

Kinetics of nitrogen uptake by *Populus tremuloides* in relation to atmospheric CO₂ and soil nitrogen availability

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Summary Sustained increases in plant production in response to elevated atmospheric carbon dioxide (CO₂) concentration may be constrained by the availability of soil nitrogen (N). However, it is possible that plants will respond to N limitation at elevated CO₂ concentration by increasing the specific N uptake capacity of their roots. To explore this possibility, we examined the kinetics of ¹⁵NH₄⁺ and ¹⁵NO₃⁻ uptake by excised roots of *Populus tremuloides* Michx. grown in ambient and twice-ambient CO₂ concentrations, and in soils of low- and high-N availability. Elevated CO₂ concentration had no effect on either NH₄⁺ or NO₃⁻ uptake, whereas high-N availability decreased the capacity of roots to take up both NH₄⁺ and NO₃⁻. The maximal rate of NH₄⁺ uptake decreased from 12 to 8 μmol g⁻¹ h⁻¹, and K_m increased from 49 to 162 μmol l⁻¹, from low to high soil N availability. Because NO₃⁻ uptake exhibited mixed kinetics over the concentration range we used (10–500 μmol l⁻¹), it was not possible to calculate V_{max} and K_m. Instead, we used an uptake rate of 100 μmol g⁻¹ h⁻¹ as our metric of NO₃⁻ uptake capacity, which averaged 0.45 and 0.23 μmol g⁻¹ h⁻¹ at low- and high-N availability, respectively. The proximal mechanisms for decreased N uptake capacity at high-N availability appeared to be an increase in fine-root carbohydrate status and a decrease in fine-root N concentration. Both NH₄⁺ and NO₃⁻ uptake were inversely related to fine-root N concentration, and positively related to fine-root total nonstructural carbohydrate concentration. We conclude that soil N availability, through its effects on fine-root N and carbohydrate status, has a much greater influence on the specific uptake capacity of *P. tremuloides* fine roots than elevated atmospheric CO₂. In elevated atmospheric CO₂, changes in N acquisition by *P. tremuloides* appeared to be driven by changes in root architecture and biomass, rather than by changes in the amount or activity of N-uptake enzymes.

Keywords: ammonium, elevated CO₂, global change, nitrate, soil N availability, specific N uptake, total nonstructural carbohydrate, uptake kinetics, uptake regulations.

Introduction

The ability of plants to sustain growth responses to increasing atmospheric carbon dioxide (CO₂) concentration may be constrained by the availability of soil resources, particularly nitrogen (N) (Bazzaz 1990). Currently, there is much interest in determining whether plants can respond to elevated atmospheric CO₂ concentration by increasing their acquisition of N (Norby 1994). Such a response could be manifested morphologically, either through increased root proliferation or greater mycorrhizal infection, or physiologically as an increase in the specific uptake capacity of roots, or as some combination of the two.

Most studies on roots of plants grown in elevated atmospheric CO₂ concentration have focused on morphological changes and have consistently shown an increase in fine-root biomass (Norby et al. 1986, van Veen et al. 1991, Rogers et al. 1992, Zak et al. 1993, Pregitzer et al. 1995). However, we do not have a complete picture of the nutrient-acquisition response of plants to elevated CO₂ because it is not known if changes in the specific uptake capacity of roots accompany the changes in fine-root biomass or root demography.

Several recent studies have investigated the response of N uptake capacity to elevated CO₂ concentration in *Pinus taeda* L. and *Pinus ponderosa* Dougl. ex P. Laws & C. Laws (BassiriRad et al. 1996a, 1996b, 1997). These studies showed a general depression of NH₄⁺ uptake and stimulation of NO₃⁻ uptake in response to elevated CO₂. Because the uptake and assimilation of NO₃⁻ requires a greater investment of energy than uptake and assimilation of NH₄⁺, BassiriRad et al. (1997) attributed this shift in NH₄⁺ versus NO₃⁻ preference to an increase in respiratory substrate at elevated CO₂. Although gymnosperms and angiosperms are believed to vary in their relative preferences for NH₄⁺ and NO₃⁻ (Smirnov and Stewart 1985), we are not aware of any studies that have analyzed the kinetics of the N uptake response to elevated CO₂ in deciduous trees.

