Increases in nitrogen uptake rather than nitrogen-use efficiency support higher rates of temperate forest productivity under elevated CO₂

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Forest ecosystems are important sinks for rising concentrations of atmospheric CO₂. In previous research, we showed that net primary production (NPP) increased by 23 ± 2% when four experimental forests were grown under atmospheric concentrations of CO₂ predicted for the latter half of this century. Because nitrogen (N) availability commonly limits forest productivity, some combination of increased N uptake from the soil and more efficient use of the N already assimilated by trees is necessary to sustain the high rates of forest NPP under free-air CO₂ enrichment (FACE). In this study, experimental evidence demonstrates that the uptake of N increased under elevated CO₂ at the Rhinelander, Duke, and Oak Ridge National Laboratory FACE sites, yet fertilization studies at the Duke and Oak Ridge National Laboratory FACE sites showed that tree growth and forest NPP were strongly limited by N availability. By contrast, nitrogen-use efficiency increased under elevated CO₂ at the POP-EUROFACE site, where fertilization studies showed that N was not limiting to tree growth. Some combination of increasing fine root production, increased rates of soil organic matter decomposition, and increased allocation of carbon (C) to mycorrhizal fungi is likely to account for greater N uptake under elevated CO₂. Regardless of the specific mechanism, this analysis shows that the larger quantities of C entering the below-ground system under elevated CO₂ result in greater N uptake, even in N-limited ecosystems. Biogeochemical models must be reformulated to allow C transfers below ground that result in additional N uptake under elevated CO₂.

Increasing rates of forest NPP under elevated CO₂, and whether responses were consistent among the four forest FACE experiments. Indeed, because N availability often limits primary productivity through its effect on photosynthesis (13) and on the synthesis of proteins required for the construction and maintenance of living tissue, some combination of increased N uptake from the soil and more efficient use of the N already assimilated by trees is necessary to sustain the high rates of forest NPP observed under elevated CO₂.

Nitrogen-use efficiency (NUE) measures the amount of biomass produced per unit of N taken up from the soil (14), defined as

\[ \text{NUE} = \frac{\text{NPP}}{N_{\text{uptake}}} \]  

where NPP and N_{\text{uptake}} are measured in units of dry matter production or N taken up from soil per square meter of land surface per unit of time (i.e., g of DM·m⁻²·yr⁻¹ or g of Nm⁻²·yr⁻¹). Furthermore, NUE can be decomposed into two processes of considerable ecological interest (15, 16): (i) the rate at which dry matter is produced per unit of N in tree biomass per unit of time (i.e., N productivity, g of DM·g of N⁻¹·yr⁻¹), and (ii) the amount of time N is available for use in C fixation before it is recycled into the soil system [i.e., the mean residence time of N in biomass in years (MRT)]. Thus, NUE can be rewritten as

\[ \text{NUE} = \frac{\text{NPP}}{MRT} \times \frac{N_{\text{uptake}}}{N_{\text{uptake}}} \]  

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errestrial ecosystems, and particularly forests, exchange large quantities of carbon with the atmosphere each year; ~15% of the atmospheric pool of CO₂ is exchanged between plants and the atmosphere annually (1, 2). Globally, trees represent 80% of plant biomass (3–5) and 50–60% of annual net primary production (NPP) in terrestrial ecosystems (6, 7). Given their large contribution to terrestrial productivity and carbon (C) storage, forest ecosystems are important sinks for anthropogenic emissions of CO₂ to the earth’s atmosphere (8, 9).

Previous research (10) shows that enhanced rates of forest NPP under free-air CO₂ enrichment (FACE) were similar among four forest sites differing in species composition, climate, and soil fertility (Table 1). At one of these sites (Duke FACE), spatial variation in soil nitrogen (N) availability correlated with increasing rates of forest NPP under present-day and elevated concentrations of atmospheric CO₂ (11, 12). This observation raised the question of how forest trees acquired N to support high

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where NPP and N uptake are as in Eq. 1 and N content is the mass of N in biomass per square meter of land surface (g of N m⁻²).

