Global Change Biology (2003) **9,** 1–9

Widespread foliage $\delta^{15}N$ depletion under elevated CO_2: inferences for the nitrogen cycle

HORMOZ BASSIRIRAD*, JOHN V. H. CONSTABLE†, JOHN LUSSENHOP*, BRUCE A. KIMBALL‡, RICHARD J. NORBY§, WALTER C. OECHEL¶, PETER B. REICH‖, WILLIAM H. SCHLESINGER**, STEPHEN ZITZER††, HARBANS L. SEHTIYA*, and SALIM SILIM*

*Department of Biological Sciences, University of Illinois at Chicago, 845 West Taylor Street, Chicago, IL 60607, USA, †Department of Biology, California State University, Fresno, CA 93740, USA, ‡Water Conservation Laboratory, Agricultural Research Service, USDA Phoenix, AZ 85040, USA, §Oak Ridge National Laboratory, Oak Ridge, TN 37831, USA, ¶Department of Biology, San Diego State University, San Diego, CA 92182, USA, ‖Department of Forest Resources, University of Minnesota, St Paul, MN 55108, USA, **Nicholas School of the Environment and Earth Sciences, Duke University, Durham, NC 27708, USA, ††Desert Research Institute, Las Vegas, NV 89119, USA*

Abstract

Leaf ^{15}N signature is a powerful tool that can provide an integrated assessment of the nitrogen (N) cycle and whether it is influenced by rising atmospheric CO_2 concentration. We tested the hypothesis that elevated CO_2 significantly changes foliage $\delta^{15}N$ in a wide range of plant species and ecosystem types. This objective was achieved by determining the $\delta^{15}N$ of foliage of 27 field-grown plant species from six free-air CO_2 enrichment (FACE) experiments representing desert, temperate forest, Mediterranean-type, grassland prairie, and agricultural ecosystems. We found that within species, the $\delta^{15}N$ of foliage produced under elevated CO_2 was significantly lower ($P < 0.038$) compared with that of foliage grown under ambient conditions. Further analysis of foliage $\delta^{15}N$ by life form and growth habit revealed that the CO_2 effect was consistent across all functional groups tested. The examination of two chaparral shrubs grown for 6 years under a wide range of CO_2 concentrations (25–75 Pa) also showed a significant and negative correlation between growth CO_2 and leaf $\delta^{15}N$. In a select number of species, we measured bulk soil $\delta^{15}N$ at a depth of 10 cm, and found that the observed depletion of foliage $\delta^{15}N$ in response to elevated CO_2 was unrelated to changes in the soil $\delta^{15}N$. While the data suggest a strong influence of elevated CO_2 on the N cycle in diverse ecosystems, the exact site(s) at which elevated CO_2 alters fractionating processes of the N cycle remains unclear. We cannot rule out the fact that the pattern of foliage $\delta^{15}N$ responses to elevated CO_2 reported here resulted from a general drop in $\delta^{15}N$ of the source N, caused by soil-driven processes. There is a stronger possibility, however, that the general depletion of foliage $\delta^{15}N$ under high CO_2 may have resulted from changes in the fractionating processes within the plant/mycorrhizal system.

Keywords: elevated CO_2, FACE, foliage ^{15}N, nitrogen cycle

Received 21 August 2002; revised version received 17 July 2003 and accepted 18 July 2003

Introduction

The ecosystem cycling of nitrogen (N) and carbon (C) are so interlinked that changes in the availability of one is likely to affect and be affected by the availability of the other. Therefore, as the atmospheric CO_2 concentration rises, key processes of the N cycle (e.g. decomposition, mineralization, nitrification, denitrification) are likely to be affected. To date, experimental evidence points to a diversity of responses in such processes that are driven by equally diverse mechanisms (Zak *et al*., 2000). Feedback from relatively poor quality tissue produced under high CO_2 could limit N availability by tightening the N cycle (Diaz *et al*., 1993), but equally plausible is the possibility that higher C input could stimulate microbial activity, open the N cycle, and improve plant N availability (Zak *et al*., 1993). Elevated

Correspondence: Hormoz BassiriRad, tel. 312-996-8674, fax 312-996-2805, e-mail: hormoz@uic.edu

CO_2 can also enhance N cycling and availability via its indirect effect on improved soil water regime (Hungate *et al.*, 1997). Whether elevated CO_2 has a marked effect on processes of the N cycle, however, remains a matter of considerable debate.

Recent theoretical and empirical studies of plant ^{15}N natural abundance offer some possibility of gaining an integrated/qualitative understanding of the N cycle under ambient conditions (Nadelhoffer & Fry, 1994; Handley & Scrimgeour, 1997; Högberg, 1997; Robinson, 2001). Used cautiously (Handley & Scrimgeour, 1997; Handley *et al.*, 1998), ^{15}N signature may be a powerful tool to assess whether processes of the N cycle are influenced by rising atmospheric CO_2 concentration. More importantly, the pattern of changes in foliage $\delta^{15}N$ in response to high CO_2 could guide future studies designed to pinpoint the exact processes of the N cycle that respond to a future climate.

