Litterfall ^{15}N abundance indicates declining soil nitrogen availability in a free-air CO_2 enrichment experiment

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Abstract. Forest productivity increases in response to carbon dioxide (CO_2) enrichment of the atmosphere. However, in nitrogen-limited ecosystems, increased productivity may cause a decline in soil nitrogen (N) availability and induce a negative feedback on further enhancement of forest production. In a free-air CO_2 enrichment (FACE) experiment, the response of sweetgum (*Liquidambar styraciflua* L.) productivity to elevated CO_2 concentrations [CO_2] has declined over time, but documenting an associated change in soil N availability has been difficult. Here we assess the time history of soil N availability through analysis of natural ^{15}N abundance in archived samples of freshly fallen leaf litterfall. Litterfall δ^{15}N declined from 1998 to 2005, and the rate of decline was significantly faster in elevated [CO_2]. Declining leaf litterfall δ^{15}N is indicative of a tighter ecosystem N cycle and more limited soil N availability. By integrating N availability over time and throughout the soil profile, temporal dynamics in leaf litterfall δ^{15}N provide a powerful tool for documenting changes in N availability and the critical feedbacks between C and N cycles that will control forest response to elevated atmospheric CO_2 concentrations.

Key words: carbon–climate feedbacks; elevated CO_2; forest litterfall; free-air CO_2 enrichment (FACE); Liquidambar styraciflua; *nitrogen availability; progressive nitrogen limitation; stable isotope; sweetgum.*

Introduction

The long-term sustainability of increased ecosystem production and C capture from a CO_2-rich atmosphere has been a topic of considerable interest, especially in forested systems, which are widely limited by soil N availability (Vitousek and Howarth 1991). Experimental increases in atmospheric [CO_2] in a number of forested ecosystems have resulted in increased aboveground dry matter production (Ainsworth and Long 2005), net primary productivity (NPP; Norby et al. 2005), and nitrogen (N) uptake from the soil (Finzi et al. 2007). However, in N-limited ecosystems, enhanced sequestration of N into long-lived biomass and soil organic matter in association with growth stimulation by CO_2 enrichment may accelerate declines in soil N availability and eventually limit or nullify increases in forest production under elevated [CO_2] (i.e., "progressive N limitation" [PNL]; Berntson and Bazzaz 1996, Luo et al. 2004).

A better understanding of how N may limit the response of terrestrial NPP to elevated atmospheric [CO_2] is essential for the improvement of coupled climate–carbon (C) cycle models (Thornton et al. 2007). However, proposed biometric indicators of PNL in forest ecosystems are acknowledged to have relatively poor sensitivity (Luo et al. 2004), and

investigations on the occurrence of PNL in forest ecosystems are faced with difficulties in assessment and interpretation (Norby and Iversen 2006). Furthermore, while declining soil N availability is the key process that sets in motion the eventual negative feedback on increased forest production under elevated [CO_2], soil N availability is a notoriously difficult parameter to measure (as reviewed in Frank and Groffman 2009). This is especially true in long-term experiments in which changes in N availability over time are expected to be subtle and depend on the interplay between plant N uptake and microbial N recycling.

In the free-air CO_2 enrichment (FACE) experiment at Oak Ridge National Laboratory (ORNL), Tennessee, USA, NPP of sweetgum trees is known to be limited by available soil N (Iversen and Norby 2008), but during the first six years of the experiment, tree N uptake increased in response to elevated [CO_2] to meet the higher N demands associated with CO_2 enrichment of NPP. There was no evidence that the N limitation was exacerbated by elevated [CO_2], resulting in a reduced tree growth response; hence, Norby and Iversen (2006) concluded that PNL was not occurring during the first six years of the experiment because stimulation of forest NPP was sustained and indicators of PNL based on N cycling (Luo et al. 2004) were absent. There was no indication of (1) declining N stocks in the forest canopy, (2) declining whole-plant N content, or (3) declining plant N uptake in response to elevated [CO_2] (Norby and Iversen 2006). Since that assessment, however, NPP

and the response of NPP to elevated $[CO_2]$ have declined (Norby et al. 2008), and the loss of response was tentatively attributed to N limitation. Whether the N limitation was associated with declining N availability in soil and whether soil N availability was reduced in response to elevated $[CO_2]$, however, have been difficult to resolve.