Previous work on several aspen species (genus *Populus*) in northern Lower Michigan, USA, has shown that root biomass and demography respond strongly to elevated atmospheric CO₂ concentration as well as to soil N availability (Curtis et al. 1994, Pregitzer et al. 1995). We initiated this experiment to determine if *Populus tremuloides* Michx. also responds physiologically to elevated atmospheric CO₂ concentration, through changes in N uptake kinetics of fine roots. Because an increase in root carbohydrate status in response to elevated CO₂ concentration may have effects on root N uptake capacity (BassiriRad et al. 1996a, 1997), we investigated the influence of elevated CO₂ concentration and soil N availability on fine-root total nonstructural carbohydrates (TNC), and the relationship between TNC and N uptake. In addition, we investigated the potential for elevated CO₂ concentration to modify N uptake capacity through changes in fine-root N concentration. Our rationale was that plant N concentration often declines in response to elevated atmospheric CO₂ concentration (McGuire and Melillo 1995). There is also a well-established negative relationship between root N concentration and the capacity of roots to take up N (Lee and Drew 1986, Chapin et al. 1988, BassiriRad et al. 1993). We hypothesized that the specific N uptake capacity of *P. tremuloides* fine roots would increase with increasing atmospheric CO₂ concentration and decreasing soil N availability. Our basis for predicting a positive CO₂ effect was that increased C fixation at elevated CO₂ concentration should result in a greater pool of energy available for N uptake and assimilation, thus decreasing the relative limitation of N on plant growth. At low-N availability, increased demand for N should result in increased specific N uptake rates.

Methods

Plants and growth conditions

In June 1994, cuttings from six local genotypes of *Populus tremuloides* were planted in either high-N or low-N soil in ambient or elevated atmospheric CO₂ concentration at the University of Michigan Biological Station, Pellston, Michigan (45°34' N, 84°40' W). Two cuttings of each genotype were planted in open-bottom root boxes containing either the A horizon of Kalkaska Sand (Typic Haplorthod; high-N availability) or a 1:4 mixture of Kalkaska A-horizon and C-horizon of Rubicon Sand (Entic Haplorthod; low-N availability). Transparent, cylindrical open-top chambers (3.6 m tall × 3 m diameter) were placed over each root box and a blower system providing supplemental CO₂ was used to raise the CO₂ concentration in the elevated treatment to approximately twice that of ambient air (see Curtis et al. 1999 for details). Mean daytime CO₂ partial pressures were 35.7 Pa and 70.7 Pa in the ambient and elevated concentration treatments respectively, and mean net N mineralization was 318 ng N g⁻¹ day⁻¹ in the high-N soil treatment and 62 ng N g⁻¹ day⁻¹ in the low-N soil treatment (Zak et al. 1999b). Each treatment was replicated five times in a randomized block design.

¹⁵N Uptake and fine-root N concentration

On June 24 and 25, 1996, just before the final harvest, enough soil cores (50 cm deep and 10 cm diameter) were collected from random locations within each chamber to obtain at least 2 g of fine roots. All live fine roots (≤ 1 mm diameter) were removed by hand and rinsed free of soil particles with 0.5 mM CaCl to maintain membrane integrity (Epstein et al. 1963). Roots from all cores per chamber were pooled in a beaker containing 0.5 mM CaCl at 20 °C. Ten 0.1-g fresh weight subsamples were removed to assay for ¹⁵N uptake. One subsample per chamber was suspended for 15 min at 20 °C in about 100 ml of 10, 50, 100, 250, or 500 μM of either K¹⁵NO₃ or ¹⁵NH₄Cl (99 atom % ¹⁵N). All of the uptake solutions contained 0.5 mM CaCl and 1% sucrose. After incubation, roots were washed three times (about 100 ml each time) with 5 mM KCl and 0.5 mM CaCl to remove any adsorbed ¹⁵N. The washed roots were oven-dried for 24 h at 70 °C. Oven-dried roots were ground to a fine powder with a mortar and pestle, and then total N and ¹⁵N abundance were determined by mass spectrometry (Europa Scientific Roboprep + Tracermass, Europa Scientific, Franklin, OH). An additional subsample of fine roots from each chamber was used to determine their natural abundance of ¹⁵N. Ammonium and NO₃⁻ uptake rates were determined by subtracting the natural abundance of ¹⁵N from total ¹⁵N after the incubation, and are reported in μmol per gram (dry weight) of tissue per hour. The Michaelis-Menten kinetic parameters (maximum velocity, V_{max}, and Michaelis-Menten constant, K_m) were calculated from a Hane's plot transformation of the ¹⁵N uptake rates (Armstrong 1989). Initial N concentrations of each root sample were determined from the mass spectrometry data by subtracting the ¹⁵N taken up during the experiment from the total N in the sample.