Here, we tested the hypotheses that elevated CO_2 significantly alters foliage ^{15}N signature and that such an effect will be independent of plant functional type (e.g. C3 vs. C4 and woody vs. herbs). Such findings provide additional information on whether the fractionating processes of the N cycle are influenced by elevated CO_2. We also examined soil $\delta^{15}N$ and foliage N concentration as potential mechanisms that may broadly explain the CO_2-induced pattern of response in the ^{15}N of foliage. The test of these hypotheses came from a cross-comparison of the natural abundance of foliage $\delta^{15}N$ of 27 species from six free-air CO_2 enrichment (FACE) experiments. The plant species and the FACE experiments represent a diversity of ecosystems and plant functional groups. Because the FACE experiments allow the examination of CO_2 effects on foliage $\delta^{15}N$ at only one enrichment concentration, no conclusions could be drawn about the pattern and/or direction of such responses over a wide range of CO_2 concentrations. Therefore, we also examined leaf $\delta^{15}N$ of two dominant chaparral shrubs that had been grown for 5 years at six CO_2 concentrations ranging from 25 to 75 Pa.

Materials and methods

Site descriptions and CO_2 exposure facilities

This study was conducted in the summer of 1999 at six FACE experimental sites in the United States. The sites were distributed across a range of climates and vegetation types including deciduous (TN) and coniferous temperate forest (NC), C3 and C4 grassland (MN), Mediterranean-type vegetation (CA), warm desert ecosystems (NV), and agro-ecosystems (AZ). All foliage samples were collected from fully expanded and fully lit foliage from the upper portions of the canopy. The general characteristics and experimental arrangement of the sites are described below, and the species sampled at each site are presented in Table 1.

Maricopa Agricultural Center, University of Arizona, AZ
The site maintained four 25 m diameter FACE rings in an agricultural field at both ambient and elevated CO_2 conditions (ambient + 20 Pa) and have been fumigated since 1986 (Conley *et al.*, 2001). Foliage samples were collected in fully expanded sun leaves from the upper 20% of the canopy from 4–6 individuals in fully irrigated treatments ($n = 4$) only. Soils at the site are a Trix clay loam (hyperthermic Typic Torrifluvents) (Kimball *et al.*, 1999).

Sky Oaks Biological Station, San Diego State University, CA
Experiment 1 (FACE experiment). The site consists of two 15 m diameter FACE rings, one at ambient and another at elevated CO_2 conditions (55 Pa), that have been in operation since 1995. The site is located in a natural Mediterranean-type/chaparral community dominated by *Adenostoma fasciculatum* and *Ceanothus greggii* (perennial woody shrubs). Foliage samples were collected from three individuals in each ring from fully expanded leaves in the upper 50% of the canopy. The site has a loamy, mixed, and mesic soil (Ultic Haploxeroll).
Experiment 2 (the null-balance system). At this site, we also examined foliage $\delta^{15}N$ responses in a series of naturally illuminated CO_2- and temperature-controlled null-balance chambers (null-balance system, Oechel *et al.*, 1992). Twelve closed $2 \times 2 \times 2$ m (total volume of 8000 L) chambers were each centered around individual *A. fasciculatum* and surrounding herbaceous plants, including at least one individual of *C. greggii*. Atmospheric CO_2 concentrations within the chambers were maintained at levels ranging from 25 to 75 Pa in 10 Pa increments ($n = 2$). The sampling schemes for the null-balance plants were similar to those of the FACE plants at this site. Leaf samples from each chamber represent pooled samples from two *A. fasciculatum* plants and one *C. greggii* plant.

BioCON, Cedar Creek Natural History Area, University of Minnesota, MN
The experimental design is a $2 \times 2 \times 4$ factorial, with ambient and 55 Pa CO_2 levels produced within 20 m diameter FACE rings; nested within each ring are additional treatments of ambient and high (fertilized with 4 g $NH_4NO_3\,m^{-2}\,yr^{-1}$) soil N availability, and four levels of species richness (1, 4, 9, 16 species) as treatments. The site focuses on 16 common native grassland species that are evenly divided among four functional groups (C3 grasses, C4 grasses, legumes, and forbs). The complexity of the experiment prevents all

Table 1 List of species whose foliage δ^{15}N are given in Fig. 1; the values in parentheses indicate the number of replications for each species

Species ID	FACE sites	Species	Functional group	Ecosystem type
1	Maricopa Center, AZ	*Sorghum bicolor* (4)	C4 grass	Agricultural field
2	Oak Ridge, TN	*Liquidamber styraciflua* (3)	Deciduous tree	Forest overstory
3		*Moss spp.* (3)	Moss	Forest understory
4		*Microstegium vimineum* (3)	C4 grass	Forest understory
5		*Rubus spp.* (3)	Deciduous tree	Forest understory
6		*Acer negundo* (3)	Deciduous tree	Forest understory
7	Duke Forest, NC	*Liriodendron tulipifera* (3)	Deciduous tree	Forest understory
8		*Ulmus alata* (3)	Deciduous tree	Forest understory
9		*Acer rubrum* (3)	Deciduous tree	Forest understory
10		*Juniperus virginiana* (3)	Coniferous shrub	Forest understory
11		*Liquidambar styraciflua* (3)	Deciduous tree	Forest understory
12		*Pinus taeda* (3)	Coniferous tree	Forest overstory
13	Cedar Creek, MN	*Petalostemum villosum* (2)	Legume	Planted monoculture
14		*Anemone cylindrica* (2)	C3 Forb	Planted monoculture
15		*Amorpha canescens* (2)	Legume	Planted monoculture
16		*Lespedeza capitata* (2)	Legume	Planted monoculture
17		*Achillea millefolium* (2)	C3 Forb	Planted monoculture
18		*Solidago rigida* (2)	C3 Forb	Planted monoculture
19		*Poa pratensis* (2)	C3 grass	Planted monoculture
20		*Agropyron repens* (2)	C3 grass	Planted monoculture
21		*Schizachyrium scoparium* (2)	C4 grass	Planted monoculture
22		*Sorghastrum nutans* (2)	C4 grass	Planted monoculture
23		*Andropogon gerardii* (2)	C4 grass	Planted monoculture
24		*Bromus inermis* (2)	C3 grass	Planted monoculture
25	Sky Oaks, CA	*Adenostoma fasciculatum* (1)	C3 evergreen shrub	Natural Chaparral
26		*Ceanothus greggii* (1)	C3 evergreen shrub	Natural chaparral
27	Desert FACE, NV	*Pleuraphis rigida* (3)	C4 grass	Natural desert
28		*Achnatherum hymenoides* (3)	C3 grass	Natural desert

