ATMOSPHERIC CO2, SOIL-N AVAILABILITY, AND ALLOCATION OF BIOMASS AND NITROGEN BY POPULUS TREMULOIDES

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Abstract. Our ability to predict whether elevated atmospheric CO2 will alter the cycling of C and N in terrestrial ecosystems requires understanding a complex set of feedback mechanisms initiated by changes in C and N acquisition by plants and the degree to which changes in resource acquisition (C and N) alter plant growth and allocation. To gain further insight into these dynamics, we grew six genotypes of Populus tremuloides Michx. that differ in autumnal senescence (early vs. late) under experimental atmospheric CO2 (35.7 and 70.7 Pa) and soil-N availability (low and high) treatments. Atmospheric CO2 concentrations were manipulated with open-top chambers, and soil-N availability was modified in open-bottom root boxes by mixing different proportions of native A and C horizon soil. Net N mineralization rates averaged 61 ng N·g⁻¹·d⁻¹ in low-N soil and 319 ng N·g⁻¹·d⁻¹ in high-N soil. After 2.5 growing seasons, we harvested above- and belowground plant components in each chamber and determined total biomass, N concentration, N content, and the relative allocation of biomass and N to leaves, stems, and roots.

Elevated CO2 increased total plant biomass 16% in low-N soil and 38% in high-N soil, indicating that the growth response of P. tremuloides to elevated CO2 was constrained by soil-N availability. Greater growth under elevated CO2 did not substantially alter the allocation of biomass to above- or belowground plant components. At both levels of soil-N availability, elevated CO2 decreased the N concentration of all plant tissues. Despite declines in tissue N concentration, elevated CO2 significantly increased whole-plant N content in high-N soil (ambient = 137 g N/chamber; elevated = 155 g N/chamber), but it did not influence whole-plant N content in low-N soil (36 g N/chamber). Our results indicate that plants in high-N soil obtained greater amounts of soil N under elevated CO2 by producing a proportionately larger fine-root system that more thoroughly exploited the soil. The significant positive relationship between fine-root biomass and total-plant N content we observed in high-N soil further supports this contention. In low-N soil, elevated CO2 did not increase fine-root biomass or production, and plants under ambient and elevated CO2 obtained equivalent amounts of N from soil. In high-N soil, it appears that greater acquisition of soil N under elevated CO2 fed forward within the plant to increase rates of C acquisition, which further enhanced plant growth response to elevated CO2.

Key words: allocation of tree biomass; atmospheric CO2 and soil-N availability; carbon cycling and storage in forests; global climate change; forest response; nitrogen, allocation and concentration in tissues; plant biomass; Populus tremuloides; soil-N availability; tree response to elevated CO2.

INTRODUCTION

The recent, and relatively rapid, rise in atmospheric CO2 has the potential to alter the cycling and storage of carbon (C) in terrestrial ecosystems. Understanding the response of forest ecosystems to rising atmospheric CO2 is important, because forests cover a substantial proportion of Earth’s land surface (43%), contain 40% of the C in living and dead biomass, and account for 72% of global net primary productivity (NPP; Melillo et al. 1993). Recent empirical evidence and theoretical analyses suggest that temperate forests are a globally important sink for atmospheric CO2 (Wofsy et al. 1993, Ciais et al. 1995, Tans et al. 1995, Goulden et al. 1996). Some have suggested that current rates of C storage in temperate forests may be sufficient to moderate the anthropogenic rise in atmospheric CO2, thus potentially slowing an increase in global temperature (Schimel...
Elevated atmospheric CO$_2$ can substantially increase C assimilation and growth in many tree species under experimental conditions (Ceulemans and Mousseau 1994, Wullschleger et al. 1995, Curtis 1996, Curtis and Wang 1998), presenting the possibility that greater tree growth responses to elevated CO$_2$ could further increase C storage in temperate forests. Several reviews suggest that tree growth responses to elevated CO$_2$ can be substantial, but vary widely among species, genotypes, and experimental conditions (0% to 290%, Ceulemans and Mousseau 1994, Ceulemans et al. 1995, McGuire et al. 1995, Curtis and Wang 1998). Greater C assimilation and plant growth under elevated CO$_2$ are accompanied by substantial declines in leaf N concentration (McGuire et al. 1995, Cotrufo et al. 1998, Curtis and Wang 1998), and it has been argued that plants grown under elevated CO$_2$ allocate less N to carboxylating enzymes and more to other metabolic functions (Drake and González-Meler 1997). Therefore, C assimilation and growth increase under elevated CO$_2$ while the amount of N allocated to leaves is thought to decline. Regardless of changes in N allocation to leaves under elevated CO$_2$, there are functional relationships between leaf N concentration and photosynthesis that may limit plant growth response to elevated CO$_2$. Low soil-N availability constrains leaf N concentration, which in turn limits C assimilation and plant growth (Field and Mooney 1986). Although more C is assimilated by leaves with lower N concentrations under elevated CO$_2$, this relationship suggests low soil-N availability could potentially limit plant growth response to elevated CO$_2$. Furthermore, elevated atmospheric CO$_2$ could modify whole-plant N allocation if large quantities of N are allocated to plant tissues (e.g., fine roots) other than leaves as atmospheric CO$_2$ rises.