Fine-root carbohydrate status

Additional subsamples of the pooled fine roots from each chamber were dried to a constant weight at 70 °C and then ground to a fine powder with a mortar and pestle. These roots were used for determination of total nonstructural carbohydrate (TNC) concentration. One-hundred-mg samples of ground roots were extracted twice with 10 ml of 90% ethanol at 60 °C to separate soluble sugars (Farrar 1993), then treated with 10 ml of 35% perchloric acid to hydrolyze starch (Sutton et al. 1981). Total nonstructural carbohydrate was determined by colorimetric reaction with phenol and sulfuric acid (Dubois et al. 1956) against a sucrose standard.

Statistical analyses

Parameters of NH₄⁺ and NO₃⁻ uptake, tissue N concentrations, and TNC were compared by analysis of variance (ANOVA) for a randomized complete block design with two factorial treatments of soil N availability and atmospheric CO₂ concentration. Individual treatment means were compared by Fisher's LSD procedure. The relationships between N uptake, fine-root N concentration, and fine root carbohydrate status at the chamber level were analyzed by simple linear regression. Significance for all statistical analyses was accepted at *P* ≤ 0.05.

All statistical analyses were performed with SYSTAT (Wilkinson 1990).

Results

Ammonium uptake by excised roots of *P. tremuloides* followed Michaelis-Menten kinetics and was several times more rapid than NO_3^- uptake at all concentrations (Figure 1). Nitrate uptake rates of excised *P. tremuloides* roots did not conform well to Michaelis-Menten kinetics over the entire range of concentrations (Figure 1B). Nitrate uptake by higher plants typically shows dual-phase kinetics, with an initial saturation of a high affinity transporter followed by a linear response at higher concentrations (Pilbeam and Kirkby 1990). Excised *P. tremuloides* roots exhibited an initial saturation at approximately $100 \mu\text{M}$. We used rates of NO_3^- uptake at $100 \mu\text{M}$ as our index of NO_3^- uptake capacity for all statistical analyses, because we had too few data points between 10 and $100 \mu\text{M}$ to estimate V_{max} and K_m reliably.

Elevated atmospheric CO_2 concentration had no effect on the kinetic parameters of NH_4^+ uptake; however, maximum uptake velocity for NH_4^+ was significantly lower and the K_m indicated that the NH_4^+ concentration bringing about half maximum velocity was significantly lower at low N availability

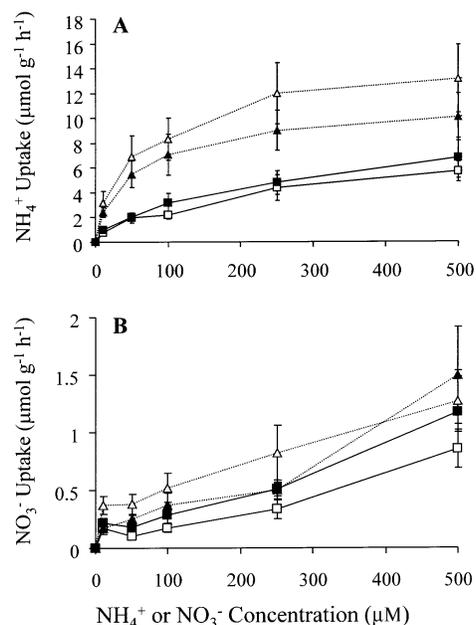


Figure 1. Ammonium (A) and NO_3^- (B) uptake by excised fine roots of *Populus tremuloides*. Each value represents the mean (± 1 SE) from five chambers. Ammonium uptake followed Michaelis-Menten kinetics and was several times more rapid than NO_3^- uptake. Nitrate uptake was more variable, but appeared to show dual-phase kinetics with an initial saturation at approximately $100 \mu\text{M}$ NO_3^- . Neither NO_3^- or NH_4^+ uptake showed any response to atmospheric CO_2 . In contrast, at all solution concentrations, NH_4^+ uptake was greater in roots from the low fertility treatments than from the high fertility treatments. Open symbols represent ambient CO_2 and filled symbols represent elevated CO_2 . Triangles with dashed lines represent low-N availability and squares with solid lines represent high-N availability.

than at high N availability (Table 1). The pattern was similar for NO_3^- uptake; atmospheric CO_2 concentration had no effect on NO_3^- uptake, but NO_3^- uptake capacity was greater at low-N availability than at high-N availability (Table 1).

