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RCM

Belowground fate of ^{15}N injected into sweetgum trees (*Liquidambar styraciflua*) at the ORNL FACE Experiment†

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Nitrogen (N) cycling can be an important constraint on forest ecosystem response to elevated atmospheric CO_2. Our objective was to trace the movement of ^{15}N, injected into tree sap, to labile and stable forms of soil organic matter derived partly from the turnover of tree roots under elevated (545 ppm) and ambient (394 ppm) atmospheric CO_2 concentrations at the Oak Ridge National Laboratory (ORNL) FACE (Free-Air Carbon Dioxide Enrichment) Experiment. Twenty-four sweetgum trees, divided equally between CO_2 treatments, were injected with 3.2 g ^{15}N-ammonium sulfate (99 atom %), and soil samples were collected beneath the trees over a period of 89 weeks. For 16 cm deep soil samples collected beneath the study trees, there was 28% more fine root (less than or equal to 2 mm diameter) biomass under elevated CO_2 ($P = 0.001$), but no significant treatment effect on the amounts of necromass, coarse root biomass, or on the N concentrations in tree roots and necromass. Nitrogen-15 moved quickly into roots from the stem injection site and the ^{15}N content of roots, necromass, and labile organic matter (i.e. particulate organic matter, POM) increased over time. At 89 weeks post-injection, approximately 76% of the necromass ^{15}N originated from fine root turnover. Nitrogen-15 in POM had a relatively long turnover time (47 weeks) compared with ^{15}N in roots (16 to 22 weeks). Over the 1.7 year period of the study, ^{15}N moved from roots into slower cycling POM and the disparity in turnover times between root N and N in POM could impose progressive limitations on soil N availability with stand maturation irrespective of atmospheric CO_2, especially if the release of N through the decomposition of POM is essential to sustain forest net primary production. Published in 2009 by John Wiley & Sons, Ltd.

Changes in belowground processes are some of the more important but least understood responses of forest ecosystems to elevated atmospheric CO_2 concentrations and climate change. In particular, increased fine root production under elevated CO_2 is a widely observed response of forest stands in free-air CO_2 enrichment (FACE) experiments, and this is usually matched by increases in annual fine root mortality.[1] One consequence of higher root production and larger root systems under elevated CO_2 is an increase in plant nitrogen (N) requirement and thus greater soil N uptake by trees.[2,3] At Oak Ridge National Laboratory's (ORNL) FACE Experiment in Oak Ridge, Tennessee,[4] and other FACE sites,[3] increased root mortality leads to increased soil carbon (C) and N inputs under elevated CO_2. A higher N input to soil under elevated CO_2 could have important consequences for the long-term sustainability of higher rates of biomass production under elevated CO_2 in N-limited forest ecosystems, such as the ORNL FACE Experiment.[4]

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Prior research indicates no apparent effect of elevated CO_2 on forest soil processes that directly govern soil N availability at a variety of FACE experiments,[5] and Norby and Iversen[2] reported no indications of progressive N limitation[6] at the ORNL FACE experiment after 6 years of sweetgum (*Liquidambar styraciflua*) exposure to elevated CO_2 (550 ppm). Elevated atmospheric CO_2 generally increases aboveground production in trees[7,8] and progressive N limitation of this response is hypothesized to occur when the N becomes increasingly sequestered in long-lived plant tissues (e.g. wood) and soil organic matter (SOM).[6] Where progressive N limitation occurs, N becomes increasingly associated with more stable forms of SOM that are resistant to microbial decomposition Therefore, soil N becomes increasingly unavailable to supply the N required to sustain higher plant production under elevated CO_2.

Injection of radioactive[9,10] and stable[11] isotopes into trees has been an effective technique for examining the fate of materials in forest element cycles. In particular, Horwath *et al.*[11] proposed the use of tree injections as a minimally invasive method for studying root turnover and the fate of root-derived N in forest soils. Using this technique, we conducted a study of the fate of N beneath ^{15}N-labeled sweetgum trees at the ORNL FACE Experiment over a

period of 1.7 years. Our objective was to compare the movement of N from ^{15}N-labeled trees into roots and both labile and stable forms of SOM via the turnover of fine roots under elevated and ambient CO_2. Necromass and particulate organic matter (POM) are labile fractions of SOM that are highly vulnerable to decomposition by soil microorganisms and are expected to have turnover times on the order of years, or less, in temperate and tropical soils.[12] By comparison, mineral-associated organic matter (MOM) includes organic matter bound to soil silt and clay and is generally considered to be a more stabilized form of surface SOM that has a turnover time of the order of decades.[13] A rapid transfer of ^{15}N to more stable forms of SOM would be indicative of soil N sequestration that could contribute to progressive N limitation of tree growth under elevated atmospheric CO_2 concentrations.