Here, we present a simple indicator of soil N availability based on natural abundance N isotope ratios in leaf litterfall to address this question: Has elevated $[CO_2]$ led to declining soil N availability over time in a long-term CO_2 enrichment experiment in a sweetgum plantation? Our approach overcomes problems with both measurement sensitivity and spatial integration of plant-available soil N for trees growing under ambient and elevated $[CO_2]$. First, natural abundance measurements of ^{15}N in leaf litterfall integrate over temporal changes in N availability that may go undetected using conventional methods. Second, leaf $\delta^{15}N$ reflects the isotopic composition of N that is acquired by tree roots over the entire rhizosphere, whereas conventional measurements of soil N availability are usually confined to surface soil horizons (Zak et al. 2003) and/or measured in incubations that preclude the effects of roots (Frank and Groffman 2009).

Methods

The ORNL FACE experiment was established in a sweetgum plantation on the Oak Ridge National Environmental Research Park (35°54′ N, 84°24′ W) near Oak Ridge, Tennessee, USA (Norby et al. 2006). Five 25-m diameter plots (rings) were laid out in 1997, and four of them were instrumented with FACE apparatus (Hendrey et al. 1999). At the start of the experiment, the trees were 10 years old and 12 m tall. There were ~90 trees per plot with stand basal area of 28 m^2/ha and leaf area index of 5.5. Elevated CO_2 exposures (544 ppm) commenced in the spring of 1998 in rings 1 and 2 and continued through autumn 2009; rings 3, 4, and 5 were exposed to ambient air (average daytime $[CO_2]$ of 391 ppm). Additional details about the experiment have been published elsewhere (Norby et al. 2006).

During the experimental manipulation, leaf litterfall was collected each autumn (soon after falling to minimize decomposition) in seven baskets (0.2 m^2) placed in each FACE ring. Collections from each basket were oven-dried and combined at the end of the year. After thorough mixing of the pooled collections, the dry leaf litterfall was subsampled, ground to a fine powder in a sample mill, and stored in glass vials. In 2009, archived litter samples, from one centrally located litter basket in each FACE ring, were analyzed for stable N isotope ratios ($^{15}N/^{14}N$) and nitrogen concentrations using continuous-flow isotope ratio mass spectrometry (Integra CN, SerCon, Crewe, UK). Nitrogen-15 abundance in samples of surface mineral soil (0–16 cm deep, no O-horizon) was also measured in April 2006 ($n = 6$ soil

cores per FACE ring). Nitrogen-15 abundance was expressed as a delta value ($\delta^{15}N$, per mil, ‰) relative to atmospheric N_2: $\delta^{15}N = [(R_{smpl} - R_{atm})/R_{atm}] \times 1000$, where $R = {}^{15}N/{}^{14}N$ and R_{atm} is atmospheric N_2. The isotope ratio mass spectrometer was calibrated using a secondary standard (ammonium sulfate) traceable to reference material (NIST 8547) from the National Institute of Standards and Technology (NIST, Gaithersburg, Maryland, USA). Internal standards (ammonium sulfate, $n = 8$) used in the analysis of ^{15}N natural abundance deviated from their expected $\delta^{15}N$ value, on average, by only −0.06‰ with a standard deviation of 0.20‰.

Unpublished data on ^{13}C abundance indicated that litter collections from the centrally located baskets were representative of leaf litterfall from each ring. For example, in 2003, the $\delta^{13}C$ for green leaves and leaf litterfall in the elevated CO_2 treatment was identical (−39.8‰ ± 0.2‰) and reflected the ^{13}C-depleted CO_2 source used in the FACE experiment. However, foliar $\delta^{13}C$ values in the elevated CO_2 treatment were different from leaves outside the plots (−29.7‰ ± 0.5‰). Similar results were found in prior years (2000) when $\delta^{13}C$ in leaf litterfall in the elevated CO_2 treatment (−41.1‰ ± 0.2‰) was indistinguishable from the depleted CO_2 source but significantly different from leaf litterfall collected under ambient CO_2 (−29.1‰ ± 0.01‰). Thus, there was no indication that leaves from outside the FACE rings were inadvertently captured by the litterfall baskets, based on both visual observations during leaf fall and analysis of stable carbon isotopes.

