8.9	65	<	0.72	<	0.33	<	2.7	52.8
5950	B3 S R·15	32	9.4	0.47	<	<	<	10.0	131.1	7.29	0.16	45.4	8.1	57	<	0.74	<	0.32	<	2.8	50.5
5951	B4 S R·15	14	8.6	0.48	<	<	<	9.1	60.4	7.46	0.15	43.6	8.4	71	<	0.70	<	0.32	<	2.9	48.0
5952	B4 S NR·15	14	6.7	0.44	<	<	<	9.4	57.1	7.58	0.14	42.3	7.9	77	<	0.60	<	0.30	<	2.8	47.7
5953	B3 R R	19	<	0.06	<	<	<	8.1	400.5	1.29	0.03	6.8	5.7	41	<	0.37	<	0.20	<	1.1	28.0
5954	B3 R NR	105	<	0.36	<	<	3.5	16.3	268.4	3.46	0.10	20.4	8.1	136	<	0.54	<	0.41	<	3.4	79.2
5955	B4 R R	32	<	0.05	<	<	<	8.7	487.2	1.38	0.03	8.7	6.8	53	<	0.41	<	0.22	<	1.0	33.0
5956	B4 R NR	107	<	0.28	<	<	2.7	13.6	1408.2	4.10	0.07	20.5	7.6	271	<	0.58	<	0.40	<	2.8	62.1
5957	C1 SB	244	8.0	0.23	<	<	<	70.7	381.3	5.19	0.09	36.2	8.3	141	<	0.57	<	0.40	<	3.2	47.1
5958	C1 S R·16	26	9.0	0.46	<	<	<	10.3	55.6	7.32	0.17	52.9	9.1	65	<	0.69	<	0.38	<	2.9	31.3
5959	C3 S R·16	23	11.6	0.37	<	<	<	8.8	48.7	6.78	0.16	49.9	8.6	69	<	0.67	<	0.36	<	2.5	31.7
5960	C4 S	26	9.7	0.39	<	<	<	8.8	49.8	6.73	0.16	50.0	8.9	65	<	0.64	<	0.33	<	2.6	29.9
5961	C3 S NR	25	8.6	0.42	<	<	<	10.1	53.9	7.28	0.17	46.2	7.9	93	<	0.68	<	0.40	<	2.8	32.2
5962	C1 R R	69	<	0.07	<	<	<	10.8	662.7	1.10	0.03	8.1	4.4	78	<	0.39	<	0.19	<	1.7	26.5
5963	C1 R NR	395	6.6	0.22	<	<	2.1	18.6	1835.6	3.87	0.07	18.0	6.1	147	<	0.72	<	0.36	<	3.4	64.8
5964	C3 R R	31	<	0.05	<	<	<	9.7	590.6	1.62	0.03	7.9	3.9	44	<	0.50	<	0.22	<	1.0	29.8
5965	C3 R NR	94	<	0.35	<	<	2.1	19.7	741.3	3.89	0.09	21.1	7.3	175	<	0.71	<	0.43	<	3.7	97.9