Changes in belowground plant growth and allocation to roots (i.e., percentage of total biomass) under elevated CO$_2$ have important implications for the acquisition of soil resources. Although plant growth often increases under elevated CO$_2$, the amount of biomass plants allocate to roots vs. shoots appears somewhat more variable. Curtis and Wang (1998) concluded biomass allocation in woody plants was relatively unaltered by elevated atmospheric CO$_2$. That is, growth increases under elevated CO$_2$ were equally partitioned above- and belowground; others have argued for small shifts in biomass allocation to roots (Ceulemans and Mousseau 1994, McGuire et al. 1995). Notwithstanding these contrasting views, it is well understood that biomass allocation in woody plants varies during the growing season (Pregitzer et al. 1990, Horwath et al. 1994), during ontogeny, and with soil-resource availability (Waring and Pitman 1985, Pregitzer et al. 1995). Biomass allocation also is under genetic control and varies among genotypes, especially in fast-growing trees (Ragolou and Jarvis 1990, Ceulemans et al. 1995, Ceulemans and Isebrands 1996). The observation that elevated CO$_2$ could increase root allocation, while greater soil-N availability could decrease the allocation of biomass to roots (McGuire et al. 1995), suggests that both atmospheric CO$_2$ and soil-N availability could modify patterns of biomass allocation.

We have observed that *Populus tremuloides* Michx. grown under elevated CO$_2$ assimilated more C and produced more fine roots than plants grown under ambient CO$_2$, a response that was dampened by low soil-N availability (Curtis et al. 2000, Pregitzer et al. 2000). In this paper, we describe how elevated atmospheric CO$_2$ and soil-N availability influence growth and allocation in *P. tremuloides*. We predicted that plant biomass would increase under elevated atmospheric CO$_2$, but low soil-N availability would constrain this response. We further hypothesized that elevated CO$_2$ should shift the allocation of N from leaves to fine roots, a response that would enable plants to more effectively forage for limiting soil resources (i.e., N). Our experiment included six genotypes of *P. tremuloides*, which differ in autumnal leaf senescence, to explore genotypic variation in growth and allocation of biomass and N in response to atmospheric CO$_2$ and soil-N availability.

**METHODS**

**Biomass harvest**

Our experiment was conducted at the University of Michigan Biological Station (45°34’N, 84°40’W), near Pellston, Michigan, USA. We investigated the influence of atmospheric CO$_2$ and soil-N availability on *Populus tremuloides* using a randomized complete-block design with factorial atmospheric CO$_2$ (35.7 and 70.7 Pa) and soil-N availability (low and high) treatments. Our experiment consisted of five blocks, each containing the four atmospheric-CO$_2$–soil-N-availability treatment combinations. Soil-N availability was modified in open-bottom roots boxes (10.9 m$^2 \times 0.4$ m deep) by mixing different proportions of native A horizon and C horizon soil. High-N-availability soil was 100% A horizon, and low-N-availability soil was 20% A horizon and 80% C horizon. A horizon material was excavated from a Typic Haplorthod (Kalsaska series) and the C horizon material was collected from an Ertic Haplorthod (Rubicon series), both commonly occurring forest soils in northern Lower Michigan. Differences in organic matter (low N = 3559 mg C/kg; high N =
12489 mg C/kg) resulted in net N mineralization of 61 ng N g\(^{-1}\) d\(^{-1}\) in low-N soil and 319 ng N g\(^{-1}\) d\(^{-1}\) in high-N soil (see Zak et al. [2000] for details). Mean growing-season rates of net N mineralization in Lake States forests, estimated by isotope dilution and incubation techniques, range from 50 ng N g\(^{-1}\) d\(^{-1}\) in xeric oak-dominated forests on outwash sands to 1000 ng N g\(^{-1}\) d\(^{-1}\) in mesic sugar maple-dominated forests on finer-textured glacial moraines (calculated from Pastor et al. 1984, Zak et al. 1989, Zak and Pregitzer 1990, Holmes and Zak 1999). *Populus tremuloides* commonly establishes across this range of soil conditions following harvest, fire, or other stand-destroying disturbance. Nitrogen mineralization rates in our experiment fall well within the range that this species encounters under field conditions. A complete summary of soil physical, chemical, and biological properties is presented in Curtis et al. (2000) and Zak et al. (2000).

In early spring 1994, open-top chambers (2.3 m in diameter, 3 m in height) were placed over the 20 open-bottom root boxes in order to control atmospheric CO\(_2\) over the duration of our experiment. Further details regarding our CO\(_2\)-exposure system and its performance can be found in Curtis et al. (2000).

On 4 June 1994 we planted two softwood cuttings of *P. tremuloides* Michx. genotypes into each root box. Softwood cuttings were propagated from roots of locally occurring genotypes that differed in autumnal leaf senescence; three genotypes senesce in early autumn and three senesce in late autumn. Rooted cuttings were graded for size prior to planting; height averaged 17.3 ± 0.45 cm (mean ± 1 SE) and the number of leaves averaged 12.3 ± 0.33 leaves/plant. Leaf litter produced following the 1994 and 1995 growing seasons fell on the soil surface, where it resided during the remaining portion of our experiment. Additional details regarding plant growth conditions can be found in Curtis et al. (2000).

After 2.5 growing seasons (7 June 1994 to 8 July 1996), we destructively harvested above- and belowground tree biomass. One week prior to harvest, we collected 12 soil cores (10 cm in diameter, 45 cm deep) from each open-bottom root box to determine the biomass and N concentration of fine roots (see Pregitzer et al. [2000] for details). At harvest, the largest trees were ~3.5 m tall and had basal stem diameters of 3–4 cm. Aboveground components were harvested by cutting each stem at the root collar. Each tree canopy was divided into three portions, and we immediately determined the fresh mass of leaves in the top, middle, and bottom canopy positions. We then composited all leaves and determined leaf area on a subsample of known mass using a LI-COR LI-3000 leaf-area meter (LI-COR, Lincoln, Nebraska). After the leaves were harvested, we located terminal bud scars, and harvested branch and stem portions produced during the 1994, 1995, and 1996 growing seasons; the fresh mass of each was immediately determined. Approximately 5% of the fresh mass of the leaves, and the branch and stem fractions, were removed and oven-dried at 70°C.

