

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

Adrien C. Finzi^{a,b}, Richard J. Norby^c, Carlo Calfapietra^d, Anne Gallet-Budynek^a, Birgit Gielen^e, William E. Holmes^f, Marcel R. Hoosbeek^g, Colleen M. Iversen^h, Robert B. Jacksonⁱ, Mark E. Kubiske^j, Joanne Ledford^c, Marion Liberloo^e, Ram Orenⁱ, Andrea Polle^k, Seth Pritchard^l, Donald R. Zak^f, William H. Schlesinger^{b,i}, and Reinhart Ceulemans^e

^aDepartment of Biology, Boston University, Boston, MA 02215; ^cEnvironmental Sciences Division, Oak Ridge National Laboratory, Oak Ridge, TN 37831; ^dDepartment of Forest Environment and Resources, University of Tuscia, I-01100 Viterbo, Italy; ^eResearch Group of Plant and Vegetation Ecology, Department of Biology, University of Antwerp, B-2610 Wilrijk, Belgium; ^fSchool of Natural Resources and Environment and Department of Ecology and Evolutionary Biology, University of Michigan, Ann Arbor, MI 48109; ^gDepartment of Environmental Sciences, Wageningen University, 6700AA-47 Wageningen, The Netherlands; ^hDepartment of Ecology and Evolutionary Biology, University of Tennessee, Knoxville, TN 37996; ⁱSchool of the Environment and Earth Sciences, Duke University, Durham, NC 27708; ^jNorth Central Research Station, U.S. Department of Agriculture Forest Service, Rhinelander, WI 54501; ^kInstitute for Forest Botany, University of Göttingen, 37077 Göttingen, Germany; and ^lDepartment of Biology, College of Charleston, Charleston, SC 29424

Contributed by William H. Schlesinger, July 11, 2007 (sent for review March 12, 2007)

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.

global change | net primary production

Terrestrial 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

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

$$NUE = \frac{NPP}{N_{\text{uptake}}}, \quad [1]$$

where NPP and N_{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 N·m⁻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

$$NUE = \frac{NPP}{N_{\text{productivity}}} \times \frac{N_{\text{content}}}{N_{\text{uptake}}}, \quad [2]$$

Author contributions: A.C.F., R.J.N., C.C., A.G.-B., B.G., W.E.H., M.R.H., C.M.I., M.E.K., M.L., R.O., A.P., D.R.Z., W.H.S., and R.C. designed research; A.C.F., R.J.N., C.C., A.G.-B., B.G., W.E.H., M.R.H., C.M.I., M.E.K., M.L., R.O., A.P., D.R.Z., W.H.S., and R.C. performed research; R.B.J., J.L., and S.P. contributed new reagents/analytic tools; A.C.F., R.J.N., C.C., A.G.-B., B.G., W.E.H., M.R.H., C.M.I., M.E.K., M.L., R.O., A.P., D.R.Z., and R.C. analyzed data; and A.C.F., R.J.N., C.M.I., R.O., and D.R.Z. wrote the paper.

The authors declare no conflict of interest.

Abbreviations: NPP, net primary production; FACE, free-air CO₂ enrichment; NUE, nitrogen-use efficiency; MRT, mean residence time of N in biomass in years; ORNL, Oak Ridge National Laboratory; SOM, soil organic matter.

†To whom correspondence may be addressed. E-mail: afinzi@bu.edu or schlesingerw@ecostudies.org.

This article contains supporting information online at www.pnas.org/cgi/content/full/0706518104/DC1.