treatments being present in all rings, and therefore $n = 2$ among CO_2 treatments. This study centered on three species from each functional group at ambient N availability under monoculture conditions. Foliage tissue samples were collected from 8–15 individuals growing in monoculture under ambient conditions of soil N availability from each species (Table 1) in each ring ($n = 2$). Leaf samples were collected from fully illuminated mature leaves. The site is located on a 35-year-old abandoned field and soils are categorized as Entisols (Grigal *et al*., 1974).

FACTS-1, Duke Forest, Duke University, NC

The FACE site is in a 17-year-old plantation of *Pinus taeda* (coniferous tree) along with numerous invading understory deciduous tree and shrub species. The site has maintained three FACE rings (25 m diameter) at both ambient and elevated (ambient + 20 Pa) CO_2 since 1996. Foliage samples were from current and 1-year-old fascicles of *P. taeda* collected from fully illuminated conditions in the upper 20% of the canopy from 3–5 individuals per ring ($n = 3$). Collections from understory species were obtained from 1–5 shaded individuals of each species per ring and pooled ($n = 3$). Soils at the site had a clayey loam in the upper 30 cm overlying clay and were identified as acidic Hapludalf (Andrews & Schlesinger, 2001).

Nevada Desert FACE Facility (NDFF), Nevada Test Site, University of Nevada System, NV

The NDFF site is situated in a natural Mojave desert community codominated by evergreen *Larrea tridentata* and deciduous *Ambrosia dumosa*. The site consists of nine 23 m rings: three at ambient CO_2, three at elevated CO_2 (55 Pa), and three nonring control plots that have been in operation since April 1997. Foliage was collected from a single individual in each ring ($n = 3$) of each species (Table 1) under natural light and water availability. The soils at the site are typic Calciorthids and display a well-developed cryptobiotic crust. Target plants included a C3, *Achnatherum hymenoides*, and a C4, *Pleuraphis rigida*, perennial grass species.

Oak Ridge National Laboratory, TN

The FACE rings (25 m diameter) are located in a closed-canopy *Liquidambar styraciflua* plantation that was established in 1988 (Norby *et al.*, 2001). The CO_2 treatment began in 1998 with two FACE rings set to 57 Pa and three rings maintained at ambient CO_2. Foliage samples for *L. styracifolua* were collected from 2–4 individuals per ring ($n = 2$ or 3) from the upper 20% of the canopy under fully illuminated conditions. Understory species were also sampled from the top 20% of the canopy. The soils of the site were silty clay loam and classified as Aquic Hapludult.

Foliage and soil $\delta^{15}N$ determination

Foliage samples were dried to constant mass at 60 °C and finely ground in a tissue homogenizer. Samples were analyzed for both leaf N concentration and $\delta^{15}N$. Approximately 10 mg ground tissue was packed in tin cups for analysis of ^{15}N at the Mass Spectrometer Facility in the Department of Crop and Soil Science at Michigan State University. The analysis was carried out by continuous-flow isotope ratio mass spectrometry (Europa Scientific, Northwich, England, Model 20-20) with a standard precision of ± 0.2‰. Soil samples were collected within each ring at 10 cm depth using a 19 mm diameter soil corer, one to three subsamples were collected within each ring and were pooled. Sampling locations were adjusted to plant scale and were collected either adjacent to (NV and MN), ~0.25 m (CA and AZ), or ~0.50 m (NC and TN) from the plant stem. We were able to collect soil samples from 10 cm depth for 19 of the target species but for a few species we were also able to collect soil samples from 20 cm depth. Samples were manually cleaned to remove large root and foliage fragments, dried at 85 °C to constant mass, and sequentially sifted through 1.2, 0.5, and 0.21 mm sieves. Soil particles < 0.21 mm were collected and 25–45 mg (depending upon soil N concentration) was packed in tin cups for analysis of total N and ^{15}N using the method as described for foliage sampling.