After all aboveground biomass was removed from a chamber, the open-bottom root box was disassembled, and root systems were freed from the sandy soils by hydro-excavation. To accomplish this task, we used a fire hose (50-mm diameter) to wash the contents of each root box and underlying soil into an adjacent 1.5-m-deep trench. This approach allowed us to recover the intact coarse root (>1 mm diameter) system of each tree, while imposing little damage to the outer bark (D. R. Zak, personal observation). Root systems were separated from one another by hand, and all roots <1 mm in diameter were removed. The remaining coarse-root systems were weighed and 5% of the fresh mass of each was oven-dried at 70°C. Our destructive harvest was completed on 8 August 1996 ~4 wk after it began.

Oven-dried leaf, branch, stem, and root tissues from each tree were ground to a fine powder using a Tecator Cyclotec 1093 sample mill (Perstorp Analytical, Silver Springs, Maryland, USA) and their N concentration was determined with a CE Elantech NC 2500 elemental analyzer (Elantech, Lakewood, New Jersey, USA). The biomass and N content of leaves, stems, coarse roots, fine roots, and very fine roots was expressed on a chamber basis (grams per chamber); the relative allocation (percentage of total) of biomass and N to leaves, stem, and roots also was summarized on a chamber basis. To evaluate genotypic responses to atmospheric CO\(_2\) and soil-N availability, we compiled the biomass and N content of leaves, stems, and coarse roots on an individual-tree basis. However, we could not include fine and very fine roots in this analysis, because these biomass components were estimated on a chamber basis, not on an individual-tree basis.

**Statistical analyses**

We analyzed total-plant biomass using an analysis of variance (ANOVA) for a fixed effects, randomized block design with two factorial treatments: atmospheric CO\(_2\) and soil-N availability. Elevated CO\(_2\) can accelerate plant ontogeny so that differences in biomass allocation, N allocation, and N concentration between ambient- and elevated-CO\(_2\)-grown plants can be explained by differences in plant size (Bazzaz et al. 1993, Coleman et al. 1993, Gebauer et al. 1996). We used total-plant biomass as a covariate to remove the potentially confounding effect of plant size in our analysis of biomass allocation, N allocation, and tissue N concentration. Biomass data expressed on an individual-tree basis were analyzed using a complete-block, split-plot design with two factorial treatments. In this analysis, atmospheric CO\(_2\)-soil-N-availability treatment combinations are split by genotype (fixed effect). We also used an analysis of covariance to remove the influence of plant size on our individual-tree-based anal-
Table 1. Harvest biomass (kg/chamber) of Populus tremuloides plants after 2.5 growing seasons under experimental atmospheric-CO₂ and soil-N-availability treatments.

<table>
<thead>
<tr>
<th>Plant components†</th>
<th>Interaction means (n = 5)</th>
<th>Main-effect means (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low soil N</td>
<td>High soil N</td>
</tr>
<tr>
<td></td>
<td>Ambient CO₂</td>
<td>Elevated CO₂</td>
</tr>
<tr>
<td>Leaves [**]</td>
<td>1.01±</td>
<td>1.12±</td>
</tr>
<tr>
<td></td>
<td>(0.043)</td>
<td>(0.144)</td>
</tr>
<tr>
<td>Stems [**]</td>
<td>1.75±</td>
<td>1.93±</td>
</tr>
<tr>
<td></td>
<td>(0.166)</td>
<td>(0.245)</td>
</tr>
<tr>
<td>Roots§</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coarse (diameter</td>
<td>1.54±</td>
<td>1.92±</td>
</tr>
<tr>
<td>&gt; 1.0 mm) [**]</td>
<td>(0.144)</td>
<td>(0.236)</td>
</tr>
<tr>
<td>Fine (diameter</td>
<td>0.07±</td>
<td>0.08±</td>
</tr>
<tr>
<td>0.5–1.0 mm) [**]</td>
<td>(0.007)</td>
<td>(0.011)</td>
</tr>
<tr>
<td>Very fine (diameter</td>
<td>0.24±</td>
<td>0.28±</td>
</tr>
<tr>
<td>&lt; 0.5 mm) [**]</td>
<td>(0.011)</td>
<td>(0.035)</td>
</tr>
<tr>
<td>Biomass</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total above-</td>
<td>2.76±</td>
<td>3.05±</td>
</tr>
<tr>
<td>ground [**]</td>
<td>(0.207)</td>
<td>(0.387)</td>
</tr>
<tr>
<td>Total below-</td>
<td>1.85±</td>
<td>2.28±</td>
</tr>
<tr>
<td>ground [**]</td>
<td>(0.156)</td>
<td>(0.244)</td>
</tr>
<tr>
<td>Total [*]</td>
<td>4.61±</td>
<td>5.33±</td>
</tr>
<tr>
<td></td>
<td>(0.355)</td>
<td>(0.619)</td>
</tr>
</tbody>
</table>

Notes: Data are means with 1 SE in parentheses. Within rows, interaction means with the same lowercase superscript letter are not significantly different at P ≤ 0.05.
* P ≤ 0.05; ** P ≤ 0.01; NS = not significant.
† Significance of N × CO₂ interaction is given in brackets.
‡ Percentage change is calculated as the second mean relative to the first. For example, the change in total biomass from low to high N = 100(16.05 – 4.97)/4.97 = 223%.
§ Fine-root and very-fine-root biomass were calculated from Pregitzer et al. (2000).