The objective of this study was to determine the relative importance of increases in the uptake of N from the soil and increases in NUE as processes supporting higher rates of NPP under elevated CO₂ compared with present-day concentrations of atmospheric CO₂ among the four forest FACE experiments. To meet this objective, we assembled plot-specific data on plant tissue N concentrations (foliage, wood, fine roots, above-ground litterfall, and fine root turnover) and NPP from these experiments. We calculated the rate of N uptake from the soil, NUE, the N content of biomass, N productivity, and the MRT of N in biomass per square meter of land surface (g of N m⁻² yr⁻¹) as in Eq. 1.

The content of N in biomass was significantly higher under elevated CO₂ than at present-day concentrations of atmospheric CO₂ at the Rhinelander, Duke, and ORNL FACE sites but not at the POP-EUROFACE site (Fig. 3A). At all sites, N productivity was significantly higher under elevated CO₂ (Fig. 3B). At the Rhinelander, Duke, and ORNL FACE sites, the increase in N productivity occurred because elevated CO₂ stimulated forest NPP (Fig. 2A) to a greater degree than the content of N in biomass (Fig. 3A). At the POP-EUROFACE site, the increase in N productivity was only due to greater forest NPP under elevated CO₂ (Fig. 2A); there was no effect of elevated CO₂ on the N content of biomass at this site (Fig. 3A).

The MRT of N in biomass at the Rhinelander and ORNL FACE sites was significantly lower under elevated CO₂ than at present-day CO₂ concentrations. NPP at present-day CO₂ concentrations, NPP was substantially higher at POP-EUROFACE compared with the other three sites. NPP at POP-EUROFACE was higher in elevated CO₂, but N uptake was not; hence, the data points for plots from elevated and present-day CO₂ concentrations align along different NUE isopleths, suggesting greater NUE in elevated CO₂. By contrast, the data points for present-day and elevated CO₂ from the Rhinelander, Duke, and Oak Ridge National Laboratory (ORNL) FACE sites generally plotted along the same NUE isopleth because NPP and N uptake were increased to a similar degree under elevated CO₂. NUE in the two established stands (Duke and ORNL) was greater than that of the developing stands (Rhinelander and POP-EUROFACE). These relationships will now be explored in a statistical framework.

Compared with NPP at present-day CO₂ concentrations, NPP was significantly higher under elevated CO₂ (Fig. 2A) (10). The average annual uptake of N from the soil increased significantly under elevated CO₂ at the Rhinelander, Duke, and ORNL FACE sites but not at the POP-EUROFACE site (Fig. 2B). By contrast, NUE was significantly higher under elevated CO₂ at the POP-EUROFACE site but not at the Rhinelander, Duke, or ORNL FACE sites (Fig. 2C).
under present-day CO2 concentrations (Fig. 3C). A similar trend was observed at the Duke FACE site under elevated CO2, but the effect was not statistically significant (Fig. 3C). There was no effect of elevated CO2 on the MRT of N in biomass at the POP-EUROFACE site.

Discussion

Forest ecosystems are important sinks for rising concentrations of atmospheric CO2. In a previous data synthesis of four forest FACE experiments (10), forest NPP increased on average by 23 ± 2% when the forests were grown under atmospheric concentrations of CO2 predicted for the latter half of this century. Because N often limits temperate forest productivity (17–19), some combination of increased uptake of N from the soil and more efficient use of the N already assimilated by trees is necessary to sustain the high rates of forest NPP observed under elevated CO2 (i.e., rearranging Eq. 1, \( NPP = N_{\text{uptake}} \times NUE \)). In this data synthesis, we show that increases in forest productivity under elevated CO2 at the Rhinelander, Duke, and ORNL FACE sites were supported by significant increases in the quantity of N taken up from the soil, not by increases in NUE (Fig. 2B). By contrast, the increase in forest productivity under elevated CO2 at the POP-EUROFACE site was supported by an increase in NUE, not greater N uptake from the soil. Thus, increased uptake of N from the soil was the more typical mechanism supporting high rates of forest NPP under elevated CO2.