The proportion of inorganic N taken up by plant roots as either NO_3-N or NH_4-N potentially affects foliar N isotope ratios (Kahmen et al. 2008). Therefore, ion exchange resins were used to measure the relative amounts of NO_3-N and NH_4-N in the soil over time. Resin access tubes (Warrington Ecological Systems Analysis, Saint Ignatius, Montana, USA) were installed at eight locations in each FACE ring to a depth of 15 cm in July 2001 (Johnson et al. 2004). Mixed-bed resin capsules (PST-1, Unibest, Bozeman, Montana, USA) were placed in each tube beginning in August 2005 and were incubated for 1–3 months thereafter during 2005 (two sampling events) and 2006 (seven sampling events). Resins were generally incubated only during the growing season in 2007 (two sampling events) and 2008 (four sampling events). Upon retrieval, resins were rinsed with distilled water to remove adhering soil particles and serially extracted three times each with 20 mL of 2 mol/L potassium chloride (for a total of 60 mL). During each extraction, the resin capsule and extractant were shaken in a plastic bottle at low speed on a reciprocating shaker for 30 min. The extractants were filtered through Whatman number 1 filters that had been pre-leached with distilled water and then frozen at −20°C until analysis for NH_4-N (EPA method number 12-107-06-1-A) and NO_3-N (EPA method number 12-107-04-1-B) on

a Lachat QuikChem 8500 autoanalyzer (Lachat Instruments, Loveland, Colorado, USA).

Time history trends in leaf litterfall $\delta^{15}N$ were analyzed using linear regression analysis (GraphPad Prism, GraphPad Software, La Jolla, California, USA). Analysis of ^{15}N at natural abundance levels was precluded after 2005 in rings 1, 2, 4, and 5 by a N-cycling tracer experiment that began in May 2006 (Garten and Brice 2009). However, the enriched ^{15}N tracer was not used in ring 3, and trends in leaf litterfall ^{15}N in ring 3 were analyzed by regression analysis from 1998 to 2008. Resin-based NO_3-N to NH_4-N ratios from 2005 through 2008 were analyzed using repeated-measures ANOVA. The ^{15}N added to the FACE rings in May 2006 was highly enriched (99 atom%) and had no effect on available soil N. Throughout this paper, summary statistics are reported as the mean ± SE or, in the case of regression analysis, slope ± SE.

RESULTS

Natural abundance ^{15}N ($\delta^{15}N$) in leaf litterfall collected under both ambient and elevated [CO_2] declined significantly ($P \leq 0.05$) from 1998 to 2005 in all five FACE rings, and the trend continued through 2008 in ring 3 (Fig. 1a). In addition, there was a significant difference ($P \leq 0.001$) in the rate of decline in $\delta^{15}N$ for leaf litterfall collected under elevated [CO_2] (rings 1 and 2) and ambient [CO_2] (rings 3, 4, and 5) (Table 1). Compared to ambient CO_2 exposures, the rate of decline in leaf litterfall $\delta^{15}N$ was nearly twice as fast under CO_2 enrichment from 1998 to 2005. Whole-soil $\delta^{15}N$ measured in April 2006 at the ORNL FACE experiment averaged 4.2‰ ± 0.15‰ ($n = 24$) and did not differ significantly among rings or between CO_2 treatments (Table 1); therefore enrichment factors (EF = leaf $\delta^{15}N$ − soil $\delta^{15}N$) were not calculated. Variation in calculated leaf litterfall $\delta^{15}N$ values at the start of the experiment, corresponding to $\delta^{15}N$ in 1997 (Table 1), was probably related to heterogeneity in initial soil conditions across the plantation. There was a strong correlation between litterfall $\delta^{15}N$ values in 1997 and whole-soil N concentration in the top 30 cm of the soil profile ($r = +0.94$).

Leaf litterfall N concentrations from all rings at the ORNL FACE experiment also declined significantly ($P \leq 0.05$) from 1998 to 2008 (Fig. 1b), but in contrast to $\delta^{15}N$, the slopes from the regressions against time were not significantly different ($P > 0.05$) among rings. Overall, litterfall N concentrations declined each year at a rate of ~0.27 g N/kg dry leaf litterfall mass under both ambient and elevated [CO_2].