Results

Plant biomass

Atmospheric CO₂ and soil-N availability interacted to influence the leaf, stem, root, and total biomass of Populus tremuloides, wherein elevated CO₂ produced a much greater increase in high-N-availability soil, than in low-N-availability soil (Table 1: interaction means). Elevated CO₂ increased total-plant biomass by 16% in low-N soil and by 38% in high-N soil; the increase in low-N soil was not statistically significant. Of all biomass components, coarse roots displayed the largest relative increase (24%) to elevated CO₂ in low-N soil, whereas the biomass of very fine roots displayed the largest response (65% increase) to elevated CO₂ in high-N soil (Pregitzer et al. 2000; Table 1: interaction means).

Atmospheric CO₂ and soil-N availability were significant main effects in the chamber-level analysis of plant biomass (i.e., kilograms per chamber); both substantially increased the total, leaf, stem, and root biomass of P. tremuloides. Total biomass increased by 223% from low- to high-N-availability soil, whereas elevated CO₂ increased total plant biomass by 32% relative to plants grown under ambient CO₂ (Table 1: main-effect means). Stem biomass was most responsive to soil-N availability and exhibited a 326% increase from low- to high-N-availability soil. This differs substantially from the growth response of P. tremuloides to atmospheric CO₂, in which the biomass of very fine roots exhibited the largest increase to elevated CO₂ (52% Table 1: CO₂ main effect).

On an individual-tree basis, we observed a significant interaction between genotype and soil-N availability, in which the increase in leaf, stem, coarse-root, and total biomass from low- to high-N-availability soil was greatest for late-senescing clones (Fig. 1A). In contrast, the biomass of early- and late-senescing genotypes responded in a similar manner to atmospheric CO₂ (Fig. 1B), so that there was no significant genotype × atmospheric-CO₂ interaction.

Plant N concentration and content

Atmospheric CO₂ and soil-N availability did not interact to influence tissue N concentrations of leaves, stems, and roots (in milligrams of N per kilogram of
干物质 [mg N/kg]; 表 2: 交互作用均值)。尽管大气 CO2 和土壤氮素可用性是显著的主要效应,但在我们的分析中,它们对植物组织的氮素浓度产生相反的影响。正如我们所预期,从低氮到高氮可用性土壤 (表 2: 土壤氮素主效应) 中,叶、茎和根的氮素浓度增加。然而,升高的大气 CO2 持续降低了所有植物组织的氮素浓度,其中叶、粗根和非常细根的下降幅度最大 (表 2: CO2 主效应)。这些减少导致整个植物的氮素浓度下降了 18%。总生物量不是任何室水平分析的组织氮素浓度的显著协变量,表明在升高的 CO2 下氮素浓度的减少不能归因于植物大小的差异。这与我们的分析一致,我们没有找到 CO2 X 氮素可用性交互作用对一个…

**Table 2.** Nitrogen (mg N/kg) of *Populus tremuloides* plant tissues grown under atmospheric-CO2 and soil-N-availability treatments.

<table>
<thead>
<tr>
<th>Plant components‡</th>
<th>Interaction means (n = 5)</th>
<th>Main-effect means (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low soil N</td>
<td>High soil N</td>
</tr>
<tr>
<td>Leaves [ns]</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ambient CO2</td>
<td>Elevated CO2</td>
</tr>
<tr>
<td></td>
<td>17 024 (241)</td>
<td>13 839 (325)</td>
</tr>
<tr>
<td>Stems [ns]</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4 222 (267)</td>
<td>3 893 (284)</td>
</tr>
<tr>
<td>Roots§</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coarse (diameter &gt; 1.0 mm) [ns]</td>
<td>4 004 (336)</td>
<td>4 296 (356)</td>
</tr>
<tr>
<td>Fine (diameter 0.5–1.0 mm) [ns]</td>
<td>7 582 (142)</td>
<td>7 632 (222)</td>
</tr>
<tr>
<td>Very fine (diameter &lt; 0.5 mm) [ns]</td>
<td>19 318 (614)</td>
<td>16 724 (1 247)</td>
</tr>
<tr>
<td>Biomass</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total above-ground [*]</td>
<td>8 980 (88)</td>
<td>7 564 (325)</td>
</tr>
<tr>
<td>Total below-ground [*]</td>
<td>6 119 (342)</td>
<td>5 941 (685)</td>
</tr>
<tr>
<td>Total [*]</td>
<td>7 841 (355)</td>
<td>6 876 (451)</td>
</tr>
</tbody>
</table>

**Notes:** Total aboveground and belowground N tissue concentration represent weighted averages based on the mass and N concentration of the component tissues. Data are means with 1 SE in parentheses. Within rows, interaction means with the same lowercase superscript letter are not significantly different at \( P = 0.05 \).

† \( P < 0.10; * P < 0.05; ** P < 0.01; \( NS \) = not significant.
†‡ Significance of N x CO2 interaction is indicated in brackets.
§ The concentrations of N in fine and very fine roots were calculated from Pregitzer et al. (2000).
individual-tree basis, and no interaction genotype between atmospheric CO₂ or soil-N availability (data not shown); individual-tree biomass (i.e., leaf + stem + coarse roots) also was not a significant covariate in this analysis.