R = ROOTS

S = SHOOTS

S+B = STEMS + BASES

Trial 2

USU #	Al	As	B	Ca	Cd	Co	Cr	Cu	Fe	K	Mg	Mn	Mo	Na	Ni	P	Pb	S	Se	Si	Sr	Zn
	mMol/kg																					
7127	8.63	0.00	1.16	53.16	0.00	<	0.00	0.32	5.46	881.1	56.12	0.30	0.17	13.85	0.00	159.34	0.00	93.51	0.00	7.57	0.06	0.91
7128	2.21	0.00	1.28	105.61	0.00	<	0.00	0.16	2.52	1321.3	49.53	0.20	0.17	2.91	0.00	243.78	0.00	99.89	0.00	4.07	0.04	0.54
7129	0.87	0.00	0.98	88.96	0.00	<	0.00	0.14	1.20	1347.2	49.67	0.17	0.14	1.56	0.00	241.66	0.00	94.50	0.00	6.50	0.03	0.51
7130	0.67	0.00	0.86	91.05	0.00	<	0.00	0.14	1.17	1385.4	49.50	0.17	0.17	1.64	0.00	253.27	0.00	94.52	0.00	5.18	0.03	0.53
7131	0.58	0.00	0.86	87.44	0.00	<	0.00	0.13	1.14	1327.7	58.88	0.18	0.16	2.96	0.00	243.50	0.00	95.22	0.00	6.87	0.03	0.50
7132	1.58	0.00	1.02	109.57	0.00	<	0.00	0.14	1.37	1305.1	54.05	0.20	0.19	3.40	0.00	264.85	0.00	104.03	0.00	7.25	0.04	0.56
7133	0.96	0.00	0.67	110.03	0.00	<	0.00	0.64	1.76	1326.9	36.34	0.19	0.15	3.28	0.00	262.58	0.00	96.30	0.00	5.09	0.04	0.78
7134	0.89	0.00	0.79	50.04	0.00	<	0.03	0.17	7.47	705.7	1.47	0.12	0.08	2.89	0.00	151.50	0.00	60.08	0.00	4.73	0.05	0.91
7135	3.65	0.00	0.00	67.63	0.00	<	0.07	0.19	19.65	807.4	17.07	0.11	0.13	7.39	0.00	131.17	0.00	65.02	0.00	8.57	0.05	0.76
7136	1.04	0.00	0.00	17.26	0.00	<	0.04	0.00	10.36	658.0	33.65	0.00	0.00	0.00	0.00	110.35	0.00	50.60	0.00	3.67	0.03	0.42
7137	1.32	0.00	0.00	35.73	0.00	<	0.05	0.00	12.93	1048.2	12.33	0.00	0.00	4.27	0.00	140.76	0.00	58.16	0.00	4.58	0.04	0.74
7138	1.12	0.00	0.00	13.37	0.00	<	0.03	0.00	8.22	444.4	38.10	0.00	0.00	2.68	0.00	103.10	0.00	45.86	0.00	3.27	0.02	0.25
7139	2.71	0.00	0.00	65.12	0.00	<	0.06	0.00	18.90	1213.2	41.74	0.00	0.00	9.72	0.00	145.39	0.00	78.02	0.00	5.39	0.05	0.81
7140	4.30	0.00	0.00	72.18	0.00	<	0.00	0.19	4.52	972.7	36.04	0.13	0.00	15.18	0.00	166.50	0.00	107.20	0.00	7.93	0.07	1.27
7141	1.29	0.00	0.00	31.10	0.00	<	0.06	0.07	15.87	490.8	26.01	0.00	0.00	10.64	0.00	141.15	0.00	78.30	0.00	5.20	0.04	0.45
7142	0.58	0.00	0.00	15.81	0.00	<	0.03	0.00	8.81	331.3	67.79	0.00	0.00	9.49	0.00	125.16	0.00	64.40	0.00	2.06	0.02	0.16
7143	3.62	0.00	0.00	58.04	0.00	<	0.17	0.10	30.87	621.0	41.86	0.00	0.07	42.28	0.00	161.25	0.00	88.36	0.00	4.59	0.07	0.57
7144	0.99	0.00	0.00	32.53	0.00	<	0.05	0.07	12.79	490.6	1.69	0.00	0.00	14.36	0.00	139.82	0.00	77.55	0.00	1.28	0.05	0.39
7145	0.69	0.00	0.00	16.85	0.00	<	0.03	0.17	6.70	436.0	1.10	0.07	0.09	13.87	0.00	148.83	0.00	73.72	0.00	0.81	0.03	0.34