EXPERIMENTAL

Site description

The ORNL FACE Experiment has been described in several prior publications,[2,14,15] and only a brief description is presented here for convenience. The experiment is located on the U.S. Department of Energy's Oak Ridge Reservation (35°54′N; 84°20′W) in Oak Ridge, TN, USA. The mean annual temperature at the site is 14.2°C and the mean annual precipitation is 1390 mm $year^{-1}$. Sweetgum trees were planted in 1988 at a 2.3 × 1.2 m spacing in five 25 m diameter rings, four of which had FACE apparatus installed, on a slightly acidic (pH 5.5–6.0), silty clay loam soil (Wolftever series) that is classified as an Aquic Hapludult. The alluvial soil contains approximately 1 kg N m^{-2} and soil N availability apparently limits tree production.[15] When the measurements started, the closed canopy stand basal area was approximately 45.1 m^2 ha^{-1}, the leaf area index was 5.5, and the tree height was approximately 17 m. Elevated CO_2 exposures (average daytime concentration of 545 ppm over 10 years) were initiated in two of the five FACE rings in 1998. Ambient daytime CO_2 concentrations in the control rings average 394 ppm.

Stem injections

Two clusters of three nearest neighbor trees were located for study within each FACE ring (24 trees total) prior to bud break in April 2006. A polygon was delineated on the ground within each cluster by defining the base of each tree in a cluster as a corner. The size of each polygon varied between clusters, but was of the order of 2 to 3 m^2. The surface of the ground inside each cluster was covered with heavy-duty, black, plastic sheeting (0.15 mm thick), secured with landscape staples, to minimize ^{15}N inputs to the soil surface via canopy leaching by precipitation and falling leaves. Individual trees in each cluster were marked with unique numbers for identification during soil sampling.

Each tree in a cluster was labeled with ^{15}N in mid-May 2006 (9^{th} growing season) by stem injection. An 11 mm diameter hole was drilled near the base of the tree approximately 5 to 6 cm deep at a slight downward angle under a constant stream of artificial sap solution (5 mM KCl and 0.4 mM malic acid adjusted to pH 5.4 with KOH).[11] The hole was immediately plugged with a tapered plastic connector (VWR, West Chester, PA, USA; part #46600-078), filled with artificial sap solution, and connected to a 0.6 m long section of Nalgene® PVC tubing (7.9 mm i.d.). The open end of the tubing was elevated, attached to the tree with string, and partially filled with artificial sap solution. The injection site was considered acceptable when there were no leaks and the fall in the level of the sap solution in the tubing indicated uptake by the tree. At that time, 25 mL of ^{15}N tracer solution containing 3.2 g ^{15}N-ammonium sulfate (99 atom %) dissolved in deionized water was added to the tubing with a 50 mL syringe. When the fluid level in the tubing approached the plastic fitting, the injection was followed by another 25 mL of artificial sap solution. Upon completion of the labeling, the tubing and the fitting were removed and the hole was plugged with a closed-foam, expandable ear-plug. With this technique we were able to successfully inject 24 trees with ^{15}N tracer over the course of 2 days.

Soil sampling and processing

Baseline soil sampling within each cluster of trees was carried out prior to tree labeling with ^{15}N, in mid-April 2006 (25 days prior to tree injection). Following the tree injections, the soils were sampled in August 2006, January, April, and August 2007, and January 2008. The sampling events corresponded to 14, 34, 49, 66, and 89 weeks of elapsed time post-injection. At each sampling event, the plastic sheeting on the ground in each cluster of labeled trees was removed and three soil cores were collected to a 16 cm depth from the area covered by plastic sheeting using a 5.4 cm diameter bucket auger. One core was collected at approximately 30 to 60 cm distance from the base of each ^{15}N-labeled tree. The plastic sheeting was replaced inside each cluster of trees after the sampling was completed. Each soil sample was emptied into a zip-lock plastic bag, transported to the laboratory, and refrigerated (5°C) prior to sample processing. In late September 2008, leaf and twig samples were accessed throughout the canopy of injected trees under both ambient ($n = 3$) and elevated ($n = 6$) CO_2 by climbing. Leaf and twig samples were transported to the laboratory, dried at 70°C and milled to a fine powder prior to isotope analysis.

In the laboratory, fresh soil samples were weighed and sieved through a 6.3 mm sieve to remove roots. Thereafter, additional, visible roots were removed from the sieved soil using forceps to obtain a 'root-free' soil sample. A subsample of the sieved soil was air-dried to a constant weight to determine a dry mass-to-fresh mass conversion factor. The roots were washed briefly in a 5% solution of sodium hexametaphosphate to remove soil adhering to root surfaces and to disperse silt and clay. The root mixture was then washed, with distilled water, through a 1 mm sieve stacked onto a 0.5 mm sieve. Roots recovered on the 1 mm sieve were sorted by hand into fine (diameter <2 mm) and coarse (diameter >2 mm) size classes. Organic debris captured on the 0.5 mm sieve that was not attached to identifiable roots was classified as necromass and consisted of dead and decaying plant parts. Coarse and fine roots and necromass samples from each soil core were dried (70°C), weighed, milled to a powder, and stored in airtight glass vials. Root, necromass, leaf, and twig samples were analyzed for N

concentrations and ^{15}N using an Integra-CN continuous flow isotope ratio mass spectrometer (SerCon Ltd., Crewe, UK). The working standard for the analysis was ammonium sulfate calibrated against reference material (NIST 8547) from the National Institute of Standards and Technology (NIST, Gaithersburg, MD, USA).