The TNC concentration in fine roots ranged from 94 to 218 mg g^{-1} dry weight, and was significantly lower at high-N availability than at low-N availability (Table 1). Fine-root TNC concentration was unaffected by atmospheric CO_2 concentration. The N concentration of fine roots ranged from 11 to 22 mg g^{-1} dry weight. It was significantly greater at high-N availability than at low-N availability and greater at ambient CO_2 than at elevated CO_2 concentration (Table 1). The concentration of N in fine roots was inversely related to their TNC concentration ($r^2 = 0.551$; $P < 0.001$). To examine what proportion of the changes in N concentration were a result of a dilution effect by TNC, we expressed N concentration on a TNC-free basis and found results similar to those for N concentration on a mass basis (Table 1). In addition, there remained a significant, but weaker, negative relationship between TNC and N concentration expressed on a TNC-free basis ($r^2 = 0.353$; $P = 0.006$).

When analyzed on a chamber basis, there was a strong negative relationship between the N concentration of fine roots and the maximal velocity of NH_4^+ uptake (Figure 2A), and a similar, but weaker, relationship between N concentration and NO_3^- uptake capacity (Figure 2C). In addition, there was a significant positive relationship between the maximal velocity of NH_4^+ uptake by fine roots and their TNC concentration (Figure 2B), and, again, a weaker correlation between the rate of NO_3^- uptake and TNC concentration (Figure 2D). Expressing N concentration on a TNC-free basis had little effect on the relationships between N concentration and either NH_4^+ or NO_3^- uptake (data not shown).

Discussion

Contrary to our hypothesis, a twice-ambient atmospheric CO_2 concentration had no effect on either the NH_4^+ or NO_3^- uptake capacity of fine roots of *P. tremuloides*, whereas soil N availability had marked effects on both processes. This pattern of responses to elevated CO_2 concentration and soil N availability appears to be the result of changes in the N concentration and carbohydrate status of fine roots, because varying soil N availability had a much greater effect on these parameters than changes in atmospheric CO_2 concentration. The relationships between N uptake and both TNC concentration and N concentration are consistent with what is known about the regulation of N uptake, and suggest that, in *P. tremuloides*, N uptake capacity is down-regulated at high soil N availability. In contrast, despite indications of greater N acquisition at elevated CO_2 concentration (Zak et al. 1999a), there was no change in N uptake capacity in response to elevated CO_2 concentration. This finding suggests that any compensatory response to stimulate N uptake in *P. tremuloides* must come about through some other mechanism, most likely an increase in fine root production (Zak et al. 1999a).

The lack of an effect of elevated CO_2 concentration on N uptake in excised roots of *P. tremuloides* differs from the re-

Table 1. Capacity for NO_3^- uptake (at $100 \mu\text{mol l}^{-1}$), kinetic parameters of NH_4^+ uptake, N concentration, TNC-free N concentration, and TNC concentration of fine roots of *Populus tremuloides*. Values are means with standard errors in parentheses. Interaction means (soil N \times atmospheric CO_2 concentration) within a row followed by the same letter were not statistically different at $P \geq 0.05$. Significance of the main effect means (soil N effects averaged across CO_2 treatments, and CO_2 effects averaged across soil N treatments) is indicated as: ns = not significant; * = $P \leq 0.05$; ** = $P \leq 0.01$.