7146	2.82	0.00	0.00	68.41	0.00	<	0.10	0.11	29.22	527.1	100.62	0.00	0.07	23.65	0.00	154.74	0.00	91.95	0.00	7.19	0.11	0.52
7147	0.32	0.00	0.00	189.81	0.00	<	0.00	0.00	2.15	1633.0	114.03	0.00	0.03	1.00	0.00	242.95	0.00	111.79	0.00	9.23	0.08	0.57
7148	0.31	0.00	0.00	214.12	0.00	<	0.00	0.00	0.66	1706.2	107.97	0.00	0.00	0.00	0.00	260.83	0.00	119.66	0.00	8.53	0.08	0.60
7149	0.00	0.00	0.00	187.61	0.00	<	0.00	0.00	0.60	1772.9	106.97	0.00	0.00	0.58	0.00	263.82	0.00	119.73	0.00	8.34	0.07	0.61
7150	0.00	0.00	0.00	204.78	0.00	<	0.00	0.00	0.55	1738.2	119.12	0.00	0.00	1.32	0.00	255.94	0.00	117.53	0.00	8.63	0.08	0.62
7151	0.00	0.00	0.00	213.42	0.00	<	0.00	0.00	0.62	1779.4	3.49	0.00	0.00	1.70	0.00	281.88	0.00	132.05	0.00	3.62	0.08	0.68
7152	0.37	0.00	0.00	175.83	0.00	<	0.00	0.16	0.70	1452.8	43.87	0.13	0.11	2.98	0.00	223.87	0.00	88.29	0.00	4.79	0.07	0.63
7153	4.78	0.00	0.00	68.47	0.00	<	0.00	0.15	2.86	1303.6	15.42	0.15	0.00	10.41	0.00	177.06	0.00	78.29	0.00	9.37	0.06	0.85
7154	1.30	0.00	0.00	13.39	0.00	<	0.04	0.00	7.05	541.0	14.10	0.00	0.00	0.76	0.00	134.11	0.00	51.21	0.00	4.47	0.02	0.31
7155	0.58	0.00	0.00	10.56	0.00	<	0.04	0.00	7.00	499.9	33.78	0.00	0.00	0.40	0.00	130.30	0.00	49.80	0.00	2.92	0.02	0.27
7156	0.90	0.00	0.00	36.84	0.00	<	0.04	0.10	8.55	1043.0	42.15	0.00	0.00	2.86	0.00	158.11	0.00	59.63	0.00	3.26	0.04	0.60
7157	2.75	0.00	0.00	76.08	0.00	<	0.09	0.14	19.59	1032.2	34.34	0.08	0.00	10.01	0.00	176.95	0.00	76.20	0.00	2.60	0.05	0.71
7158	2.92	0.00	0.00	33.19	0.00	<	0.06	0.11	12.34	1021.6	13.48	0.00	0.00	3.27	0.00	158.58	0.00	57.68	0.00	5.55	0.04	0.63
7159	0.93	0.00	0.00	10.43	0.00	<	0.04	0.00	8.79	451.0	57.73	0.00	0.00	0.62	0.00	129.13	0.00	47.13	0.00	3.65	0.00	0.22
7160	0.64	0.00	0.00	122.74	0.00	<	0.00	0.06	1.35	1964.7	61.07	0.04	0.00	2.52	0.00	245.56	0.00	83.53	0.00	6.26	0.04	0.47
7161	0.85	0.00	0.00	133.88	0.00	<	0.00	0.08	0.99	1956.0	61.34	0.04	0.00	2.68	0.00	255.98	0.00	85.74	0.00	8.73	0.05	0.48
7162	0.50	0.00	0.00	132.95	0.00	<	0.00	0.07	0.94	1866.3	59.43	0.04	0.00	3.82	0.00	250.73	0.00	82.02	0.00	12.32	0.05	0.43
7163	0.58	0.00	0.00	131.49	0.00	<	0.00	0.06	0.89	1802.8	62.99	0.04	0.00	2.00	0.00	233.45	0.00	81.70	0.00	11.52	0.05	0.41
7164	1.71	0.00	0.77	121.87	0.00	<	0.00	0.05	1.04	1752.4	56.33	0.06	0.00	1.82	0.00	228.32	0.00	84.39	0.00	9.97	0.04	0.41
7165	0.43	0.00	0.00	123.05	0.00	<	0.00	0.05	0.95	1781.4	0.49	0.04	0.00	1.64	0.00	238.11	0.00	84.42	0.00	4.97	0.04	0.44