A portion of the sieved, root-free, air-dry soil was used for physical separation of the bulk soil into POM and MOM.[16] Samples of soil (20 g) were shaken overnight in a 100 mL solution of sodium hexametaphosphate (5 g L^{-1}). The mixture was wet-sieved through a 0.053 mm sieve. POM was defined as matter captured on the 0.053 mm sieve while MOM was organic matter associated with silt and clay that passed through the sieve. Both parts were oven dried (70°C). POM consists of free organic debris in the soil, partially decomposed, and larger fragments of organic matter (greater than or equal to 0.053 mm diameter) released by dispersion of soil aggregates. It was distinguished from necromass both on the basis of size and on the more direct association of necromass with plant roots. MOM included organic matter bound to silt and clay size particles (<0.053 mm diameter) and smaller fragments of organic matter released by dispersion of soil aggregates. Each fraction was milled to a powder and stored in airtight glass vials. Samples of bulk soil, POM, and MOM were analyzed for total C and N concentrations using a LECO CN-2000 elemental analyzer (LECO Corp., St. Joseph, MI, USA) and for ^{15}N using the Integra-CN isotope ratio mass spectrometer (as above). The elemental analyzer was calibrated using LECO standards traceable to NIST.

Calculations

The base of each individual tree was repeatedly sampled through time. Thus, the 24 trees were divided into two treatment groups (ambient and elevated CO_2) and compared with a repeated measures analysis of variance (ANOVA). In those cases where there were missing values, as was the case for a few samples that contained no coarse roots, a straight two-way ANOVA that included the main effects of CO_2 treatment and time was performed. Two of 124 measurements of coarse root biomass were judged to be outliers and were replaced in the ANOVA by the monthly mean value for the treatment. All other data were retained. The means and standard errors were used as summary statistics, unless stated otherwise. Fisher's least significant difference (LSD) was used to compare treatment group means after ANOVA.

Because the enrichment of ^{15}N in roots and soil beneath the labeled trees was relatively low, the N isotope data are presented as $\delta^{15}N$ values with units of parts per thousand (‰):

$$\delta^{15}N = [(^{15}N/^{14}N_{sample} \div {}^{15}N/^{14}N_{standard}) - 1] \times 1000,$$

where the standard was atmospheric N_2 (0.0 ‰). The contribution of fine root ^{15}N to necromass, POM, and MOM was also calculated for each sampling event using a two-pool

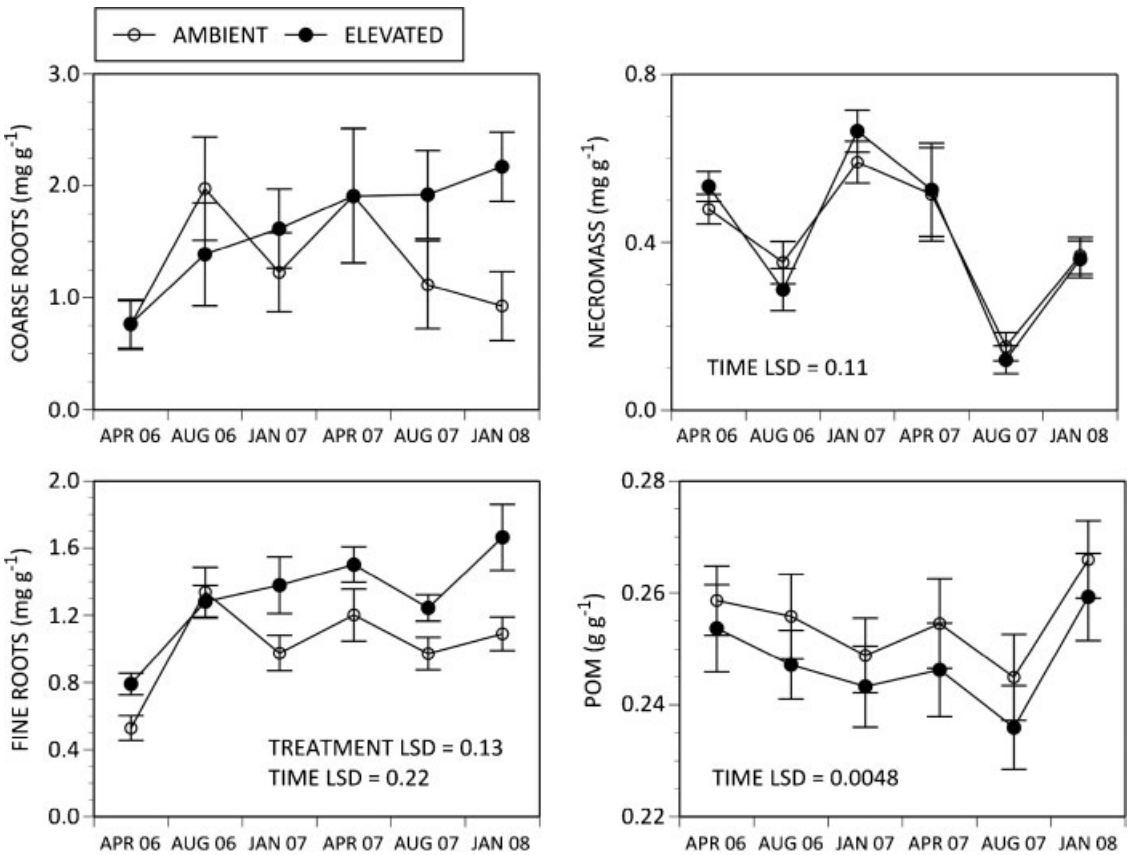

Figure 1. Amounts of coarse roots, fine roots, necromass, and POM from surface soil (16 cm deep) beneath sweetgum trees growing under ambient and elevated atmospheric CO_2 concentrations, in the ORNL FACE Experiment, before (April 2006) and after ^{15}N stem injection (May 2006 onwards). Error bars indicate ±1 SE about the mean; LSD = Fisher's least significant difference.