Repeated-measures ANOVA indicated no difference ($F_{4,56} = 0.659$, $P = 0.62$) among the five rings in the relative amount of soil N present as NO_3-N vs. NH_4-N (Table 1). The ratio of NO_3-N and NH_4-N, as indicated by extractions of ion exchange resins, was 1.0 ± 0.17 ($n = 75$). There were no systematic trends over time in NO_3-N:NH_4-N ratios. In addition, data from 75
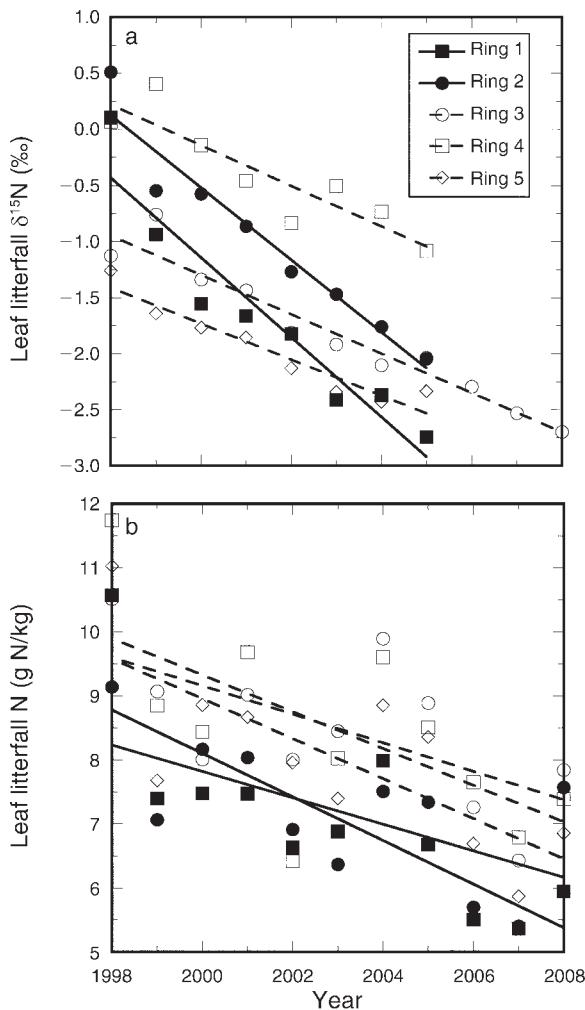

FIG. 1. Change in (a) leaf litterfall $\delta^{15}N$ values and (b) leaf litterfall N concentrations over time in different treatment rings at the free-air CO_2 enrichment (FACE) experiment in a sweetgum (*Liquidambar styraciflua*) plantation at the Oak Ridge National Laboratory, Oak Ridge, Tennessee, USA. Solid symbols designate measurements under elevated [CO_2] (544 ppm), and open symbols designate measurements under ambient [CO_2] (391 ppm). See *Methods* for a description of the treatment rings. Slopes for the linear regression of $\delta^{15}N$ against time are presented in Table 1.

sampling events were not normally distributed; so there was a 50%, 70%, and 80% chance, respectively, that the NO_3-N:NH_4-N ratio was ≤ 0.3, ≤ 1.0, and ≤ 2.0 at our study site.

DISCUSSION

We have found that temporal trends in sweetgum leaf litterfall ^{15}N abundance provide strong evidence that N availability in the ORNL FACE plots declined over time and declined faster in plots exposed to elevated [CO_2]. Coupled with the observations of declining response of forest NPP to CO_2 enrichment in this experiment (Norby et al. 2008), we conclude that elevated [CO_2] is

TABLE 1. Soil N data and parameters for linear regressions of natural ^{15}N abundance in leaf litterfall against years of CO_2 exposure (1997 is year 0).

[CO$_2$] (ppm) and treatment ring	Soil N (g N/kg)	Soil δ^{15}N (‰) (n = 6)	Soil NO$_3$-N:NH$_4$-N ratio	Regression parameters†		
				δ^{15}N in 1997 (‰)	Slope (‰ per yr)	r^2
Elevated (544 ppm)						
1	1.02	4.1 ± 0.3	1.1 ± 0.5	−0.08 ± 0.25	−0.355 ± 0.050	0.90
2	1.06	3.8 ± 0.2	1.0 ± 0.3	0.45 ± 0.17	−0.322 ± 0.034	0.94
Ambient (391 ppm)						
3	0.90	. . .	1.3 ± 0.6	−0.77 ± 0.10	−0.176 ± 0.015	0.93
4	1.02	4.4 ± 1.3	0.8 ± 0.3	0.40 ± 0.18	−0.181 ± 0.036	0.80
5	0.90	4.3 ± 0.2	0.9 ± 0.2	−1.25 ± 0.10	−0.160 ± 0.020	0.92

Notes: Initial (1997) soil N data (0–30 cm) are from Jastrow et al. (2005). Values for soil δ^{15}N, soil NO$_3$-N:NH$_4$-N ratio, δ^{15}N in 1997, and slope are given as mean ± SE. See *Methods* for a description of the treatment rings. Data are from the free-air CO_2 enrichment (FACE) experiment in a sweetgum (*Liquidambar styraciflua*) plantation at the Oak Ridge National Laboratory, Oak Ridge, Tennessee, USA.