Trial 2

USU #	Ident.
7127	White room harvest #2 B1 stem bases
7128	B1 Shoots 16 rows Not rinsed
7129	B2 Shoots 16 rows Not rinsed
7130	B3 Shoots 16 rows Not rinsed
7131	B2 Shoots 16 rows Rinsed
7132	B1 Shoots 16 rows Rinsed
7133	B3 Shoots 16 rows Rinsed
7134	B1 Root DI H20 rinse
7135	B1 Root Non rinsed
7136	B1 Root Acid rinsed
7137	B3 Root B1 H20 rinse
7138	B3 Root Acid Rinse
7139	B3 Root Non rinse
7140	C1 Stem Bases
7141	C4 Root DI H20 rinse
7142	C4 Root Acid rinse
7143	C4 Root Non rinse
7144	C3 Root DI H2O rinse
7145	C3 Root Acid rinse
7146	C3 Root Non rinse
7147	C1 Shoots 16 rows Non rinse
7148	C3 Shoots 16 rows Non rinse
7149	C4 Shoots 15 Rows Non rinse
7150	C1 Shoots 16 Rows Rinse
7151	C3 Shoots 16 Rows Rinse
7152	C4 Shoots 16 Rows Rinse
7153	A3 Stem Bases
7154	A2 Root Non rinsed
7155	A2 Root Acid rinse
7156	A2 Root DI H20 rinse
7157	A4 Root Non rinsed
7158	A4 Root DI H20 rinse
7159	A4 Root Acid rinse
7160	A1 Shoots 16 Rows Rinse
7161	A2 Shoots 16 Rows Rinse
7162	A4 Shoots 16 Rows Rinse
7163	A4 Shoots 16 Rows Non rinsed
7164	A1 Shoots 15 Rows Non rinsed
7165	A4 Shoots 15 Rows Non rinsed

Trial 3

Plant digests by HNO3/H2O2

USU #	Ident.	Al	B	Ca	Cd	Co	Cr	Cu	Fe	K	Mg	Mn	Mo	Na	Ni	P	Pb	S	Se	Sr	Zn
		mg/kg	mg/kg	%	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	%	%	mg/kg	mg/kg	mg/kg	mg/kg	%	mg/kg	%	mg/kg	mg/kg	mg/kg
4422	3A	27	13.6	0.38	0.0	0.0	0.0	8.1	78.2	7.61	0.15	12.5	8.3	66	0	0.85	0	0.34	0	3.3	29.3
4423	3B	29	12.7	0.34	0.0	0.0	0.0	8.7	57.5	7.50	0.26	10.9	8.7	112	0	0.90	0	0.36	0	3.3	51.1
4424	3C	15	13.6	0.54	0.0	0.0	0.0	8.7	52.2	7.76	0.16	10.6	5.2	82	0	0.89	0	0.34	0	4.1	29.7

NOTE: A value of zero means below detection limit

NOTE: Potassium values are with dilution

Trials 4-6

Plant samples
NITRIC/PEROXIDE DIGEST

USU #	Ident.	Al	B	Ca	Cd	Co	Cr	Cu	Fe	K	Mg	Mn	Mo	Na	Ni	P	Pb	S	Se	Sr	Zn
		mg/kg	mg/kg	%	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	%	%	mg/kg	mg/kg	mg/kg	mg/kg	%	mg/kg	%	mg/kg	mg/kg	mg/kg
4342	4A	51.04	8.13	0.69	0.00	0.00	0.00	13.81	59.37	8.16	0.20	4.84	7.07	195.76	0.00	0.99	0.00	0.39	0.00	5.10	47.51
4344	4B	80.35	8.32	0.67	0.00	0.00	0.00	10.64	98.00	8.68	0.17	4.23	6.81	102.29	0.00	1.02	0.00	0.41	0.00	4.57	47.60
4346	4C	17.99	0.00	0.64	0.00	0.00	0.00	11.65	64.20	8.04	0.15	4.96	6.40	98.88	0.00	1.10	0.00	0.40	0.00	5.71	46.96
4343	5A	30.67	0.00	0.61	0.00	0.00	0.00	12.65	70.51	7.69	0.19	6.46	8.00	106.82	0.00	0.98	0.00	0.40	0.00	4.69	44.77
4347	5B	54.89	9.84	0.42	0.00	0.00	0.00	13.81	62.97	8.27	0.32	4.35	5.87	82.99	0.00	1.17	0.00	0.41	0.00	2.58	53.56
4345	5C	21.96	0.00	0.67	0.00	0.00	0.00	12.65	65.79	7.94	0.16	5.73	3.74	107.81	0.00	0.99	0.00	0.33	0.00	4.89	37.72
4339	6A	34.18	0.00	0.68	0.00	0.00	0.00	14.66	70.84	7.24	0.19	5.40	3.87	104.78	0.00	1.02	0.00	0.38	0.00	3.05	57.13
4340	6B	28.18	0.00	0.50	0.00	0.00	0.00	13.66	62.45	8.05	0.15	5.36	3.07	113.60	0.00	1.06	0.00	0.39	0.00	3.46	40.37
4341	6C	38.14	0.00	0.45	0.00	0.00	0.00	12.65	76.89	7.66	0.16	5.44	4.27	96.06	0.00	1.02	0.00	0.42	0.00	3.34	37.17

APPENDIX E: STATISTICAL ANALYSIS

ANOVA indications of significance (P = 0.05) agreed closely with results displayed in graphs with standard errors of the mean. 95% confidence intervals masked significance and were too conservative. For example, 95% confidence intervals suggested that the only significant effect of CO_2 or N on percent root mass was an increase in percent root mass of the 100 μM N treatment in elevated CO_2. However, an ANOVA demonstrated that both CO_2 and levels were significant (Table E-4).