Statistical analysis

The data set consisted of replicated foliage $\delta^{15}N$ values for plants of the same species growing under ambient and elevated CO_2 concentrations. These data were verified for normality using plots of predicted values vs. studentized residuals, and the Shapiro–Wilk statistic. The response of the $\delta^{15}N$ of each species to atmospheric CO_2 was analyzed with a nested ANOVA (Proc GLM, SAS 2000, SAS Institute Inc., Cary, NC, USA), with the unit of statistical analysis being the plant species. The nested ANOVA contrasted ambient with elevated plants within the same species (total species number = 28, *L. styraciflua*, occurs in two sites), then species within FACE sites, and finally FACE sites. Type three sums of squares were used to analyze the data because of the unequal numbers of replications. We also compared the responses of individual species with a one-way ANOVA followed by Dunnett's multiple range test. The association between foliage $\delta^{15}N$ and N was analyzed by regression using ANOVA, as was the relationship between growth CO_2 and leaf $\delta^{15}N$ in the null-balance experiment. In addition, an ANCOVA was used to test for differences in the slope of the foliage $\delta^{15}N$ and the covariate, N, for each CO_2 treatment. A parallel line analysis was used to compare their *y*-intercepts.

Results

Overall, the average $\delta^{15}N$ differed significantly among FACE sites ($F_{5,81} = 64.22$, $P < 0.0001$). Species within each FACE also differed significantly in their $\delta^{15}N$ signature ($F_{23,81} = 4.86$, $P < 0.0001$). When the CO_2 effect within species was compared, the mean $\delta^{15}N$ was significantly ($F_{27,81} = 1.69$, $P < 0.038$) depleted for foliage produced under elevated (0.083‰) as opposed to ambient CO_2 (0.984‰) conditions (Fig. 1). Analyzed individually (by a one-way ANOVA followed by Dunnett's multiple range test), we found that foliage $\delta^{15}N$ was significantly enriched in three species and depleted in 19 species in plants grown under high compared to ambient CO_2 (Fig. 2). Foliage $\delta^{15}N$ was unaffected by growth CO_2 in six species. Among functional types, CO_2 had a similar qualitative effect on the foliage $\delta^{15}N$ for woody vs. herbs and C3 vs. C4 species (Fig. 3).

Foliage $\delta^{15}N$ of the species in the null-balance experiment, experiencing six CO_2 treatments, was also negatively correlated with growth CO_2 concentration (Fig. 4). The slope of this relationship was significantly different from zero for *A. fasciculatum* ($P > 0.0001$) and *C. greggii* ($P > 0.0002$).

We found that across species, there was a significant positive correlation between foliage $\delta^{15}N$ and N concentration at ambient ($P < 0.0002$) and elevated ($P < 0.007$) CO_2 levels (Fig. 5). An analysis of covariance indicated that the relationship between leaf %N and $\delta^{15}N$, as indicated by the slope of the linear regression, did not change significantly in response to CO_2 enrichment, but the *y*-intercept was significantly ($P < 0.009$) lower at elevated compared to ambient CO_2 levels. We also found that there was no significant correlation between soil and foliage $\delta^{15}N$ for either ambient or elevated CO_2 plants (Fig. 5, inset).

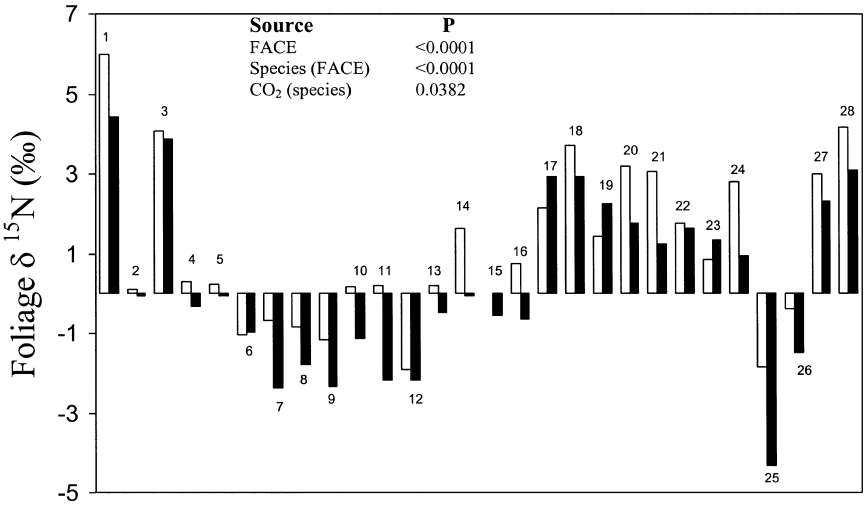

Fig. 1 Foliage δ^{15}N of 27 species grown under field conditions and treated at either ambient (open bars) or elevated (closed bars) CO_2 levels. Species identifications and their corresponding FACE site information are given in Table 1. Data were collected from six FACE sites in the US. For each species, foliage samples of individual plants from a given ring were pooled for ^{15}N analysis and were used to calculate the means from 2–3 rings, depending upon the design of each FACE site. There are 28 pairs of values reported here (one more than the number of species) because *L. styraciflua* occurs at both the ORNL (#2) and the Duke (#11) FACE sites. *L. styraciflua* from ORNL are mature dominant overstory plants, but those from Duke FACE are understory saplings. Means for ambient and elevated CO_2 treatments were compared using a nested two-way ANOVA, and the results are shown in the inset table.