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mixing model with initial $\delta^{15}N$ values in the unlabeled soil pool (prior to tree labeling) as the first end member and the measured $\delta^{15}N$ value of fine roots as the second end member. Calculations were performed using ISOERROR 1.04.[17] The mean residence time (i.e. turnover time) of ^{15}N in tree roots, necromass, and POM was calculated from a one-phase exponential association ($Y = Y_{max} \bullet e^{-kt}$) where Y is the $\delta^{15}N$ value at different times, Y_{max} is the plateau in $\delta^{15}N$, t is time (weeks), k is the rate coefficient (per week), and 1/k is the turnover time (weeks).

RESULTS

Differences in necromass and root dry matter

The fine root biomass was significantly ($P = 0.001$) greater under elevated CO_2 (1.02 ± 0.05 and 1.31 ± 0.06 mg g^{-1} soil under ambient and elevated CO_2, respectively), but the effects of elevated CO_2 on necromass, coarse root biomass, and POM were not statistically significant. The amounts of POM, necromass and fine roots, but not coarse roots, varied significantly ($P = 0.001$) over time (Fig. 1). There was less necromass in the soil in late summer (August) than in early winter (January). Fine and coarse root biomass beneath trees exposed to elevated CO_2 tended to increase over the sampling period. Similar to fine roots, coarse root biomass was significantly ($P = 0.05$) greater under elevated CO_2 at the last sampling event (Fig. 1).

Nitrogen concentrations

Elevated CO_2 had no statistically significant effect on N concentrations in necromass, fine roots, or coarse roots. For this reason, measurements from different treatments were combined and analyzed over time (Fig. 2). Nitrogen concentrations in fine roots were relatively constant throughout the sampling period, but necromass N concentrations increased ($P = 0.05$) with elapsed time (ET). Changes in necromass N were best described by a logarithmic equation (mg N $g^{-1} = 5.64 + 1.98$ log(ET), $r = 0.91$). The N concentrations in coarse roots (4.3 ± 0.12 mg g^{-1}) were approximately half (or less) of those in fine roots (9.1 ± 0.15 mg g^{-1}). Despite significant differences over time, there were no consistent differences between dormant and growing season measurements of necromass and root N concentrations (Fig. 2).

The nitrogen concentrations in POM and MOM were significantly ($P = 0.05$) greater in soils collected beneath labeled trees exposed to elevated CO_2 (Fig. 3). The carbon concentrations in POM from soils beneath trees in the elevated CO_2 treatment were also significantly ($P = 0.01$) greater than those in the ambient CO_2 treatment, but the treatment effects were not significant for MOM carbon. Although changes in POM and MOM N and C concentrations were statistically significant over time, there were no distinguishable, systematic temporal trends (Fig. 3).

Measurements of ^{15}N abundance

There was no significant difference between $\delta^{15}N$ values under ambient and elevated CO_2 for any of the plant and soil components sampled. Therefore, measurements from different treatments were pooled and analyzed over time. Three

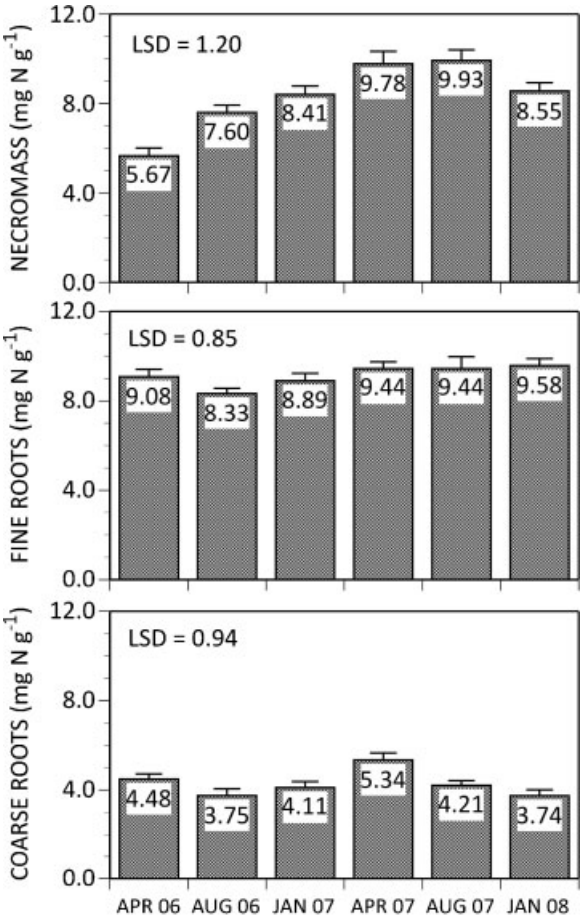

Figure 2. Nitrogen concentrations in necromass, fine roots, and coarse roots from surface soil beneath sweetgum trees at the ORNL FACE Experiment before (April 2006) and after ^{15}N stem injection (May 2006 onwards). Error bars indicate ± 1 SE about the mean; LSD = Fisher's least significant difference.