† See Fig. 1.

causing progressive N limitation as hypothesized by Luo et al. (2004). Here, we provide the rationale for associating the ^{15}N signal in leaf litterfall with soil N availability in this ecosystem and we explain its relationship to productivity and other measures of ecosystem N cycling.

Foliar ^{15}N abundance is indicative of soil N availability

Numerous studies (Garten 1993, Johannisson and Högberg 1994, Emmett et al. 1998, Martinelli et al. 1999, Koba et al. 2003, Garten et al. 2007, Craine et al. 2009) have established associations between foliar δ^{15}N and soil N availability. For example, a chronosequence study (10–100 years) by Compton et al. (2007) showed declining foliar N and δ^{15}N during progressive tightening of the N cycle with forest stand maturation in Rhode Island. Moreover, in a recent review, Craine et al. (2009) found increasing foliar δ^{15}N with increasing soil N availability (as indexed by various measures) in 13 of 15 multisite studies. Mathematical modeling of soil N dynamics and plant N uptake has also been used to explain foliar δ^{15}N dynamics, or enrichment factors (equal to the difference between foliar δ^{15}N and soil δ^{15}N), as a function of varying plant uptake of soil NH$_4$-N and NO$_3$-N, the isotopic composition of soil N pools, and relative rates of soil N transformations (Garten and Van Miegroet 1994). The isotopic signature of inorganic N taken up from the available soil pool depends on the balance between immobilization and nitrification of the NH$_4$ pool. As the relative amount of immobilization increases, indicating a tighter N cycle and less available soil N, the available N pool becomes isotopically lighter (Garten 1993). Plant mycorrhizal associations have also been found to drive foliar δ^{15}N values in the same direction, that is, declines in foliar ^{15}N abundance are related to decreasing N availability (Garten et al. 2007, Craine et al. 2009), because mycorrhizal fungi create ^{15}N-depleted compounds for transfer to plants (Hobbie and Hobbie 2008). Hence, irrespective of the proximate cause, within-site measures of foliar δ^{15}N can be used as an indicator of temporal changes in soil N availability.

CO_2 effects on ^{15}N abundance

In the sweetgum plantation, leaf litterfall δ^{15}N declined through time, indicating declining N availability and a progressive tightening of the N cycle. This is an expected response given that the forest stand is N limited (Iversen and Norby 2008) and that atmospheric N deposition rates to the site (1.2–1.5 g N/m^2; Johnson et al. 2004) are less than the rate of N sequestration into perennial biomass (Norby and Iversen 2006) and soil organic matter (Jastrow et al. 2005). The steeper decline of leaf litterfall δ^{15}N in elevated [CO$_2$] plots indicates a specific effect of CO_2 enrichment on soil N availability. This is the first such documentation of either declining N availability through time or an effect of elevated [CO$_2$] on N availability in this FACE experiment. Previous work at the ORNL FACE experiment had not detected an effect of elevated [CO$_2$] on N mineralization or microbial immobilization of N in surface soil samples (Zak et al. 2003, Austin et al. 2009) or deeper in the soil profile (Iversen et al. 2010). Also, Johnson et al. (2004) reported no effect of elevated [CO$_2$] on intermittent measures of soil N availability as assessed using in situ soil incubations and ion exchange resin techniques. The absence of a CO_2 enrichment effect in previous assessments of N availability is not necessarily at odds with our measured response in the δ^{15}N data set. Previous measurements could not be used to test a hypothesis of changing N availability over time as they employed different sampling and analytical protocols, whereas leaf litterfall δ^{15}N integrates N availability over the entire period of active N uptake and over the entire rooting volume. It is through the analysis of the entire time series of leaf litterfall δ^{15}N that we were able to detect a CO_2 enrichment effect. Differences between treatments in any given year were not statistically significant and were confounded with initial differences related to site heterogeneity. For example, eight years into the ORNL FACE experiment (2005), there was a tendency for less ^{15}N in leaf litterfall under elevated [CO$_2$] (−2.4‰) relative to ambient [CO$_2$] (−1.8‰) (BassiriRad et al. 2003), but a statistically significant