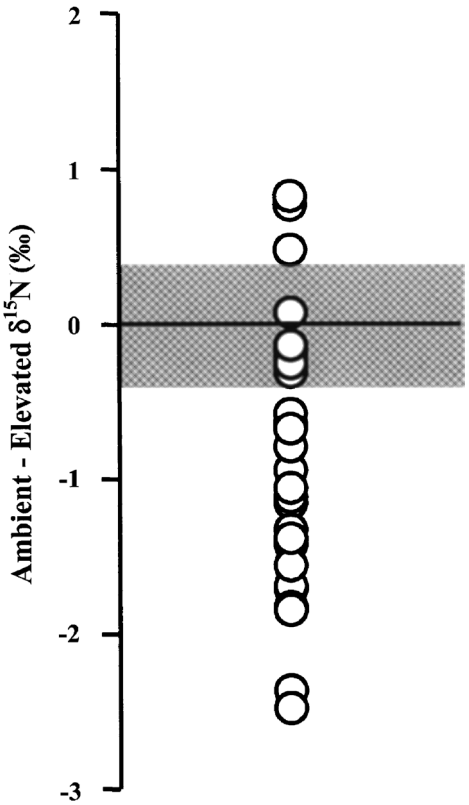

Fig. 2 Differences in foliage δ^{15}N between ambient and elevated CO_2 plants analyzed by individual species. Data below the zero line indicate depletion and those above the line indicate enrichment. The width of the hatch bar indicates the 5% minimum significant difference of Dunnett's multiple range test.

Discussion

The negative correlation between leaf δ^{15}N and growth CO_2, demonstrated here for a large number of species grown under FACE conditions, as well as the two species that were grown under a wide range of CO_2 concentrations, is consistent with data reported by Hinkson (1996), who also found that δ^{15}N in the leaves of *Quercus agrifolia* seedlings grown in pots inside the null-balance system for a full growing season decreased significantly with increased CO_2 concentration. On the other hand, foliage ^{15}N of *Pinus ponderosa* (Johnson *et al*., 1996) and *Larrea tridentata* (Billings *et al*., 2002) has been shown to become enriched in response to elevated CO_2. It is not possible, however, to compare the results of these two studies directly with ours, because of the differences in sampling protocol. In the case of *P. ponderosa*, only senesced needles were analyzed, while in *L. tridentata* the samples combined leaf and stem tissues.

One possible cause of a common decrease in foliage δ^{15}N in response to a high CO_2 environment is a greater input of N from symbiotic or free-living N_2 fixation. In fact, one of the species in the null-balance system, *C. greggii*, is an actinorhizal plant capable of N_2 fixation (Bond, 1983). A number of N_2-fixing species have been shown to respond more positively to elevated CO_2 compared with nonfixing species (Soussana & Hartwig, 1996; Lüshscher *et al*., 1998; Hungate *et al*., 1999), but this effect is not consistent in all studies (Niklaus *et al*.,

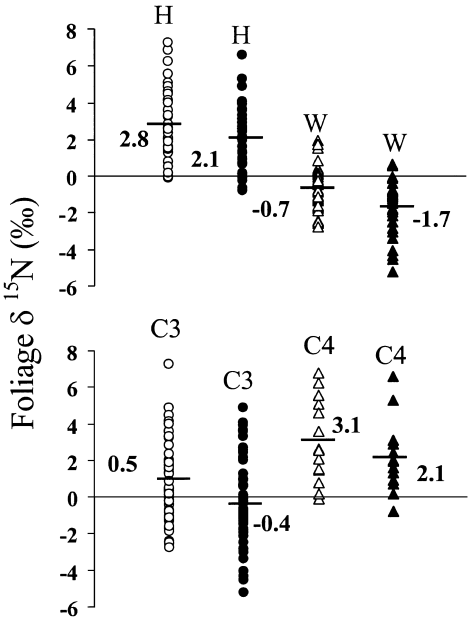

Fig. 3 Foliage $\delta^{15}N$ for individual plants at either ambient (open symbols) or elevated (closed symbols) CO_2 treatments from six US FACE sites according to functional groups. Comparison between (a) woody (W) and herbaceous (H) species and (b) C3 and C4 species. Horizontal bars represent the mean for each functional group at a given CO_2 treatment.

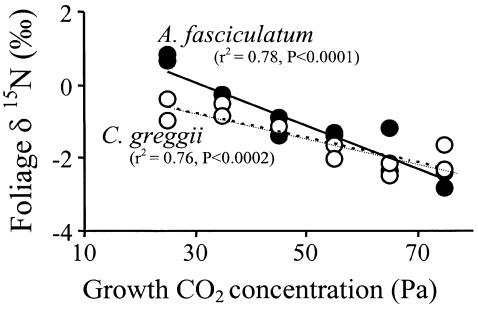

Fig. 4 Changes in leaf $\delta^{15}N$ of two chaparral shrub species (*Adenostema fasciculatum*, closed symbols; *Ceanothus greggii*, open symbols) as a function of growth CO_2 concentrations. Plants are mature shrubs that have been continuously grown in large closed-system chambers at CO_2 concentrations of either 25, 35, 45, 55, 65, or 75 Pa for about 6 years. This system is located at the Sky Oak Field Station in San Diego, CA (see the Materials and methods section). The *P*-values indicate that slopes of both lines are significantly less than zero.