Root parameter	Interaction means ($n = 5$)				Main effect means ($n = 10$)					
	Low N		High N		Soil N		CO_2			
	Ambient $[\text{CO}_2]$	Elevated $[\text{CO}_2]$	Ambient $[\text{CO}_2]$	Elevated $[\text{CO}_2]$	Low N	High N	Ambient $[\text{CO}_2]$	Elevated $[\text{CO}_2]$		
NO_3^- Uptake ¹ ($\mu\text{mol g}^{-1} \text{h}^{-1}$)	0.52 a (0.125)	0.37 ab (0.102)	0.171 c (0.032)	0.289 bc (0.074)	0.45 (0.080)	0.23 (0.043)	*	0.35 (0.084)	0.33 (0.061)	ns
$V_{\text{max}} \text{NH}_4^+$ ($\mu\text{mol g}^{-1} \text{h}^{-1}$)	13.36 a (2.656)	11.18 ab (2.315)	7.40 b (1.328)	8.70 ab (2.354)	12.27 (1.700)	8.05 (1.292)	*	10.38 (1.716)	9.94 (1.611)	ns
$K_m \text{NH}_4^+$ ($\mu\text{mol l}^{-1}$)	44.0 a (11.72)	53.1 a (11.06)	180.9 b (42.92)	142.7 b (39.12)	48.6 (7.74)	161.8 (27.87)	**	112.5 (30.77)	97.9 (24.30)	ns
N (mg g^{-1})	15.2 ab (0.73)	14.4 a (0.79)	17.7 c (0.90)	16.1 b (0.76)	14.8 (0.53)	16.9 (0.60)	**	16.5 (0.63)	15.3 (0.57)	*
TNC-free N (mg g^{-1})	18.6 a (0.88)	17.8 a (1.47)	20.9 b (1.29)	18.6 a (0.99)	18.2 (0.82)	19.7 (0.86)	*	19.7 (0.83)	18.2 (0.85)	*
TNC (mg g^{-1})	183.3 a (20.25)	187.6 ab (11.00)	154.3 ab (16.10)	137.4 b (14.39)	185.5 (15.40)	145.8 (14.95)	*	68.8 (18.55)	162.5 (16.93)	ns

sults with *Pinus* species reported by BassiriRad et al. (1996a, 1996b, 1997). These authors found down-regulation of NH_4^+ , and up-regulation of NO_3^- , at elevated CO_2 concentration. Although *P. tremuloides* generated more belowground biomass at elevated CO_2 concentration (Zak et al. 1999a, Pregitzer et al. 1999), it was physiologically indistinguishable from that produced at ambient CO_2 concentration. At low-N availability, there was a trend toward repression of N uptake capacity at

elevated CO_2 concentration; this trend was marginally significant for NO_3^- ($P = 0.098$) but far from significant for NH_4^+ ($P = 0.298$). However, the importance of this pattern is difficult to evaluate, because of the variance in our data and the small change relative to that elicited by N availability. In contrast to atmospheric CO_2 , the high-N treatment resulted in decreases in specific NH_4^+ and NO_3^- uptake rates of excised roots of approximately 50% compared with the rates measured in ex-

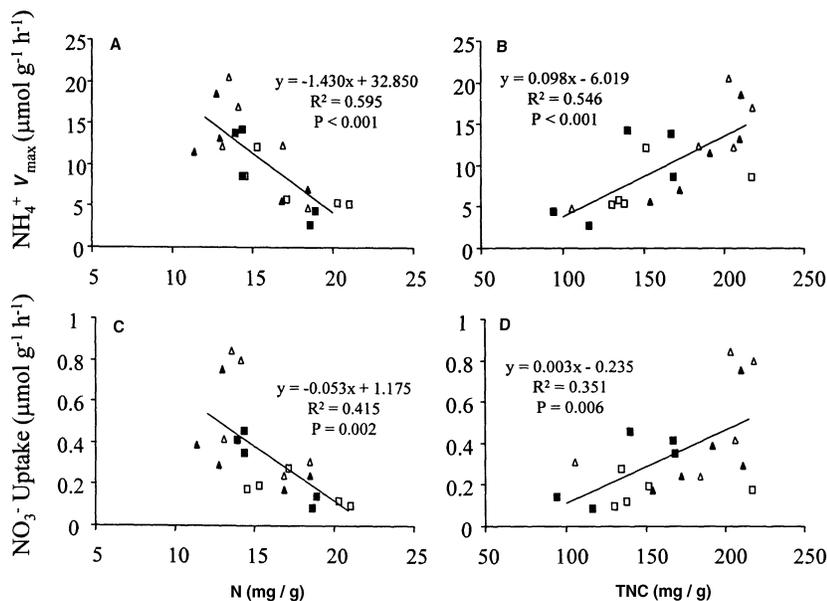


Figure 2. Relationships between mean fine-root N (A and C) or TNC (B and D) concentration in each chamber and the estimate of V_{max} for NH_4^+ (A and B) or NO_3^- uptake at $100 \mu\text{M}$ (C and D) for that chamber. For both NO_3^- and NH_4^+ , rate of uptake was negatively correlated with fine-root N concentration and positively correlated with fine-root TNC concentration, and these relationships tended to be stronger for NH_4^+ than for NO_3^- . Open symbols represent ambient CO_2 concentration and filled symbols represent elevated CO_2 concentration. Triangles represent low-N availability and squares represent high-N availability.