© 2007 by The National Academy of Sciences of the USA

Table 1. Characteristics of the four FACE experiments

Name	Rhinelander	Duke	ORNL	POP-EUROFACE
Location	Rhinelander, WI	Durham, NC	Oak Ridge, TN	Tuscania (Viterbo), Italy
Latitude, longitude	45°40'N, 89°37'W	35°58'N, 79°05'W	35°54'N, 84°20'W	42°22'N, 11°48'E
Mean annual precipitation, mm	810	1,140	1,390	818*
Mean annual temperature, °C	4.9	15.5	14.2	14.1
Growing season,† days	150	200	190	247
Soil classification (US)	Alfic Haplorthod	Ultic Hapludalf	Aquic Hapludult	Pachic Xerumbrept
Soil texture	Sandy loam	Clay loam	Silty clay loam	Loam and silt loam
Total soil N, g·kg ⁻¹	1.20	0.79	1.12	1.1–1.4
Overstory vegetation	<i>Populus tremuloides</i> Michx., <i>Acer saccharum</i> Marsh., <i>Betula papyrifera</i> Marsh	<i>Pinus taeda</i> L.	<i>Liquidambar styraciflua</i> L.	<i>Populus alba</i> L. <i>P. nigra</i> L. <i>P. x euramericana</i> Dode Guinier
Peak leaf area index,‡ m ² ·m ⁻²	2.7–3.4	3.4	5.5	4.6–7.4
Year planted	1997	1983	1988	1999
Year of treatment initiation	1997	1997	1998	1999

Data are taken from ref. 10.

*The POP-EUROFACE experiment used irrigation to avoid drought, so inferences regarding precipitation should be avoided for this site.

†For deciduous stands, the growing season is the duration that trees have leaves; for the evergreen system, it is the period of active stem growth.

‡Values of leaf area index are expressed as leaf area per ground area. Values for the Duke FACE site are projected, and values for the hardwood sites are one-sided.

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 ($\text{g of N}\cdot\text{m}^{-2}$). It is useful to estimate both N productivity and the MRT of N in biomass because a change in the magnitude of either or both quantities has important implications for the cycling of C and N under elevated CO_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 CO_2 compared with present-day concentrations of atmospheric CO_2 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 under present-day and elevated concentrations of atmospheric CO_2 .

Results

The four FACE sites varied in the relationship between NPP and N uptake (Eq. 1 and Fig. 1). Both NPP and N uptake were substantially higher at POP-EUROFACE compared with the other three sites. NPP at POP-EUROFACE was higher in elevated CO_2 , but N uptake was not; hence, the data points for plots from elevated and present-day CO_2 concentrations align along different NUE isopleths, suggesting greater NUE in elevated CO_2 . By contrast, the data points for present-day and elevated CO_2 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 CO_2 . 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 CO_2 concentrations, NPP was significantly higher under elevated CO_2 (Fig. 2A) (10). The average annual uptake of N from the soil increased significantly under elevated CO_2 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 CO_2 at the POP-EUROFACE site but not at the Rhinelander, Duke, or ORNL FACE sites (Fig. 2C).

The content of N in biomass was significantly higher under elevated CO_2 than at present-day concentrations of atmospheric CO_2 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 CO_2 (Fig. 3B). At the Rhinelander, Duke, and ORNL FACE sites, the increase in N productivity occurred because elevated CO_2 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 CO_2 (Fig. 2A); there was no effect of elevated CO_2 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 CO_2 than