Although elevated atmospheric CO₂ generally lowered the N concentration of all plant tissues, total-plant N content (grams of N per chamber) at elevated CO₂ was equivalent to (low-N soil) or greater than (high-N soil) total-plant N under ambient CO₂ (Table 3: interaction means). In high-N soil, we found a significant, linear relationship between fine-root (<1.0 mm diameter) biomass and total-plant N content (g N/chamber = 46.6 (g fine root/chamber) + 23.8; r = 0.66; n = 10 observations), indicating that increases in fine-root biomass under elevated CO₂ in part contributed to greater whole-plant N content in high-N soil. We found no relationship between fine-root biomass and whole-plant N content in low-N soil, in which we observed equivalent total-plant N contents under ambient and elevated CO₂. Moreover, atmospheric CO₂ significantly increased the N content of leaves, stem, and very fine roots in high-N soil, but it did not influence the N content of these plant components in the low-N soil (Table 3: interaction means). The N content of coarse and fine roots was unaltered by the atmospheric CO₂ at both levels of soil-N availability.

Atmospheric CO₂ and soil-N availability were significant main effects in our analysis; both produced a significant increase in total-plant N, albeit of different magnitudes (Table 3). Averaged across CO₂ treatments, total-plant N in low-N soil was 35.8 g N/chamber and 145.7 g N/chamber in high-N soil, a 307% increase from low- to high-N-availability soil. On the other hand, total-plant N content increased 11% from ambient to elevated atmospheric CO₂ (Table 2: CO₂ main effect). As expected, high-N soil (main effect) substantially and significantly increased the N content of all plant components, with stem N content exhibiting the largest (408%) increase between the N-availability treatments. Changes in the N content of plant components were variable in response to atmospheric CO₂, wherein elevated CO₂ significantly increased the N content of leaves, stem, and very fine roots but did not alter the N content of coarse roots (Table 2: CO₂ main effect).

Analysis of plant N content on an individual-tree basis revealed a significant genotype × soil-N availability interaction, in which late-senescing clones displayed a proportionately larger increase in leaf, stem, coarse-root, and total-plant N content than early-senescing clones (Fig. 2A). There was no genotype × atmospheric-CO₂ interaction, indicating that atmo-
spheric CO₂ increased the N capital (leaves, stem, and coarse root) of early- and late-senescing genotypes by the same proportion (Fig. 2B); individual-tree biomass was not a significant covariate in this analysis.

Allocation of biomass and N

Atmospheric CO₂ and soil-N availability did not interact to influence allocation to leaves, stems, and roots (Table 4: interaction means). We also found that total biomass was not a significant covariate influencing the allocation of biomass to any plant component. When partitioned into total above- and belowground allocation, elevated CO₂ increased belowground biomass allocation at both levels of soil-N availability. Nevertheless, the magnitude of this response was small and was only significant in high-N soil (Table 4: interaction means). Atmospheric CO₂ and soil-N availability were significant main effects in our analysis of biomass allocation, but each had a different effect on allocation to leaves, stems, and roots. Soil-N availability significantly increased allocation to stems and decreased allocation to all other plant components (Table 4: soil-N main effect). This pattern resulted in an overall increase in aboveground allocation (58.5% at low N and 67.4% at high N) and a subsequent decline in belowground allocation from low- to high-N-availability soil. Al-

![Figure 2](image_url)

**Fig. 2.** The influence of (A) soil-N availability and (B) atmospheric CO₂ concentration on plant N capital for early- and late-senescing *Populus tremuloides* genotypes. The increase in N capital from low- to high-N soil was proportionately greater for late-senescing genotypes, relative to the increase in N capital for early-senescing genotypes. Atmospheric CO₂ increased the N capital of early- and late-senescing genotypes in a similar manner, leading to a nonsignificant genotype × atmospheric-CO₂ interaction. Fine and very fine roots were not included in this tree-level analysis, because these components were determined only on a chamber basis. Bars with the same lowercase letter (total-plant N) are not significantly different at α = 0.05.

![Table 4](table_image_url)

**Table 4.** Relative allocation of (%) biomass to leaves, stem, and roots under experimental atmospheric-CO₂ and soil-N-availability treatments.

<table>
<thead>
<tr>
<th>Plant components†</th>
<th>Interaction means (n = 5)</th>
<th>Main-effect means (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low soil N</td>
<td>High soil N</td>
</tr>
<tr>
<td></td>
<td>Ambient CO₂</td>
<td>Elevated CO₂</td>
</tr>
<tr>
<td>Leaves [NS]</td>
<td>22.3 (0.90)</td>
<td>21.0 (0.44)</td>
</tr>
<tr>
<td>Stems [NS]</td>
<td>37.7 (0.98)</td>
<td>36.0 (0.97)</td>
</tr>
<tr>
<td>Roots Coarse (diameter &gt; 1.0 mm) [NS]</td>
<td>33.3 (0.71)</td>
<td>36.0 (1.05)</td>
</tr>
<tr>
<td>Fine (diameter 0.5–1.0 mm) [NS]</td>
<td>1.5 (0.16)</td>
<td>1.6 (0.25)</td>
</tr>
<tr>
<td>Very fine (diameter &lt; 0.5 mm) [NS]</td>
<td>5.2 (0.30)</td>
<td>5.5 (0.93)</td>
</tr>
<tr>
<td>Biomass Aboveground [*]</td>
<td>60.0* (0.75)</td>
<td>57.0* (1.21)</td>
</tr>
<tr>
<td>Belowground [*]</td>
<td>40.0* (0.75)</td>
<td>43.0* (1.21)</td>
</tr>
</tbody>
</table>

**Notes:** Values are the percentage of total biomass (means with 1 SE in parentheses) allocated to each plant component. Within rows, interaction means with the same lowercase superscript letter are not significantly different at P ≤ 0.05.