months after the tree injection, the ^{15}N had moved into the roots, necromass, and POM as indicated by $\delta^{15}N$ values that were significantly ($P = 0.05$) elevated above the pre-injection, natural abundance measurements (Fig. 4). The aboveground $\delta^{15}N$ values in leaves and twigs from the injected trees, that were not different between elevated and ambient CO_2, averaged 912 ± 54‰ ($n = 26$) and 917 ± 68‰ ($n = 27$), respectively.

Mixing model calculations indicated that at 89 weeks post-injection, approximately 76% of the necromass ^{15}N originated from fine root turnover and that the ^{15}N moved rapidly into the necromass pool (Table 1). While we expected roots to contribute a high proportion to soil organic matter, the former value may be somewhat inflated by excluding leaf litter input to soil beneath the ^{15}N-labeled trees. Over the same time period, there was less movement of ^{15}N into POM (19% contribution from fine roots) and MOM (<1% contribution from fine roots). The results were similar when necromass, rather than fine roots, was used as a contributing source to ^{15}N in POM and MOM (because of the similarity in $\delta^{15}N$ values in the fine roots and necromass).

The increase in ^{15}N in fine roots and necromass over time indicated a relatively constant infusion of tracer into belowground biomass via basipetal translocation of N

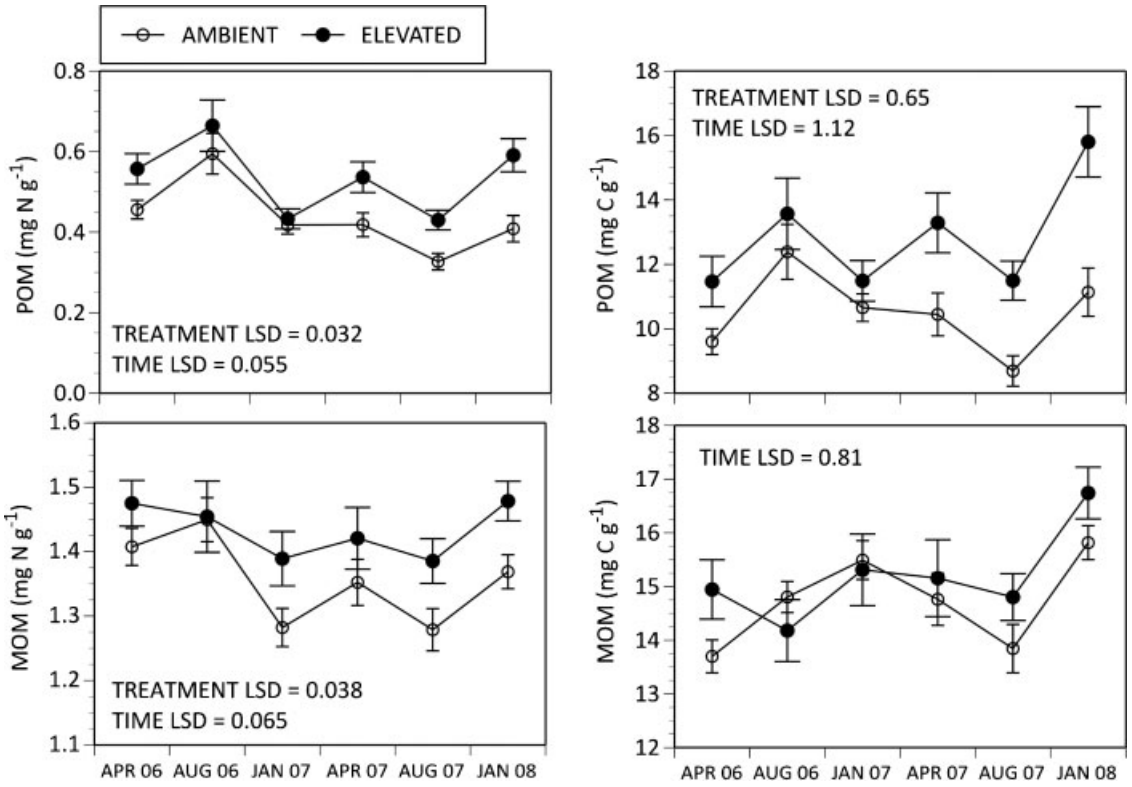

Figure 3. Nitrogen and carbon concentrations in POM and MOM from surface soil beneath sweetgum trees growing under ambient and elevated atmospheric CO_2 concentrations, in the ORNL FACE Experiment, before (April 2006) and after ^{15}N stem injection (May 2006 onwards). Error bars indicate ± 1 SE about the mean; LSD = Fisher's least significant difference.