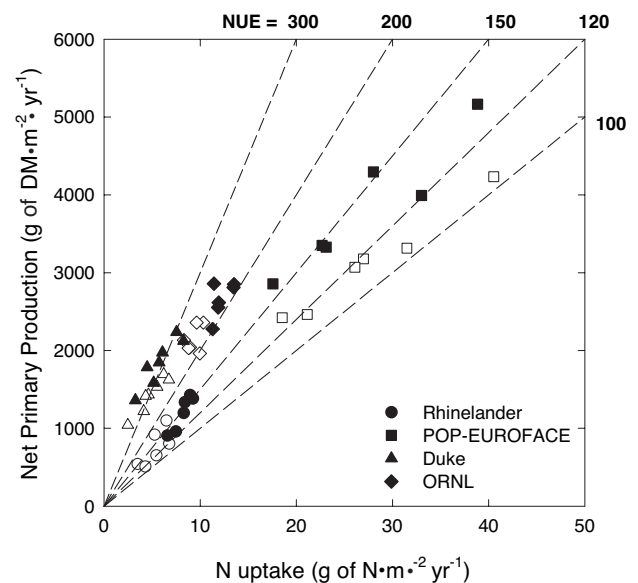


Fig. 1. Correlation between N uptake ($\text{g of N}\cdot\text{m}^{-2}\cdot\text{yr}^{-1}$) and NPP ($\text{g}\cdot\text{m}^{-2}\cdot\text{yr}^{-1}$) at each of the four forest FACE sites under present-day (open symbols) and elevated (filled symbols) CO_2 . The data points represent the mean values in present-day and elevated CO_2 in different years and in different species (POP-EUROFACE) or species assemblages (Rhinelander FACE). The dashed lines across the plot are isopleths of differing NUE ranging from 100 to 300.

(*Pinus taeda*) trees at the Duke FACE site were 13 years old, and the sweetgum (*Liquidambar styraciflua*) trees at the ORNL FACE site were 10 years old. Data from the Rhinelander FACE site were obtained from two communities composed of *Populus tremuloides* and of *P. tremuloides* interplanted with *Betula papyrifera*. Data from the POP-EUROFACE site were collected from three genotypes of *Populus* spp. that were initiated from cuttings planted into bare ground.

Each experiment used FACE technology to expose 20- to 30-m-diameter plots to high concentrations of atmospheric CO₂. The Duke, Rhinelander, and ORNL FACE sites used similar technology, where pure CO₂ was mixed with a turbulent air stream, distributed through a plenum, and released from the ground surface to the top of the tree canopy via vertical vent pipes (75). The POP-EUROFACE site used pure CO₂ delivered at supersonic velocity directly into the FACE plots on the upwind side of each plot (69). The target concentration for atmospheric CO₂ in the enriched plots at the Rhinelander and ORNL FACE sites was 550 μl/liter, at the POP-EUROFACE site was 560 μl/liter, and at the Duke site was present-day + 200 μl/liter, which are concentrations predicted for the year 2050 (76). The control plots at the Rhinelander, Duke, and ORNL sites were treated identically to the CO₂-enriched plots with the exception that air at current atmospheric CO₂ concentrations (376 μl/liter) was delivered through vent pipes. No fumigation infrastructure was built around the control plots at the POP-EUROFACE site. Detailed descriptions of the experimental methods for CO₂ exposure at each site are presented for Duke, ORNL, Rhinelander, and POP-EUROFACE in refs. 75, 77, 78 and 79, and 69, respectively.

Extensive description of the methods used to calculate NPP can be found in ref. 10. In brief, NPP was measured in units of dry matter per square meter of land surface per day, as the sum of biomass increments ($I_{\text{wood}} + I_{\text{leaf}} + I_{\text{coarse root}} + I_{\text{fine root}}$) plus inputs by major detrital pools ($D_{\text{leaves}} + D_{\text{fine roots}}$). Although methods used to estimate NPP differed slightly from site to site, increments in biomass pools were estimated by means of allometric scaling of diameter growth measurements (Duke in ref. 80, ORNL in ref. 77) or destructive harvests (Rhinelander in ref. 81, POP-EUROFACE in ref. 82). The turnover of leaves was measured from litter collected in litter baskets (83–85). Fine root production and turnover were estimated from analysis of minirhizotron images and in-growth cores (56–58, 86), or from published rates of *Populus* fine root production and mortality (31) that were applied to allometrically determined peak fine root biomass estimates at the Rhinelander FACE site (81).

The concentration of N in biomass increments and turnover was measured on an element analyzer. At the Rhinelander and POP-EUROFACE sites, the concentration of N in foliage, wood, and coarse and fine root increments was measured on samples from the destructive harvests. At the Duke and ORNL

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 provide *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>).