* *P ≤ 0.05; **P ≤ 0.01; NS = not significant.

† Significance of N × CO₂ interaction is indicated in brackets.
though atmospheric CO₂ significantly increased allocation to coarse roots and decreased allocation to stem, changes in biomass allocation with respect to atmospheric CO₂ were small (Table 3: CO₂ main effect). Overall, atmospheric CO₂ resulted in a 4% decline in aboveground allocation and a 6% increase in belowground allocation (Table 3: CO₂ main effect). Taken together, our results indicate that the growth of *Populus tremuloides* changed allometrically in response to increased soil-N availability and isometrically with respect to rising atmospheric CO₂. This response could not be attributed to differences in plant development among treatments, because plant size was not a significant covariate in any analysis of biomass allocation.

In low-N soil, the allocation of N to leaves significantly declined from 48.6% at ambient atmospheric CO₂ to 43.0% at elevated CO₂, but we did not observe a similar reduction related to atmospheric CO₂ in high-N soil (Table 5: interaction means). Allocation of N to stems was not influenced by an atmospheric-CO₂–soil-N-availability interaction, nor was allocation of N to fine and very fine roots (Table 5: interaction means). Allocation of N to coarse roots in low-N soil increased significantly with respect to rising atmospheric CO₂, but we did not observe any significant changes in the allocation of N to leaves, stems, and roots related to soil-N availability (Table 5: soil N main effect). Total-plant biomass also altered plant N allocation, but atmospheric CO₂ did not.

### DISCUSSION

Our ability to predict whether elevated atmospheric CO₂ increases, decreases, or does not alter ecosystem C-storage centers on understanding changes in the acquisition of C and soil nutrients by plants and the extent to which changes in resource acquisition alter plant growth and allocation. In our experiment, increases in plant biomass under elevated CO₂ were constrained by soil-N availability, a response consistent with that in many other woody plants (Brown 1991, Conroy et al 1992, Bazzaz and Miao 1993, Johnson et al. 1997, Kinney and Lindroth 1997, Prior et al. 1997, Johnson et al. 1998). In a 57-d experiment with *Populus tremuloides*, Kinney and Lindroth (1997) observed elevated CO₂ (65 Pa) increased total biomass by 35% in low-N soil and by 71% in high-N soil, a much larger
increase than we observed after 2.5 growing seasons. Further, they observed the growth responses of *Quercus rubra* and *Acer saccharum* to elevated CO₂ also were dependent on soil-N availability, wherein the greatest response to elevated CO₂ occurred in N-rich soil. Several studies with coniferous trees have drawn similar conclusions. Prior et al. (1997), for example, found that *Pinus palustris* exhibited virtually no growth response to elevated CO₂ (72 Pa) when soil-N availability was low, but elevated CO₂ substantially increased growth (45%) when N was in ample supply. Johnson et al. (1998) summarized a series of laboratory and field studies and concluded low soil-N availability limited the growth response of *P. ponderosa* and *P. taeda* to elevated CO₂ (70–71 Pa). Although the aforementioned studies indicate that tree growth under elevated CO₂ can be greater when soil resources are in ample supply, it will be necessary to determine the extent to which ontogenetic differences contributed to such a response. Norby et al. (1996) demonstrated that variation in plant growth response to elevated CO₂ could be attributed to ontogenetic differences in leaf-area display, regardless of variation in soil-resource availability. Nonetheless, our results indicate the greatest growth response to elevated CO₂ occurred in high-N soil, and that such a response could not be attributed to differences in plant development.

At a fundamental level, C assimilation by leaves, the accumulation of leaf area, and nutrient acquisition by fine roots and mycorrhizae control plant resource acquisition (i.e., C and N) and hence the patterns of plant growth we observed among our atmospheric-CO₂ and soil-N-availability treatments. Over the duration of our experiment, elevated atmospheric CO₂ increased leaf-level C assimilation at both levels of soil-N availability (Curtis et al. 2000). Similarly, leaf area increased under elevated CO₂ by 28% in high-N soil, but elevated CO₂ did not influence leaf area in low-N soil (Curtis et al. 2000). Although leaf-level C assimilation increased to a much smaller extent from low- to high-N soil (e.g., 22%, Curtis et al. 2000), greater C acquisition in high-N soil was facilitated by a much larger increase in leaf area (197%). Thus, greater rates of leaf-level C assimilation and leaf-area accumulation resulting from increases in atmospheric CO₂ and soil-N availability directly corresponded to differences in total biomass among our atmospheric-CO₂-soil-N-availability treatments. Moreover, the large increase in very-fine-root biomass (52%) we observed under elevated CO₂ (Table 1: CO₂ main effect), compared to the relatively smaller increases in leaf mass (30%) and area (20%; Curtis et al. 2000), suggest that fine roots are more responsive to elevated CO₂ than are leaves. This response appears to have important implications for the acquisition of soil resources under elevated CO₂.