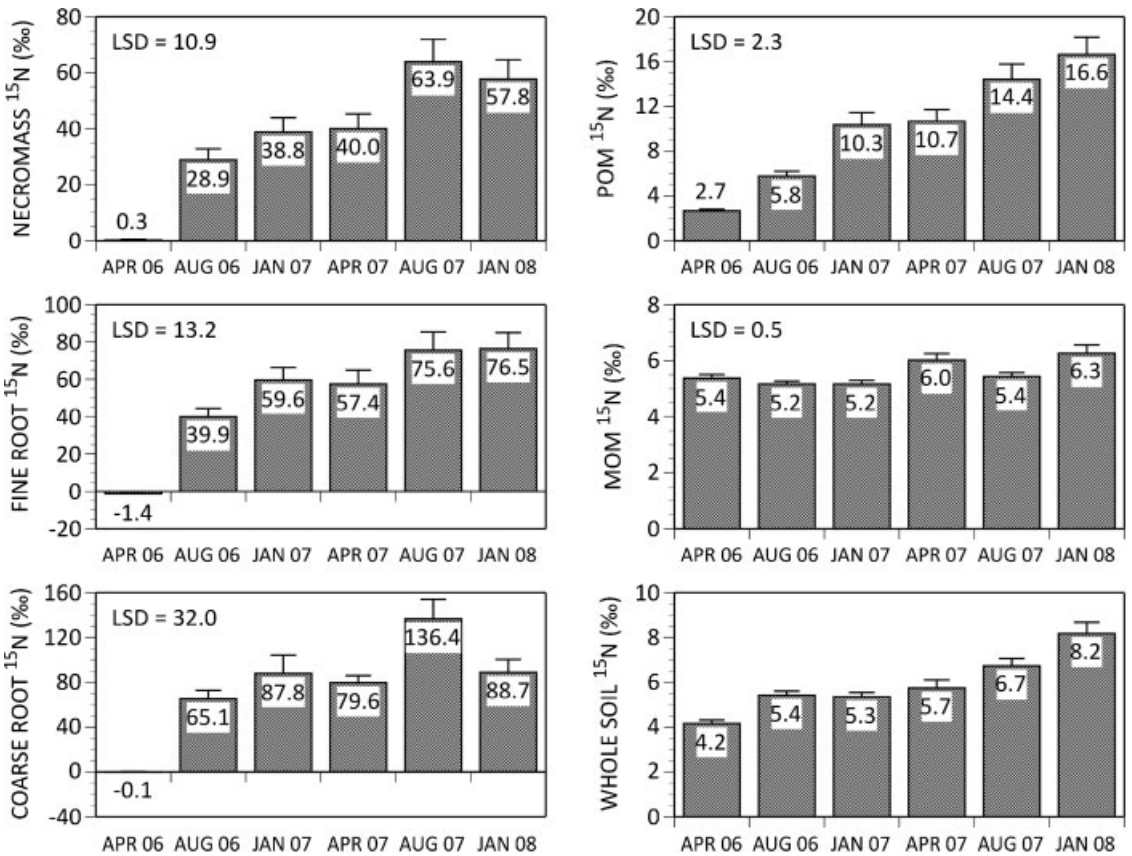

Figure 4. Nitrogen-15 ($\delta^{15}N$) in necromass, fine roots, coarse roots, POM, MOM, and whole surface soil (16 cm deep) beneath sweetgum trees at the ORNL FACE Experiment before (April 2006) and after ^{15}N stem injection (May 2006 onwards). Error bars indicate ± 1 SE about the mean; LSD = Fisher's least significant difference.

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Table 1. Mean (±SE) percent contribution of fine root ^{15}N to necromass, particulate organic matter (POM), and mineral-associated organic matter (MOM) beneath ^{15}N-labeled sweetgum trees at the ORNL FACE Experiment. Calculations were performed using ISOERROR 1.04[17]

	Weeks after tree injection with ^{15}N					
Soil pool	0	14	34	49	66	89
Necromass	0 ± 17	72 ± 13	65 ± 12	70 ± 13	85 ± 15	76 ± 12
POM	0 ± 5	8.3 ± 1.6	13.5 ± 2.5	14.6 ± 2.7	16.1 ± 2.8	18.9 ± 3.1
MOM	0 ± 2.7	0 ± 0.5	0 ± 0.4	1.3 ± 0.5	0.1 ± 0.3	0.3 ± 0.5

within the injected trees. A one-phase exponential association ($Y = Y_{max} \bullet (1 - e^{-kt})$) was fitted to the data in Fig. 4 to calculate the turnover time (1/k) of ^{15}N in belowground components. The mean residence time, or turnover time, of ^{15}N increased in the following order: coarse roots < fine roots < necromass < POM. Fits to a one-phase exponential association were statistically significant ($P = 0.05$) for the latter four belowground components (Fig. 5), but not for $\delta^{15}N$ in MOM or whole soil $\delta^{15}N$. The ^{15}N in MOM did not change significantly over time. The change in whole soil $\delta^{15}N$ (Y, ‰) was described by a linear regression against time (X, weeks): $Y = 4.25 + 0.0397\ X$ ($r^2 = 0.91$, $P = 0.05$). Overall, the ^{15}N tracer experiment indicated negligible short-term sequestration of fine root N in the MOM fraction.