difference was not detected. If the regressions based on our data (Fig. 1a) are extended into the future to point comparisons, statistically significant differences in litterfall $\delta^{15}N$ between the ambient and elevated [CO_2] treatments are not detectable until the 20th year of the experiment. Therefore, temporal dynamics in leaf litterfall $\delta^{15}N$, as indicated by the slopes of the regressions (Fig. 1a), are potentially more revealing about changes in forest N cycling than one-time comparisons of leaf litterfall $\delta^{15}N$ or other measures of N availability between ambient and elevated [CO_2] treatments. Furthermore, recent ^{15}N tracer experiments at the ORNL FACE experiment (see *Methods*) indicated that N is moving from tree roots into slower cycling particulate organic matter in near-surface soils under both the ambient and elevated [CO_2] treatments (Garten and Brice 2009). A prolonged flux of N into longer-lived belowground pools could create PNL over decadal time frames, especially if N release through soil organic matter decomposition is essential to sustaining forest NPP under elevated [CO_2] (Garten and Brice 2009). The crux of these considerations is that long-term data advance the likelihood of detecting PNL more than do treatment comparisons at particular points in time or studies of treatment differences over relatively short time periods.

Other factors possibly influencing leaf litterfall $\delta^{15}N$

We have also considered several other factors (leaf senescence, changes in soil $\delta^{15}N$ with depth, nitrate leaching, and root–mycorrhizal associations) that could affect the relationship between ^{15}N abundance in leaf litterfall and soil N availability in the ORNL FACE experiment:

(1) We used freshly fallen leaf litter in this analysis rather than green leaves collected from the canopy because this approach enabled us to integrate over the entire canopy. Comparisons of natural ^{15}N abundance in living and abscised leaves have shown that there is negligible isotopic fractionation associated with N translocation (Garten 1993, Kolb and Evans 2002). Thus, provided that it is fresh and undecomposed, leaf litterfall $\delta^{15}N$ is a time-integrated measure of ^{15}N abundance in living leaves over the time of active N uptake during the growing season.

(2) Soil $\delta^{15}N$ values generally increase with depth (Högberg 1997), and elevated [CO_2] at the ORNL FACE experiment has produced a significant increase in fine roots at soil depths from 30 to 60 cm (Norby et al. 2004, Iversen et al. 2008). Such increased deep-root production could result in mining of deeper soil N under elevated [CO_2] to supply increased plant N demands (Iversen 2010) and, in turn, cause leaf litterfall $\delta^{15}N$ to increase over time. However, this scenario is contrary to the observed steeper decline in leaf litterfall $\delta^{15}N$ over time under elevated [CO_2] (Fig. 1a) and therefore would fail to explain our results.

(3) Nitrate leaching can also affect litterfall $\delta^{15}N$. When leaching is important, ecosystem N losses are typically accompanied by increases in foliar ^{15}N content because leaching of isotopically depleted nitrate leaves residual plant-available N isotopically heavy (Högberg and Johannisson 1993, Pardo et al. 2002). We do not know whether there are significant differences in leaching between the ambient and elevated [CO_2] treatments. Our analysis of buried ion exchange resins indicated no treatment effects and no consistent, multi-year, temporal trend in NO_3-N:NH_4-N ratios. A separate study also indicated inorganic N adsorption on resin capsules was usually greater at deeper sampling depths, but no CO_2 treatment effects for N adsorbed on ion exchange resins in deeper soils (55 cm) (Iversen et al. 2010). A prior two-year record of N leaching from the ORNL FACE experiment (1999–2001), at a 20-cm soil depth, indicated a 20–25% reduction in nitrate leaching may have occurred under elevated [CO_2] (Johnson et al. 2004). If the latter trend continued, decreased nitrate leaching might indicate a progressive closing of the N cycle under CO_2 enrichment, resulting in decreased litterfall $\delta^{15}N$ values in the CO_2-enriched plots relative to the control plots. If the N cycle has become progressively more closed under CO_2 enrichment as a result of both increased N demand to sustain higher NPP and accompanying reductions in nitrate leaching, then the expected outcome is progressively more negative leaf litterfall $\delta^{15}N$ over time (Martinelli et al. 1999, Garten et al. 2007) and a faster rate of decline in litterfall $\delta^{15}N$ under elevated [CO_2].