Elevated atmospheric CO₂ enhanced the ability of *Populus tremuloides* to forage for N in our high-N soil, a contention supported in part by a substantial increase in whole-plant N content under elevated CO₂. This response did not occur in low-N soil, in which whole-plant N content was equivalent under ambient and elevated CO₂. Although the influence of elevated CO₂ on nutrient acquisition is somewhat variable among studies, others have observed that increases in whole-plant nutrient contents under elevated CO₂ were controlled by soil-nutrient availability. For example, elevated CO₂ decreased the whole-plant N content of *Eucalyptus grandis* when soil-N availability was very low, but substantially increased whole-plant N content when soil N was in ample supply (Conroy et al. 1992). Johnson et al. (1997) grew *Pinus ponderosa* over a range of soil-N availability and observed consistent increases (61%) in whole-plant N content under elevated CO₂. Others have observed equivalent or small increases in whole-plant N content under elevated CO₂, but these small increases were not statistically significant (Berntson and Bazzaz 1996, 1998). Differences in whole-plant N content among these studies, and hence the acquisition of N from soil, are undoubtedly controlled by changes in root foraging and the extent to which net N mineralization is modified by above- and belowground plant litter inputs to soil (sensu Díaz et al. 1993, Hungate et al. 1997, Berntson and Bazzaz 1998).

Several lines of evidence suggest that greater N acquisition under elevated CO₂ resulted from a more thorough exploitation of high-N soil by a larger and more productive root system with an equivalent physiological capacity to assimilate N (micromoles per gram per second). First, Rothstein et al. (2000) observed that elevated CO₂ did not alter the maximum velocity of NH₄⁺ or NO₃⁻ uptake (i.e., *V_max*, in micromoles per gram per second) in the fine roots *Populus tremuloides* growing in our experiment. However, the maximum velocity for NH₄⁺ uptake declined in high-N soil as did the affinity of the enzyme system for substrate. That is, the physiological capacity of fine roots to assimilate N declines with greater N availability, but this was not affected by elevated CO₂. Second, elevated CO₂ significantly increased the biomass and net production of fine roots in high-N soil and had no effect on the fine roots of plants grown in low-N soil (Pregitzer et al. 2000), a pattern that paralleled differences in whole-plant N content. Third, we found a significant, positive relationship between whole-plant N content and fine-root biomass in high-N soil. And finally, gross rates of N mineralization and microbial immobilization were not altered by elevated CO₂, which provided for equivalent rates of net N mineralization under conditions of ambient and elevated CO₂ (Zak et al. 2000). Taken together, plants in high-N soil further increased fine-root production under elevated CO₂ and acquired more soil N, which likely fed forward within the plant to increase leaf-level C assimilation and leaf area (Curtis et al. 2000). Additionally, the large increase in whole-
plant N content we observed from low-N to high-N soil suggests the advantage conferred through greater soil exploitation by a larger fine-root system (i.e., increased surface area) must have far surpassed the physiological reduction in ion uptake per unit of fine root (mmol NH$_4^+$ or NO$_3^-$·g$^{-1}$·h$^{-1}$).

The total biomass and N content of early- and late-senescing genotypes responded differently to soil-N availability, but both responded in the same manner to atmospheric CO$_2$. Late-senescing clones, which produced greater leaf area than early-senescing genotypes (Curtis et al. 2000), displayed proportionately larger increases in plant biomass and N content than early-senescing genotypes (Figs. 1 and 2) in response to soil-N availability. However, both genotypes increased growth to the same extent under elevated CO$_2$. *Populus* genotypes are known to vary widely in their growth response to soil-nutrient availability (Ceulemans and Isebrands 1996), and several studies have documented that growth responses to elevated CO$_2$ also differ among genotypes (Radoglou and Jarvis 1990, Ceulemans et al. 1995, 1996). Given the very limited number of genotypes in our study (i.e., six), it is difficult to draw conclusions about genotypic responses to atmospheric CO$_2$ in *Populus*. This topic clearly warrants further attention, because genotypic responses to atmospheric CO$_2$ will likely be an important factor controlling variability in the growth response of *P. tremuloides* forests to rising atmospheric CO$_2$.

While some have argued that elevated CO$_2$ will increase allocation to roots (McGuire et al. 1995, Rogers et al. 1996), we found little evidence to support this contention. In our study, elevated atmospheric CO$_2$ did not alter the allocation of biomass or N in *P. tremuloides*, and this was true in both low- and high-N soil. Moreover, total plant mass was not a significant factor influencing allocation to any biomass component, suggesting that elevated CO$_2$ did not alter the rate of ontogeny in *P. tremuloides*. Although the relatively small decrease in aboveground biomass allocation (−4%) and slight increase in belowground (6%) under elevated CO$_2$ (main effect) were statistically significant, these small changes are of little biological consequence in light of the much larger increase in plant biomass under elevated CO$_2$ (compare Tables 1 and 4). Radoglou and Jarvis (1990) also observed that elevated CO$_2$ (70 Pa) elicited no change in biomass allocation for several *Populus* genotypes, while others working with different genotypes have drawn similar conclusions (Bosac et al. 1995). Rey and Jarvis (1987) noted no shift in the biomass allocation of *Betula pendula* grown under elevated CO$_2$ (70 Pa). Understanding the response of biomass allocation to elevated CO$_2$ has important ecosystem-level implications for the cycling and storage of C and N in forest ecosystems.