cised roots from the low-N treatment. These results with excised roots are consistent with those obtained for intact *P. tremuloides* plants in an *in situ* ^{15}N -labeling experiment (Mikan et al. 1998). In that experiment, plants grown at high-N availability took up about half as much ^{15}N per unit of belowground biomass than plants grown at low-N availability (Mikan et al. 1998).

To understand better the pattern of responses to CO_2 and soil N availability, we analyzed fine-root N and carbohydrate concentrations and their relationships to N uptake. Decreased N uptake at high root N concentrations is a well-established phenomenon (Lee and Rudge 1986). We found negative relationships between fine-root N concentration and uptake rates and positive relationships between fine-root TNC concentration and uptake rates. The effects of elevated CO_2 concentration and soil N availability on N and TNC concentrations appear to account well for the effects of these treatments on N uptake rate. The actual relationship between TNC concentration and N uptake capacity is likely to be even stronger than that shown in Figure 2, because we added sucrose to the assay medium. The response of N uptake to soil N availability, but not to CO_2 , is consistent with the finding that changes in soil N availability led to changes in N and TNC concentrations, whereas changes in atmospheric CO_2 concentration had little or no effect. However, any explanation of the mechanisms underlying the pattern of response of N uptake capacity to changes in N and TNC concentrations is confounded by the fact that the TNC and N concentrations are inversely correlated with each other.

Nevertheless, there are valid theoretical reasons for predicting that both of the correlations are biologically significant. In the case of fine-root N concentration, many studies have shown that specific N uptake rates are increased in N-starved plants (Lee and Drew 1986, Chapin et al 1988, BassiriRad et al 1993, BassiriRad et al. 1996a). The most straightforward explanation for the relationship between N concentration and N uptake, is that, at high N concentration, the N uptake system is under feedback inhibition by amino acids, which are the first products of primary N metabolism (Touraine et al. 1994, Barneix and Causin 1996).

In contrast, theoretical explanations for the correlation between N uptake and TNC are more complicated. One would expect N uptake to be positively correlated with TNC concentration, because primary N metabolism requires energy for both uptake and reduction of mineral N (Pate and Layzell 1990). In addition, nonstructural carbohydrates serve as a ready source of carbon skeletons for the conversion of mineral N into organic forms. This second role of carbohydrates may explain the positive relationship between TNC and N uptake. At high TNC concentrations there are ample carbon skeletons with which to assimilate incoming N; however, at low TNC concentrations, N assimilation is limited at the stage of NH_4^+ incorporation into amino acids, leading to feedback inhibition of the uptake system. There is much evidence suggesting that internal NH_4^+ serves as a strong repressor of NH_4^+ uptake (Causin and Barneix 1993, Wang et al. 1993), and that inhibitors of NH_4^+ assimilation can lead to depression of NH_4^+ uptake

through this mechanism (Causin and Barneix 1993). It is likely, therefore, that high soil N availability inhibited N uptake directly through a buildup of amino acids in roots, and indirectly through a reduction in carbon skeletons and respiratory substrate.

In conclusion, doubling the atmospheric CO_2 concentration had no effect on the N uptake capacity of *P. tremuloides* fine roots, whereas increasing soil N availability markedly decreased their capacity to take up both NH_4^+ and NO_3^- . These results, in combination with the relationships between N uptake and N and TNC concentrations, suggest that these plants down-regulated their NH_4^+ uptake system in response to improved N status. Soil N availability had a much stronger effect on fine-root physiology and chemistry than atmospheric CO_2 concentration. The lack of an effect of elevated CO_2 concentration on fine-root TNC concentration is interesting because plant C gain increased in response to CO_2 concentration (Zak et al. 1999a). Apparently, under elevated CO_2 conditions, *P. tremuloides* allocates the extra C to increase biomass of fine-roots without increasing their carbohydrate supply or their physiological activity. We conclude that any response by this species to increase N acquisition in response to elevated atmospheric CO_2 concentration will come through changes in root biomass or morphology, or both, rather than changes in root physiology.

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