DISCUSSION

Root dynamics vary with soil depth at the ORNL FACE Experiment,[4] and the present study included a zone of active turnover of belowground biomass near the soil surface. The calculated mean turnover time of fine roots (<2 mm diameter) in surface soil at the ORNL FACE Experiment in 2006, based on data presented by Iversen *et al.*,[4] was approximately 1.5 years. Therefore, we assume that the near-surface fine root biomass beneath ^{15}N-labeled trees turned over two to three times during the 89 week long ^{15}N tracer experiment. There appear to be small,[4] or negligible,[1] differences in root turnover times under ambient and elevated CO_2. In 2006, approximately 23% of the peak standing crop root biomass (to 60 cm) resided in the surface 15 cm of soil under both ambient and elevated CO_2; however, both root production and peak root biomass in the top 15 cm of soil was three times greater under elevated CO_2.[4] The measured greater fine root biomass beneath ^{15}N-labeled sweetgum trees grown under elevated CO_2 (Fig. 1) was in agreement with results from mini-rhizotron measurements, indicating that greater belowground C allocation is a key response to elevated CO_2 at the ORNL FACE Experiment.[1,4] In addition, our measurements indicated increasing fine, and possibly coarse, root biomass under elevated CO_2 over the 89-week study.

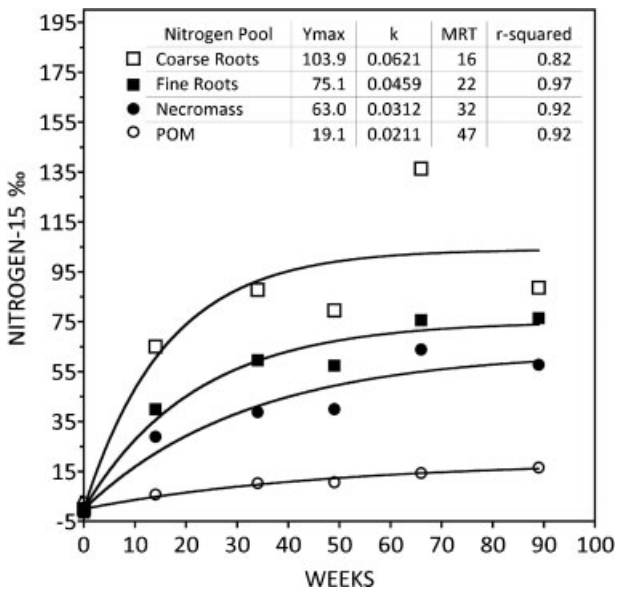

Figure 5. Analysis of the increase in $\delta^{15}N$ in different belowground N pools, based on a one-phase exponential association ($Y = Y_{max} \bullet e^{-kt}$), under sweetgum trees at the ORNL FACE Experiment following ^{15}N stem injection. The mean residence time (MRT, weeks) of ^{15}N in each pool was calculated from 1/k.

The measurements of N concentrations and $\delta^{15}N$ values indicated a strong similarity in the make-up of necromass and fine root biomass. However, the separation of necromass and fine root biomass was supported based on differences in the visual appearance of the material and the difference in ^{15}N turnover times (Fig. 5). Coarse roots are expected to have a longer mean residence time in the soil[18] and, for this reason, were assumed to make a minor contribution to necromass N over the relatively short time period of our observations. The more rapid turnover of ^{15}N in coarse roots challenged this assumption. While the ^{15}N moved quickly from the injection site in the tree to both coarse and fine roots, the one-phase exponential associations indicated that the ^{15}N content in the roots was near steady state at 89 weeks post-injection. The nitrogen-15 in necromass exhibited dynamics that were similar to those in fine roots but had a longer turnover time.

Contrary to the rapid movement of ^{15}N into roots and necromass (Table 1), there was relatively less transfer of ^{15}N to POM and MOM beneath the labeled trees. Furthermore, there was no effect of elevated CO_2 on the amounts of ^{15}N measured in different belowground components. The $\delta^{15}N$ values of necromass and POM were remarkably different indicating that our distinction between these two pools of surface soil organic matter was methodologically valid. The nitrogen-15 originating from tree roots moved primarily into the more labile components of soil organic matter (SOM) (necromass and POM) with less transfer to more stable MOM (Table 1). The calculated turnover times

supported the hypothesis that MOM nitrogen was more stable than POM nitrogen.

The nitrogen concentrations measured in POM and MOM were significantly greater under elevated CO_2 (Fig. 3), suggesting that increased belowground inputs of carbon and nitrogen[4] have partly altered the quality of SOM. The fate of the N that is released upon microbial decomposition of necromass and POM is not altogether clear, especially because there are no apparent effects of elevated CO_2 on measurements of soil N availability.[5,19] Strong ^{15}N retention by the labeled trees would be expected at this site where net annual biomass production is limited by soil N supply.[15] We hypothesize that ^{15}N released upon mineralization of labile forms of SOM is rapidly recycled back into sweetgum trees to supply the higher total N requirement for greater belowground biomass production under elevated CO_2.[2] The calculated mean residence times indicated that ^{15}N cycled through roots two to three times faster than it cycled through POM. The disparity in turnover times between root N and N in labile SOM (POM) could impose some constraints on N cycling at our site, especially if the release of N through decomposition of POM is essential for stand nutrition. Although there was no indication of declining N stores in necromass or POM over the short term and ^{15}N injected into sweetgum trees was preferentially partitioned to labile rather than stable forms of SOM, ^{15}N was transferred from fast cycling (roots) to slow cycling N pools (POM) over the 1.7 year period of our study.