We found no evidence to support the hypothesis that elevated CO$_2$ decreases the amount of N allocated to leaves, thereby lowering their N concentration. Moreover, declines in tissue N concentration under elevated CO$_2$ also cannot be attributed to differences in plant development as some have argued (Coleman et al. 1993), because total plant biomass was not a significant covariate in any analysis of tissue N concentration. McGuire et al. (1995) concluded that elevated CO$_2$ can produce substantial reductions in leaf N concentration (−21%), but often causes much smaller reductions in the N concentration of fine roots. In a more recent synthesis, Cotrufo et al. (1998) also suggested that elevated CO$_2$ caused plants to increase N allocation to roots, because the N concentration of aboveground tissues (−14%) declined to a much greater extent than that of roots (−9%). Similarly, in our study, the N concentration of leaves (−17%) produced under elevated CO$_2$ declined to a much greater extent than did the N concentration of fine roots (−5%; Table 2: CO$_2$ main effect). However, the changes in N concentration we observed did not result from shifts in N allocation. In fact, elevated CO$_2$ did not alter the proportion of N allocated to any biomass component, a result consistent with patterns of biomass allocation. Given no shift in biomass or N allocation under elevated CO$_2$, declines in tissue N concentration simply result from a 32% increase in total biomass and only a 17% increase in whole-plant N content under elevated CO$_2$ (Tables 1 and 3: CO$_2$ main effect). That is, relative allocation was not altered by elevated CO$_2$, but increases in total biomass surpassed increases in whole-plant N content, giving rise to a decline in whole-plant N tissue N concentration.

Atmospheric CO$_2$ did not substantially alter biomass allocation in *P. tremuloides*, but soil-N availability did. There was a greater amount of biomass allocated to stems in high-N soil, while allocation of biomass to all other plant components declined as N availability increased. These changes were sufficient to significantly decrease belowground allocation from 41% in low-N soil to 33% in high-N soil, a response consistent with that of many herbaceous and woody plants. Similarly, we observed greater allocation of N to stem in high-N-availability soil and a decline in N allocation to leaves, fine roots, and very fine roots, while there was no change in N allocation to coarse roots. Notwithstanding these changes, allocation of N to belowground plant components did not change from low-N (34%) to high-N soil (31%). From these results, it is clear that soil-N availability is a potent modifier of above- vs. belowground biomass allocation, but it has a much smaller influence on the allocation of N to above- vs. belowground plant components.

**Summary and implications**

Understanding the interaction between atmospheric CO$_2$ and soil-N availability on the growth and biomass allocation of *Populus tremuloides* has important im-
plications for ecosystem development and forest management. *P. tremuloides* is perhaps the most widely distributed deciduous tree species in the Northern Hemisphere and is broadly used by the forest industry in the Lake States region (USA) and Canada. Throughout the Upper Lake States (i.e., Minnesota, Wisconsin, and Michigan), this and other fast-growing, early-successional aspen (i.e., *Populus grandidentata*) occupy ~4.8 × 10^6 ha over a range of soil conditions that our experimental N-availability treatments were designed to reflect (Smith 1986, Miles et al. 1995, Leatherberry and Spencer 1996). The majority of this land area comprises relatively young (<60 yr), even-aged stands that regenerated following clear-cut harvest (Smith 1986, Miles et al. 1995, Leatherberry and Spencer 1996). Although it is difficult to directly extrapolate from our results to *P. tremuloides* forests throughout the Lake States region, the response of young trees in our experiment is relevant to these ecosystems. A substantial proportion of the forested landscape in the Upper Lake States region is covered by young *P. tremuloides* stands that are rapidly accumulating biomass, and their response to rising atmospheric CO₂ has the potential to alter fiber production and ecosystem C storage across a large spatial extent (Alban and Perala 1992).

If trees respond in the field as they did in our experiment, then elevated atmospheric CO₂ will likely increase the rate of biomass accumulation in young, aggrading *P. tremuloides* stands. Our results indicate the extent of this increase will be governed by soil-N availability, wherein the greatest responses will likely occur on the most fertile soils. Given that total biomass increased 17% even in our low-N soil, it is also likely that elevated CO₂ will increase rates of biomass accumulation on relatively poor soils with low net N-mineralization rates. Moreover, these increases cannot be attributed to more rapid rates of tree development or ontogeny under elevated CO₂, because total plant mass was not a significant covariate in our analyses of biomass allocation. Regardless of soil-N availability, plants grew larger under elevated CO₂, not just faster, with a constant proportion of leaves, stem, and roots (i.e., no change in biomass allocation).

Greater rates of biomass accumulation under elevated CO₂ present the possibility that *P. tremuloides*-dominated forests will reach both commercial and ecological maturity over a shorter period of time. Such a response could lessen the commercial rotation age and accelerate rates of ecosystem development. However, it is unclear whether the growth response of individual trees to elevated CO₂ will be maintained as canopy closure occurs aboveground and root closure occurs belowground. Although elevated atmospheric CO₂ enabled *P. tremuloides* to accumulate more N from our high-N soil (i.e., greater whole-plant N content), it is likely that such a response will not be maintained once roots fully exploit the soil. If such a response is not maintained, it will be important to determine whether this will eventually result in a reduction in enhanced biomass accumulation under elevated CO₂ on fertile soils. It also will be important to determine if greater growth will be maintained on soils of low N availability in which plants obtain equivalent quantities of soil N under ambient and elevated CO₂. The extent to which growth responses to elevated CO₂ diminish following canopy and root closure will be an important determinant of whether elevated atmospheric CO₂ increases, decreases, or does not alter the cycling and storage of C in forest ecosystems. Achieving this understanding can only occur through longer-term experiments in which trees have fully exploited both above- and belowground resources.

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Eucalyptus grandis