1998). Any increase in N_2 fixation contribution to plant N demand should, however, drive the $\delta^{15}N$ signal closer to zero and not away from it. Therefore, N_2 fixation cannot adequately explain the results of the null-balance foliage $\delta^{15}N$ depletions or those of the

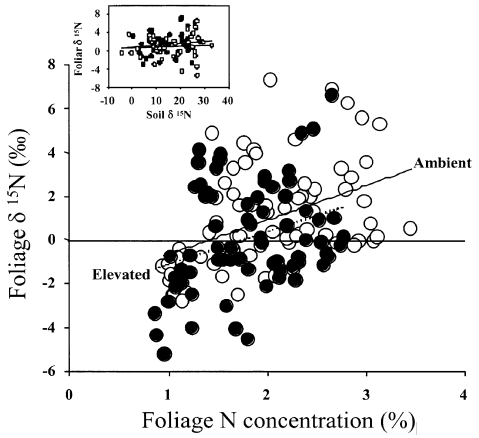

Fig. 5 Changes in foliage $\delta^{15}N$ as a function of foliage N concentration for all the individuals sampled from the FACE sites. The ANOVA indicates a significant positive correlation between leaf N and leaf $\delta^{15}N$ for both ambient ($r^2 = 0.18$, $P < 0.0002$) and elevated ($r^2 = 0.1$, $P < 0.007$) CO_2 treatments. Open and closed symbols refer to ambient and elevated CO_2 treatments, respectively. The inset shows the relationship between foliage $\delta^{15}N$ and bulk soil $\delta^{15}N$ from the top 10 cm for ambient ($r^2 = 0.02$) and elevated ($r^2 = 0.001$) CO_2 treatments. There is no significant correlation between soil and leaf $\delta^{15}N$.

FACE experiments, which moved toward numbers more negative than 0‰.

When foliage $\delta^{15}N$ changes significantly in response to an environmental factor, a common interpretation is that the fractionating processes of the N cycle, particularly those that control the isotopic signature of the N source, must have changed. There are many theoretical and empirical reasons (Handley & Scrimgeour, 1997), however, as to why natural abundance of foliage $\delta^{15}N$ should not be used as a tracer of ^{15}N between the source and sink. In fact, our data showed that the bulk soil $\delta^{15}N$ at a depth of 10 cm did not change significantly in response to high CO_2. Therefore, bulk soil $\delta^{15}N$ is unlikely to have driven changes in foliage $\delta^{15}N$ under high CO_2. This apparent lack of a relationship between soil and foliage $\delta^{15}N$ is not entirely surprising because the bulk soil $\delta^{15}N$ does not always mirror short-term changes in the available soil N pool, which could potentially alter foliage $\delta^{15}N$ without leaving a detectable signal in the bulk soil $\delta^{15}N$. Consequently, the possibility of soil-driven processes explaining the observed foliage depletion in response to high CO_2 cannot be entirely ruled out. The interpretation of our soil $\delta^{15}N$ data is further confounded by the fact that the soil samples were taken only at a depth of 10 cm. More specifically, soil ^{15}N signature varies with depth, and plant species differ significantly in the depth at which they acquire most of

their N (Handley & Scrimgeour, 1997 and references therein).

The correlation between leaf N and leaf δ^{15}N observed here is also reported in many other field studies (Vitousek *et al*., 1989; Högberg, 1990; Garten, 1993; Garten & Van Miegroet, 1994; Johannisson & Högberg, 1994; Hobbie *et al*., 2000; Kitayama & Iwamoto, 2001) and has often been interpreted to represent increased symbiotic association with mycorrhizal fungi. Leaves of mycorrhizal plants are typically ^{15}N depleted compared with the source N, and the extent of this depletion is often positively correlated with the degree of mycorrhizal infection (Handley *et al*., 1993; Pate *et al*., 1993; Högberg, 1997; Hobbie *et al*., 2000). Because elevated CO_2 generally increases root symbiotic association with mycorrhizal fungi (BassiriRad *et al*., 2001), it is likely that mycorrhizal fungi are a major mechanism causing the drop in leaf δ^{15}N under high CO_2 levels. We believe that mycorrhizal regulation of foliage δ^{15}N depletion response to high CO_2 remains a strong possibility that deserves further investigation.

Changes in foliage δ^{15}N can also be attributed to internal fractionating processes of the N cycle within the plant. A number of theoretical and empirical studies have attempted to explain why there should be differences in the δ^{15}N signal of the root and shoot (Raven, 1987; Evans *et al*., 1996; Robinson *et al*., 1998; Comstock, 2001; Yoneyama *et al*., 2001). While the exact mechanism may depend on species and environmental conditions, partitioning of nitrate assimilation, particularly an increased assimilation of nitrate by the root system, can provide a likely explanation. Because nitrate reductase, the assimilatory enzyme of NO_3^-, discriminates heavily against ^{15}N, the assimilated products of this process are considerably more depleted than the N source (Handley & Raven, 1992; Robinson, 2001). While not consistently, a large number of studies have shown a reduction in shoot assimilation of nitrate under elevated CO_2 conditions (Stitt & Krapp, 1999 and references therein). Furthermore, there is some evidence that under high CO_2 levels (Constable *et al*., 2001; Harmens *et al*., 2001; Kruse *et al*., 2002), a greater proportion of nitrate assimilation may be redirected to the roots. In a recent study, BassiriRad & Sehtiya (2002) examined the xylem sap of seedlings of eight tree species using detopped roots, and found that across species, total inorganic N concentration of the ascending sap decreased by 70% whereas total amino acid concentration increased by 71% in response to CO_2 enrichment.