CONCLUSIONS

Over the period of our study, the fate of ^{15}N beneath labeled sweetgum trees did not support a hypothesis of progressive N limitation (strictly defined as a CO_2-induced response) that could arise through the rapid sequestering of N in more stable forms of surface soil organic matter under elevated CO_2. The absence of a difference in belowground ^{15}N dynamics between ambient and elevated CO_2 and the absence of greater association of ^{15}N with more stable forms of soil organic matter (MOM) under elevated CO_2 both indicated that progressive N limitation of net primary production was not at work. Prior studies of aboveground[2] and belowground[5] process level responses to elevated CO_2 are consistent with our ^{15}N tracer study indicating that measurable progressive N limitation is not underway at the ORNL FACE Experiment; however, progressive N limitation may occur over time frames longer than a decade. During our 89 week long study, ^{15}N moved from roots into slower cycling POM. The disparity in turnover times between N in tree roots (16 to 22 weeks) and N in POM (47 weeks) could gradually impose limitations on soil N availability with stand maturation irrespective of atmospheric CO_2, especially if the release of N through decomposition of POM is essential to sustain forest net primary production. Further investigations are needed into the intricacies of N and C cycling in forest ecosystems under changing environmental conditions, and particularly the role of N in limiting forest responses to elevated CO_2. The injection of ^{15}N tracers into trees is a promising approach to such research.

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REFERENCES

1. Norby RJ, Ledford J, Reilly CD, Miller NE, O'Neill EG. *Proc. Natl. Acad. Sci. USA* 2004; **101**: 9689.
2. Norby RJ, Iversen CM. *Ecology* 2006; **87**: 5.
3. Finzi AC, Norby RJ, Calfapietra C, Gallet-Budynek A, Gielen B, Holmes WE, Hoosbeek MR, Iversen CM, Jackson RB, Kubiske ME, Ledford J, Liberloo M, Oren R, Polle A, Pritchard S, Zak DR, Schlesinger WH, Ceulemans R. *Proc. Natl. Acad. Sci. USA* 2007; **104**: 14014.
4. Iversen CM, Ledford J, Norby RJ. *New Phytol.* 2008; **179**: 837.
5. Zak DR, Holmes WE, Finzi AC, Norby RJ, Schlesinger WH. *Ecol. Appl.* 2003; **13**: 1508.
6. Luo Y, Su B, Currie WS, Dukes JS, Finzi A, Hartwig U, Hungate B, McMurtrie RE, Oren R, Parton WJ, Pataki DE, Shaw MR, Zak DR, Field CB. *Bioscience* 2004; **54**: 731.
7. Ainsworth EA, Long SP. *New Phytol.* 2005; **165**: 351.
8. Norby RJ, DeLucia EH, Gielen B, Calfapietra C, Giardina CP, King JS, Ledford J, McCarthy HR, Moore DJP, Ceulemans R, DeAngelis P, Finzi AC, Karnosky DF, Kubiske ME, Lukac M, Pregitzer KS, Scarascia-Mugnozza GE, Schlesinger WH, Oren R. *Proc. Nat. Acad. Sci. USA* 2005; **102**: 18052.
9. Witherspoon JP Jr. *Ecol. Monogr.* 1964; **34**: 403.
10. Garten CT Jr. *Oecologia* 1988; **76**: 43.
11. Horwath WR, Paul EA, Pregitzer KS. *Soil Sci. Soc. Am. J.* 1992; **56**: 316.
12. Bird MI, Chivas AR, Head J. *Nature* 1996; **381**: 143.
13. Garten CT Jr, Ashwood TL. *Global Biogeochem. Cycles* 2002; **16**: Article No. 1114.
14. Norby RJ, Wullschleger SD, Hanson PJ, Gunderson CA, Tschaplinski TJ, Jastrow JD. In *Managed Ecosystems and CO_2 Case Studies, Processes, and Perspectives*, Nosberger J, Long SP, Norby RJ, Stitt M, Hendrey GR, Blum H (eds). Springer-Verlag: Berlin, 2006; 231.
15. Iversen CM, Norby RJ. *Can. J. Forest. Res.* 2008; **38**: 1021.
16. Cambardella CA, Elliott EA. *Soil Sci. Soc. Am. J.* 1992; **56**: 777.
17. Phillips DL, Gregg JW. *Oecologia* 2001; **127**: 171.
18. Gill RA, Jackson RB. *New Phytol.* 2000; **147**: 13.
19. Johnson DW, Cheng W, Joslin JD, Norby RJ, Edwards NT, Todd DE Jr. *Biogeochemistry* 2004; **69**: 379.