We thank John Pastor and an anonymous reviewer for insightful comments on a previous version of this manuscript. The Duke, ORNL, and Rhinelander FACE experiments were supported by the U.S. Department of Energy Office of Science, Biological and Environmental Research. POP-EUROFACE was supported by EU-POPFACE (ENV4-CT97-0657), EU-EUROFACE (EVR1-CT-2002-40027), the Center of Excellence “Forest and Climate” (Italian Ministry of University and Research), and Italy–USA Bilateral Project on Climate Change of the Italian Ministry of Environment. The synthesis activity was supported by the Research Foundation–Flanders Scientific Research Network on Impact of Global Change on Terrestrial Ecosystems and by the National Science Foundation Research Coordination Network on Terrestrial Ecosystem Response to Atmospheric and Climatic Change. A.C.F. and R.B.J. acknowledge ancillary support from the U.S. National Science Foundation (Grants DEB0236356 and DEB0235425). B.G. and M.L. acknowledge the Fund for Scientific Research–Flanders (Belgium). C.M.I. acknowledges the support of a Department of Energy Global Change Education Program Fellowship.

- Amthor JS (1995) *Global Change Biol* 1:243–274.
- Schlesinger WH (1997) *Biogeochemistry: An Analysis of Global Change* (Academic, New York).
- Whittaker RH, Likens GE (1973) *Brookhaven Symp Biol*, 281–302.
- Atjay GL, Ketner P, Duvigneaud P (1979) in *The Global Carbon Cycle*, eds Bolin B, Degens ET, Kempe S, Ketner P (Wiley, Chichester, UK), SCOPE Report 13, pp 129–181.
- Olson JS, Watts JA, Allison LJ (1983) *Carbon in Live Vegetation of Major World Ecosystems* (Oak Ridge National Laboratory, Oak Ridge, TN), Report ORNL-5862.
- Houghton RA, Skole DL (1990) in *Earth as Transformed by Human Action*, ed Turner BI, II (Cambridge Univ Press, Cambridge, UK).
- Field CB, Behrenfeld MJ, Randerson JT, Falkowski P (1998) *Science* 281:237–240.
- Pacala SW, Hurtt GC, Baker D, Peylin P, Houghton RA, Birdsey RA, Heath L, Sundquist ET, Stallard RF, Ciais P, et al. (2001) *Science* 292:2316–2320.
- Janssens IA, Freibauer A, Ciais P, Smith P, Nabuurs GJ, Folberth G, Schlamadinger B, Hutjes RWA, Ceulemans R, Schulze ED, et al. (2003) *Science* 300:1538–1542.
- Norby RJ, DeLucia EH, Gielen B, Calfapietra C, Giardina CP, King JS, Ledford J, McCarthy HR, Moore DJP, Ceulemans R, et al. (2005) *Proc Natl Acad Sci USA* 102:18052–18056.
- Oren R, Ellsworth DS, Johnsen KH, Phillips N, Ewers BE, Maier C, Schafer KVR, McCarthy H, Hendrey G, McNulty SG, Katul GG (2001) *Nature* 411:469–472.
- Finzi AC, DeLucia EH, Hamilton JG, Richter DD, Schlesinger WH (2002) *Oecologia* 132:567–578.
- Field C (1983) *Oecologia* 56:341–347.
- Vitousek P (1982) *Am Nat* 119:553–572.
- Berendse F, Aerts R (1987) *Funct Ecol* 1:293–296.
- Pastor J, Bridgman SD (1999) *Oecologia* 118:50–58.
- Mitchell HL, Chandler RF (1939) *Black Rock Forest Bulletin No. 11* (Cornwall Press, Cornwall, NY).
- Miller HG (1981) *Forestry* 54:158–167.
- Magill AH, Aber JD, Berntson GM, McDowell WH, Nadelhoffer KJ, Melillo JM, Steudler P (2000) *Ecosystems* 3:238–253.
- Sinsabaugh RL, Saiya-Corka K, Long T, Osgood MP, Neher DA, Zak DR, Norby RJ (2003) *Appl Soil Ecol* 24:263–271.