In conclusion, the overwhelming majority of species tested here showed a decrease in foliage δ^{15}N in response to elevated CO_2. The results provide strong evidence that rising atmospheric CO_2 concentration will have a serious impact on N dynamics of diverse ecosystems. Such a widespread pattern of foliage δ^{15}N depletion in response to CO_2 enrichment does not automatically reveal the exact component(s) at which alterations in the N cycle occur, but the data provide strong evidence that high CO_2 levels increase net fractionation against ^{15}N between soil and foliage. Viewed in this light, a knowledge of foliage δ^{15}N and how it changes in response to rising atmospheric CO_2 concentration provides a valuable tool in narrowing the search for the components of the plant and soil N cycle that are most sensitive to a changing climate.

Acknowledgements

We have benefited greatly by discussing various aspects of ^{15}N cycling with Dave Evans and Miquel Gonzalez-Meler and by incorporating comments from two anonymous reviewers. We also thank Vince Gutschick for valuable discussions about how N nutrition affects N allocation in plants. Mark Rudy provided valuable field assistance in gathering foliage and soil samples. This work was funded by the National Science Foundation.

References

Andrews JA, Schlesinger WH (2001) Soil CO_2 dynamics, acidification, and chemical weathering in a temperate forest with experimental CO_2 enrichment. *Global Biogeochemical Cycles*, **15**, 149–162.

BassiriRad H, Gutschick VP, Lussenhop J (2001) Root system adjustments: regulation of plant nutrient uptake and growth responses to elevated CO_2. *Oecologia*, **126**, 305–320.

BassiriRad H, Sehtiya HL (2002) Root nitrogen and water fluxes to the shoot are influenced by growth CO_2. Ecological Society of America Abstracts, **87**, p. 72.

Billings SA, Schaeffer SM, Zitzer S *et al*. (2002) Alterations of nitrogen dynamics under elevated carbon dioxide in an intact Mojave Desert ecosystem: evidence from nitrogen-15 natural abundance. *Oecologia*, **131**, 463–467.

Bond G (1983) Taxonomy and distribution of non-legume nitrogen-fixing systems. In: *Biological Nitrogen Fixation in Forest Ecosystems: Foundations and Applications* (eds Gordon JC, Wheeler CT), Martinus Nijhoff/Dr W. Junk Publishers, The Hague.

Comstock JP (2001) Steady-state isotopic fractionation in branched pathways using plant uptake of NO_3 as an example. *Planta*, **214**, 220–234.

Conley MM, Kimball BA, Brooks TJ *et al*. (2001) CO_2 enrichment increases water use efficiency in sorghum. *New Phytologist*, **151**, 407–412.

Constable JVH, BassiriRad H, Lussenhop J *et al*. (2001) Influence of elevated CO_2 and mycorrhizae on nitrogen acquisition: contrasting responses in *Pinus taeda* and *Liquidambar styraciflua*. *Tree Physiology*, **21**, 83–91.

Diaz D, Grime JP, Harris J *et al*. (1993) Evidence of feedback mechanism limiting plant response to elevated carbon dioxide. *Nature*, **364**, 616–617.

Evans RD, Bloom AJ, Sukrapanna SS *et al*. (1996) Nitrogen isotope composition of tomato (*Lycopersicon esculentum* Mill. cv. T-5) grown under ammonium or nitrate nutrition. *Plant, Cell and Environment*, **19**, 1317–1323.

Garten CT Jr, VanMiegroet H (1994) Relationships between soil nitrogen dynamics and natural ^{15}N abundance in plant foliage from Great Smoky Mountains National Park. *Canadian Journal of Forestry*, **24**, 1636–1645.

Garten CT Jr (1993) Variation in foliar ^{15}N abundance and the availability of soil nitrogen on Walker Branch Watershed. *Ecology*, **74**, 2098–2113.

Grigal DG, Chamberlain LM, Finney HR, *et al*. (1974) The soils of Cedar Creek Natural History Area. University of Minnesota Agricultural Experiment Station Miscellaneous Report 123. 47pp.

Handley LL, Draft MJ, Wilson J *et al*. (1993) Effects of Ecto- and VA-mycorrhizal fungi *Hydnagium carneum* and *Glomus clarum* on the $\delta^{15}N$ and $\delta^{13}C$ values of *Eucalyptus globulus* and *Ricinus communis*. *Plant, Cell and Environment*, **16**, 375–382.

Handley LL, Raven JA (1992) The use of natural abundance of nitrogen isotopes in plant physiology and ecology. *Plant, Cell and Environment*, **15**, 965–985.

Handley LL, Scrimgeour CM, Raven JA (1998) ^{15}N at natural abundance levels in terrestrial vascular plants: a précis. In: *Stable Isotopes* (ed Griffiths H), pp. 89–109. BIOS Scientific Publishers Ltd, Oxford.

Handley LL, Scrimgeour CM (1997) Terrestrial plant ecology and ^{15}N natural abundance: the present limits to interpretation for uncultivated systems with original data from a Scottish old field. *Advances in Ecological Research*, **27**, 133–212.

Harmens H, Marshal C, Stirling CM *et al*. (2001) Partitioning and efficiency of use of N in *Dactylis glomerata* as affected by elevated CO_2: Interaction with N supply. *International Journal of Plant Science*, **162**, 1267–1274.

Hinkson CL (1996) Gas exchange, growth response, and nitrogen dynamics of *Quercus agrifolia* under elevated atmospheric CO_2 and water stress. Masters Thesis, San Diego State University.

Hobbie EA, Macko SA, Williams M (2000) Correlations between foliar $\delta^{15}N$ and nitrogen concentrations may indicate plant-mycorrhizal interactions. *Oecologia*, **122**, 273–283.