![Fig. 2](image1.png)

![Fig. 3](image2.png)
During the decomposition of soil organic matter (SOM), microorganisms release N to the soil solution, often in mineral form ("mineralization"), as well as take up N from the soil solution ("immobilization"), with the difference between mineralization and immobilization assumed to represent N available to plants. Given that the rate of N mineralization did not increase significantly more than the rate of N immobilization in the soils under elevated CO₂ at the Rhinelander, Duke, and ORNL FACE sites (20–23), greater uptake of N from the soil in response to forest growth under elevated CO₂ was unexpected. This was particularly true of the Duke and ORNL FACE sites where tree growth is demonstrably N-limited [supporting information (SI) Table 2] (11). In N-limited ecosystems, the rate at which N is converted to available forms is slow relative to the rate of N uptake by trees, and as a consequence it is assumed that there is little or no additional capacity of soils to supply N to forest trees (24–29). Indeed, most biogeochemical models predict increases in NUE in response to high rates of forest NPP under elevated CO₂ in N-limited ecosystems (26, 27, 30) and suggest that enhanced rates of N uptake under elevated CO₂ can only occur where N availability exceeds demand under present-day concentrations of atmospheric CO₂ (29, 30). Thus, the response of N uptake and NUE in these young temperate forests exposed to FACE is the opposite of that predicted by the current generation of biogeochemical models (26–28, 30).

A variety of mechanisms have been proposed to explain greater uptake of N by plants growing under elevated CO₂. The most common explanation is that significant increases in fine root production under elevated CO₂ allow trees to explore more of the soil volume for available N ["root exploration" (22, 31–35)]. The underlying assumption for this model is that N is being mineralized in excess of microbial demand in the soil and that this supply of N is only available to trees growing under elevated CO₂ because of a more extensive fine root network. The root-exploration hypothesis is attractive because it reconciles the contradiction between the observation that the rate of N mineralization does not increase under elevated CO₂ (20–22) but that more N is taken up by trees under elevated CO₂ (Fig. 2B). If the root-exploration hypothesis is correct, then enhanced NPP of forests under elevated CO₂ decreases the degree to which N limits forest productivity.

Increases in fine root production are often associated with increases in C allocation to mycorrhizal fungi and root exudation, processes that are also thought to increase plant access to soil N under elevated CO₂ (36–38). Mycorrhizal fungi play a crucial role in N cycling through the release of enzymes involved in the decomposition of SOM (39–41), the capture of organic and inorganic forms of N from the soil (42, 43), and the transfer of N to host plants (44, 45). Similarly, the addition of C substrates to the soil stimulates the decomposition of SOM, often as a result of an increase in microbial activity (reviewed in ref. 46). Because SOM contains N, the delivery of plant-derived substrates can also stimulate a more rapid mineralization of N from SOM (47–51). Rapid plant growth under elevated CO₂ is associated with greater hyphal length and fungal activity in the soil and an increase in the degree to which fine roots are colonized by ecto- and arbuscular mycorrhizal fungi (36, 52, 53). Moreover, enhanced NPP under elevated CO₂ increases the quantity of C entering soil (54, 55) by increasing fine root production and turnover (56–58) and root exudation (59, 60), processes that increase the metabolism of organic substrates by soil microbial communities (61–65) and soil respiration (66–68).

In contrast to the Rhinelander, Duke, and ORNL sites, N uptake was not significantly greater under elevated CO₂ at the POP-EUROFACE site (Fig. 2B), a response that is likely explained by the growing conditions and land-use history of this site. The POP-EUROFACE site was irrigated during exposure to elevated CO₂, so there were few constraints of water on tree growth throughout the summer growing season (Table 1) (69). Similarly, the POP-EUROFACE site was located on former agricultural land where soil N availability was high and not limiting to tree growth (SI Table 2) (35). As a result, additional uptake of soil N was not required to support high rates of NPP under elevated CO₂ (Fig. 2B).