Table E-1. ANOVA of N recovery for all 6 trials (2 N treatments.) Three studies were conducted in ambient (360 μmol mol^{-1}) and elevated CO_2 (1200 μmol mol^{-1}) at average solution NO_3^- concentrations of 100 and 1000 μM. Root health status (healthy, Mn deficient, or infected with *Pythium*) was analyzed as a block effect.

Source	DF	SS	MS	F	P
Block	2	17.5			
CO_2	1	35.4	35.4	1.62	>0.25
NO_3^-	1	72.0	72.0	3.31	>0.10
CO_2 X NO_3^-	1	14.1	14.1	0.65	>0.25
Pooled Error	6	130.6	21.8		
Total	11	269.5			

Table E-2. ANOVA of N recovery for all 6 trials (3 N treatments.) Three studies were conducted in ambient (360 μmol mol^{-1}) and elevated CO_2 (1200 μmol mol^{-1}) at average solution NO_3^- concentrations of 100 and 1000 μM, as well as a NO_3^- treament that started at 4000 μM but depleted to less than 500 μM by the end of the trial. Health denotes root health status (healthy, Mn deficient, or infected with *Pythium*).

Source	DF	MS	F	P
CO_2	1	4.1	0.27	0.632
Health	2	205.3	13.37	0.017
NO_3^-	2	222.3	14.48	0.015
CO_2 X Health	2	68.0	4.43	0.097
CO_2 X NO_3^-	2	93.7	6.11	0.061
Health X NO_3^-	4	114.9	7.48	0.038
Pooled Error	4	15.4		
Total	17			

Table E-3. ANOVA of total biomass for plants grown in ambient (360 μmol mol^{-1}) or elevated CO_2 (1200 μmol mol^{-1}) at average solution NO_3^- concentrations of 100 and 1000 μM.

Source	DF	SS	MS	F	P
CO_2	1	3868.8	3868.84	41.03	<0.01
NO_3^-	1	292.4	292.41	3.10	>0.10
CO_2 X NO_3^-	1	0.4	0.44	0.00	>0.50
Pooled Error	12	1131.5	94.30		
Total	15	5293.2			

Table E-4. ANOVA of percent root mass of wheat grown at CO_2 levels of 360 and 1200 μmol mol^{-1} and NO_3^- concentrations of 100 and 1000 μM.

Source	DF	MS	F	P
CO_2	1	9.30	10.30	<0.01
NO_3^-	1	9.92	10.98	<0.01
CO_2 X NO_3^-	1	0.02	0.02	>0.25
Error	12	0.90		
Total	15	30.09		

Table E-5. ANOVA of N uptake for 0-8 days after emergence for all 6 trials. Three studies were conducted in ambient (360 μmol mol^{-1}) and elevated CO_2 (1200 μmol mol^{-1}) at average solution NO_3^- concentrations of 100 and 1000 μM. Health describes root status (healthy, Mn deficient, or infected with *Pythium*). Day indicates days after emergence.

Source	DF	SS	MS	F	P
CO_2	1	9042.0	9042.00	104.96	0.00
Health	2	356.2	178.10	2.07	0.16
NO_3^-	1	1130.4	1130.40	13.12	0.00
Day	8	493410.7	61676.30	715.95	0.00
CO_2 X Health	2	6777.2	3388.60	39.34	0.00
CO_2 X NO_3^-	1	173.5	173.50	2.01	0.18
CO_2 X Day	8	12692.2	1586.50	18.42	0.00
Health X NO_3^-	2	50.1	25.10	0.29	0.75
Health X Day	16	8234.6	514.70	5.97	0.00
NO_3^- X Day	8	1238.7	154.80	1.80	0.15
CO_2 X Health X NO_3^-	2	1070.1	535.10	6.21	0.01
CO_2 X Health X Day	16	9186.5	574.20	6.66	0.00
CO_2 X NO_3^- X Day	8	2390.7	298.80	3.47	0.02
Health X NO_3^- X Day	16	3240.4	202.50	2.35	0.05
Pooled Error	16	1378.3	86.10		
Total	107	550371.7			

Table E-6. ANOVA for nitrogen use efficiency ($g_{biomass}\ g_{N\ removed}^{-1}$). Three studies were conducted in ambient (360 μmol mol^{-1}) and elevated CO_2 (1200 μmol mol^{-1}) at average solution NO_3^- concentrations of 100 and 1000 μM.