Högberg P (1990) Forests losing large quantities of nitrogen have elevated $^{15}N:^{14}N$ ratios. *Oecologia*, **84**, 229–231.

Högberg P (1997) Tansley Review No. 95. ^{15}N natural abundance in soil–plant systems. *New Phytologist*, **137**, 179–203.

Hungate BA, Chappin FS III, Zhong H *et al*. (1997) Stimulation of grassland nitrogen cycling under carbon dioxide enrichment. *Oecologia*, **109**, 149–153.

Hungate BA, Dijkstra P, Johnson DW *et al*. (1999) Elevated CO_2 increases nitrogen fixation and decreases soil nitrogen mineralization in Florida scrub oak. *Global Change Biology*, **5**, 781–789.

Johannisson C, Högberg P (1994) ^{15}N abundance of soils and plants along an experimentally induced forest nitrogen supply gradient. *Oecologia*, **97**, 322–325.

Johnson DW, Henderson PH, Ball JT, *et al* (1996) Effects of CO_2 and N on growth and N dynamics in ponderosa pine: results from the first two growing seasons. In: *Carbon Dioxide and Terrestrial Ecosystems* (eds Koch GW, Mooney HA), pp. 23–40. Academic Press, San Diego, CA.

Kimball BA, LaMorte RL, Pinter PJ Jr *et al*. (1999) Free-air CO_2 enrichment (FACE) and soil nitrogen effects on energy balance and evapotranspiration of wheat. *Water Resources Research*, **35**, 1179–1190.

Kitayama K, Iwamoto K (2001) Patterns of natural ^{15}N abundance in the leaf-to-soil continuum of tropical rain forest differing in N availability on Mount Kinabalu, Borneo. *Plant and Soil*, **229**, 203–212.

Kruse J, Hetzger I, Hänsch R *et al*. (2002) Elevated pCO_2 favours nitrate reduction in the roots of wild-type tobacco (*Nicotinia tabacum cv. Gat*). and significantly alters N-metabolism in transformants lacking functional nitrate reductase in the roots. *Journal of Experimental Botany*, **53**, 2351–2367.

Lüshscher A, Hendrey GR, Nösberger J (1998) Long-term responsiveness to free-air CO_2 enrichment of functional types, species and genotypes of plants from fertile permanent grassland. *Oecologia*, **113**, 37–45.

Nadelhoffer KJ, Fry B (1994) Nitrogen isotope studies in forest ecosystems. In: *Stable Isotopes in Ecology and Environmental Science* (eds Lajtha K, Michener R), pp. 23–44. Blackwell Scientific Publications, Boston, MA.

Niklaus PA, Leadley PW, Stöcklin J *et al*. (1998) Nutrient relations in calcareous grassland under elevated CO_2. *Oecologia*, **116**, 67–75.

Norby FJ, Todd DE, Fults J *et al*. (2001) Allometric determination of tree growth in a CO_2-enriched sweetgum stand. *New Phytologist*, **150**, 477–487.

Oechel WC, Riechers G, Lawrence WT *et al*. (1992) CO_2 'LT', an automated null-balanced system for studying the effects of elevated CO_2 level and global climate change on unmanaged ecosystems. *Functional Ecology*, **6**, 86–100.

Pate JS, Stewart GR, Unkovitch M (1993) ^{15}N natural abundance of plant and soil components of a *Banksia* woodland ecosystem in relation to nitrate utilization, life form, mycorrhizal status and N_2-fixation abilities of component species. *Plant, Cell and Environment*, **16**, 365–373.

Raven JA (1987) The application of mass spectrometry to biochemical and physiological studes. In: *The Biochemistry of Plants, Vol 13, Methodology* (ed Davies DD), pp. 127–180. Academic Press, London.

Robinson D (2001) $\delta^{15}N$ as an integrator of the nitrogen cycle. *TRENDS in Ecology and Evolution*, **16**, 153–162.

Robinson D, Handley LL, Scrimgeour CM (1998) A theory for $^{15}N/^{14}N$ fractionation in nitrate-grown vascular plants. *Planta*, **205**, 397–406.

Soussana JF, Hartwig UA (1996) The effects of elevated CO_2 on symbiotic N_2 fixation: a link between the carbon and nitrogen cycles in grassland ecosystems. *Plant and Soil*, **187**, 321–332.

Stitt M, Krapp A (1999) The interaction between elevated carbon dioxide and nitrogen nutrition: the physiological and molecular background. *Plant, Cell and Environment*, **22**, 583–621.

Vitousek PM, Shearer G, Kohl DH (1989) Foliar ^{15}N natural abundance in Hawaiian rainforest: patterns and possible mechanisms. *Oecologia*, **78**, 383–388.

Yoneyama T, Matsumaru T, Usui K *et al*. (2001) Discrimination of nitrogen isotopes during absorption of ammonium and nitrate at different nitrogen concentrations by rice (*Oryza sativa* L.) plants. *Plant, Cell and Environment*, **24**, 133–139.

Zak DR, Pregitzer KS, Curtis PS *et al*. (1993) Elevated atmospheric CO_2 and feedback between carbon and nitrogen cycle. *Plant and Soil*, **151**, 105–117.

Zak DR, Pregitzer KS, King JS *et al*. (2000) Elevated atmospheric CO_2, fine roots and the response of soil microorganisms: a review and hypothesis. *New Phytologist*, **147**, 201–222.