Rather, the N already assimilated by the trees at the POP-EUROFACE site was used more efficiently to support high rates of forest NPP under elevated CO₂ (Fig. 2C) (70). Although NUE did not change at all sites, the analysis of the two components of NUE, N productivity and the MRT of N in biomass, provides important insights into how these forests used assimilated N. Elevated CO₂ increased N productivity at all sites (Fig. 3B), indicating that more C was fixed per unit of N in biomass per unit time under elevated CO₂ than under present-day concentrations of atmospheric CO₂. This response is likely to have occurred as a result of the significant increase in photosynthetic NUE under elevated CO₂ at the four FACE sites (71–74). Despite the increase in N productivity under elevated CO₂, NUE did not increase at the Rhinelander, Duke, and ORNL sites because elevated CO₂ stimulated NPP and N uptake from the soil (Fig. 2B) to similar degrees, whereas N content in biomass increased less (Fig. 3A). The smaller increase in N content is explained by the more rapid turnover of the plant N pool at these sites [i.e., shorter MRT (Fig. 3C)]. The decline in the MRT of N in these stands was associated with the allocation of N to plant pools that turn over rapidly and have high N concentrations [i.e., leaves and fine roots (SI Table 3)]. Consequently, the gains in N productivity were offset by the declines in the MRT of N in biomass, resulting in no change in NUE under elevated CO₂ (Eq. 2 and Fig. 2C). Notably, the decrease in the MRT of N in biomass, and the increased production of ephemeral tissues with high N concentrations, reinforces a requirement for greater N uptake under elevated CO₂ at the Rhinelander, Duke, and ORNL FACE sites (Fig. 2B). In contrast, at the POP-EUROFACE site, where >60% of the N taken up annually was allocated to the production of wood (SI Table 3), MRT was not altered by elevated CO₂, and NUE and N productivity increased similarly.

In biogeochemical models, trees take up N from the "available" pool in the soil (26–28, 30). The available pool of N is defined as the amount of inorganic N in soil solution, a pool that increases or decreases in size through time based on microbial activity and the amount of N needed for microbial growth and maintenance. The consequence of this model construction is that increases in forest NPP under elevated CO₂ result in a significant increase in NUE in N-limited ecosystems. In contrast to the predictions of biogeochemical models, this data synthesis documents greater tree N uptake in N-limited ecosystems and increases in NUE in ecosystems that were not limited by N availability. It is likely that the combination of increasing fine root production, enhanced rates of SOM decomposition due to increased root exudation, and increased allocation of C to mycorrhizal fungi explains the ability of forest trees to take up more N from the soil under elevated CO₂. Site-specific studies must now quantify the importance of these different processes. Regardless of the specific mechanism, this analysis demonstrates that large quantities of C entering the below-ground system under elevated CO₂ result in greater N uptake, even in N-limited ecosystems. To accurately forecast the response of forest ecosystems to rising concentrations of atmospheric CO₂, biogeochemical models must be recalibrated to allow C transfers below ground (52) that result in additional N uptake under elevated CO₂.

Materials and Methods

This research synthesized data from four, temperate-forest FACE experiments. Data from the Duke and ORNL sites were collected from experiments initiated in established monoculture plantations. At the time measurements began, the loblolly pine
FACE sites, the concentration of N in wood increments was taken from tree cores collected from 5 to 10 trees per FACE plot. The concentration of N in above-ground turnover (leaves, twigs, bark) was measured from subsamples of litter. We assumed that N was not retranslocated before fine root senescence (87). The concentration of N in coarse roots was assumed to be the same as that in wood.

The concentration data were multiplied by the appropriate biomass data to calculate the N content of the different pools (g m⁻²) and fluxes (g m⁻² yr⁻¹). From these values, the rate of N uptake from the soil (g m⁻² yr⁻¹) was calculated as the sum of (i) the N content of the wood (i.e., branches, bole, and coarse roots) produced in the current year, (ii) the N content in the canopy produced in the current year minus the amount of N resorbed from the canopy in the previous year, and (iii) the content of N in the roots produced in the current year (33, 87). The N content of biomass (g m⁻²) was calculated as the sum of (i) the N content in wood, (ii) the canopy content of N at peak mass, and (iii) the N content of fine roots at peak biomass. Seasonal maxima in foliage and fine roots were determined annually at each site based on repeated analysis of foliar biomass and fine root production. NUE was calculated as NPP divided by N uptake (Eq. 1). N productivity was calculated as NPP divided by the N content of biomass (Eq. 2).

The MRT of N in biomass was calculated as the N content of biomass divided by N uptake (Eq. 2).

We used two-way ANOVA to test for site-specific changes in the pools and fluxes of N in response to forest growth under present-day and elevated CO₂ with year as a random variable. In this article, we only interpreted the main effect of CO₂ but provided P values for both effects and their interaction in SI Table 4.

The data from this synthesis activity can be downloaded from the Carbon Dioxide Information Analysis Center website (http://public.ornl.gov/face/synthesis.shtml).

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