Source	DF	MS	F	P
CO_2	1	5.34	3.44	>0.10
NO_3^-	1	2.67	1.72	>0.10
CO_2 X NO_3^-	1	2.40	1.55	>0.25
Error	8	1.55		
Total	11			

Table E-7. ANOVA of total N concentration in shoots and roots of plants grown in ambient (360 μmol mol^{-1}) or elevated CO_2 (1200 μmol mol^{-1}) at average solution NO_3^- concentrations of 100 and 1000 μM. Plant part denotes shoots or roots.

Source	DF	SS	MS	F	P
CO_2	1	17.5	17.45	7.36	0.02
NO_3^-	1	13.4	13.43	5.67	0.04
Plant part	1	2158.1	2158.08	910.56	0.00
CO_2 X NO_3^-	1	1.6	1.62	0.68	0.43
CO_2 X Plant Part	1	23.0	23.00	9.70	0.01
NO_3^- X Plant Part	1	0.1	0.09	0.04	0.85
CO_2 X NO_3^- X Part	1	12.2	12.19	5.14	0.04
Rep (CO_2 X NO_3^-)	12	43.2	3.60	1.52	0.24
Pooled Error	12	28.4	2.37		
Total	31	2297.5			

Table E-8. ANOVA of NO_3^--N concentration in shoots and roots of plants grown in ambient (360 μmol mol^{-1}) or elevated CO_2 (1200 μmol mol^{-1}) at average solution NO_3^- concentrations of 100 and 1000 μM. Plant part denotes shoots or roots.

Source	DF	SS	MS	F	P
CO_2	1	293.79	293.79	289.48	0.00
NO_3^-	1	4.31	4.31	4.24	0.06
Plant Part	1	851.61	851.61	839.13	0.00
CO_2 X NO_3^-	1	0.35	0.35	0.35	0.57
CO_2 X Plant Part	1	78.56	78.56	77.41	0.00
NO_3^- X Plant Part	1	0.79	0.79	0.78	0.39
CO_2 X NO_3^- X Part	1	0.20	0.20	0.19	0.67
Rep (CO_2 X NO_3^-)	12	1.18	1.18	1.16	0.40
Pooled Error	12	1.02	1.02		
Total	31	1255.96			

Table E-9. ANOVA of NH_2-N concentration in shoots and roots of plants grown in ambient (360 μmol mol^{-1}) or elevated CO_2 (1200 μmol mol^{-1}) at average solution NO_3^- concentrations of 100 and 1000 μM. Plant part denotes shoots or roots.

Source	DF	SS	MS	F	P
CO_2	1	168.0	168.04	64.73	0.00
NO_3^-	1	2.5	2.53	0.97	0.34
Plant Part	1	298.4	298.35	114.93	0.00
CO_2 X NO_3^-	1	3.5	3.48	1.34	0.27
CO_2 X Plant Part	1	186.6	186.58	71.87	0.00
NO_3^- X Plant Part	1	1.4	1.42	0.55	0.47
CO_2 X NO_3^- X Part	1	15.5	15.47	5.96	0.03
Rep (CO_2 X NO_3^-)	12	53.0	4.42	1.70	0.19
Pooled Error	12	31.2	2.60		
Total	31	760.1			

Table E-10. ANOVA for water use efficiency ($g_{biomass}$ $kg_{water\ transpired}$). Three studies were conducted in ambient (360 μmol mol^{-1}) and elevated CO_2 (1200 μmol mol^{-1}) at average solution NO_3^- concentrations of 100 and 1000 μM.

Source	DF	MS	F	P
CO_2	1	8.47	16.94	<0.01
NO_3^-	1	0.00	0.00	>0.90
CO_2 X NO_3^-	1	0.50	1.00	>0.25
Pooled Error	8	0.50		
Total	11			