(4) The steeper decline of leaf litterfall $\delta^{15}N$ under elevated CO_2 might be explained by a greater reliance of proliferating deep fine roots on N coming from mycorrhizae (e.g., see Hobbie and Hobbie 2008), which would cause the sweetgum trees in the ORNL FACE experiment to become progressively depleted in ^{15}N. In a 1999 survey of vegetation growing at six FACE experiments (including ORNL), BassiriRad et al. (2003) reported widespread depletion of foliar ^{15}N in 19 plant species (representing diverse functional groups) growing under elevated [CO_2]. Given that there is measureable isotopic fractionation associated with the transfer of N from mycorrhizal fungi to plants (Evans 2001, Craine et al. 2009), BassiriRad et al. (2003) speculated that increased root colonization by mycorrhizal fungi or increased root assimilation of soil NO_3-N were possible causes of depleted foliar ^{15}N under elevated [CO_2]. However, sweetgum trees at the ORNL FACE experiment are populated with arbuscular mycorrhizae, which often show less ^{15}N discrimination than other types of mycorrhizae (Hobbie and Hobbie 2008). Ultimately, even if increased reliance on mycorrhizal N uptake is a contributing mechanism to the observed temporal changes in litterfall $\delta^{15}N$ (Fig. 1a), the overall conclusion of our study that leaf litterfall $\delta^{15}N$ is a sensitive and accurate indicator of declining

soil N availability under elevated [CO_2] remains unchanged.

Progressive nitrogen limitation

Our new analysis of soil N availability based on leaf litterfall ^{15}N abundance, coupled with the decline in response of NPP to elevated [CO_2] (Norby et al. 2008), provides the missing evidence that PNL is probably occurring in the ORNL FACE experiment. This empirical evidence for declining N availability is consistent with theoretical analyses. In a modeling study Franklin et al. (2009) identified declining N availability as the causative agent for the diminishing differences in NPP between ambient and elevated [CO_2] treatments at the ORNL FACE site. According to their theoretical predictions, N availability declined during the first seven years of treatment at the ORNL FACE experiment, and the modeling analysis also indicated a threshold in N availability beyond which the NPP response to CO_2 enrichment no longer occurs (Franklin et al. 2009). Our data indicate soil N availability has been declining in both ambient and elevated [CO_2], probably as a result of stand maturation (Johnson 2006), but that declines in N availability were accelerated under elevated [CO_2]. The effect of elevated [CO_2] on N availability can be ascribed to increased sequestration of N in woody biomass (Norby and Iversen 2006) and/or soil organic matter (Jastrow et al. 2005).

CONCLUSIONS

As pointed out by Franklin et al. (2009), conventional measures of soil N availability do not necessarily correspond to plant-available N, and the difficulty in assessing changes in N availability have made evaluations of the role of N limitation to CO_2 responses in forests difficult. Measurements of leaf or leaf litterfall $\delta^{15}N$ overcome this important problem of detection. Based on changes over time in the ^{15}N content of leaf litterfall, we conclude that soil N availability has declined over time and has declined faster in response to CO_2 enrichment at the ORNL FACE site. These observations support the occurrence of PNL in the ORNL FACE experiment when conventionally proposed indicators of PNL failed to detect such changes during the first six years of tree growth under elevated [CO_2] (Norby and Iversen 2006). The extent to which our results can be generalized to other locations is unknown, and retrospective studies are encouraged at other FACE sites to test the hypothesis that leaf litterfall $\delta^{15}N$ is a sensitive and accurate indicator of soil N availability and has utility in assessments of PNL. It is important to emphasize that temporal changes in leaf litterfall $\delta^{15}N$ will be highly site-specific and that leaf litterfall $\delta^{15}N$ may not reveal between-site differences in soil N availability without appropriate normalization for between-site differences in soil $\delta^{15}N$ through the use of enrichment factors (EF = leaf $\delta^{15}N$ – soil $\delta^{15}N$) or other suitable corrections (Garten et al. 2007). Within the context of current models of ^{15}N distribution in ecosystems, temporal dynamics in leaf litterfall $\delta^{15}N$ provide a powerful tool for documenting changes in soil N availability and the critical feedbacks between the C and N cycles that will control forest response to elevated atmospheric CO_2.

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