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Plant litter chemistry and mycorrhizal roots promote a nitrogen feedback in a temperate forest

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Summary

1. Relationships between mycorrhizal plants and soil nitrogen (N) have led to the speculation that the chemistry of plant litter and the saprotrophy of mycorrhizal symbionts can function together to closely couple the N cycle between plants and soils. We hypothesized that a tannin-rich, ericoid mycorrhizal (ERM) plant promotes the retention of protein–tannin N in soil, and that this N source is accessible to saprotrophic ERM symbionts and their hosts, but remains less available to co-occurring ectomycorrhizal (ECM) and arbuscular mycorrhizal (AM) symbionts and their hosts.

2. We tested this feedback hypothesis in a southern Appalachian forest community composed of two microsites: a hardwood microsite with ECM and AM trees in the overstorey and understorey, and an AM herb layer; and a rhododendron microsite where the understorey and herb layer are replaced by ERM rhododendron. We synthesized ^{15}N-enriched protein–tannin complexes from leaf litter extracts representing each forest microsite and examined the fate of ^{15}N in soil volumes 3 months and 1 year after the complexes were placed in the field.

3. Protein–tannin complexes derived from the rhododendron microsite led to a higher retention of ^{15}N in soil organic matter and a lower recovery in dissolved N pools than those from the hardwood microsite, supporting the hypothesis that rhododendron tannins create stable complexes that increase organic N retention in soils.

4. Rhododendron complexes led to greater ^{15}N-enrichment in ERM roots than in AM and ECM roots, supporting the hypothesis that rhododendron can better access the N complexed by its own litter tannins than can co-occurring forest trees and plants. Our results suggest that both fungal saprotrophy and a high specific root length contribute to the ability of ERM roots to acquire N from complex organic sources.

5. *Synthesis.* This study provides evidence of an intricate N feedback where plant litter chemistry influences the cycle of N to maximize N acquisition by the host's mycorrhizal roots, while hindering N acquisition by mycorrhizal roots of co-occurring plants. Feedback processes such as these have the potential to drive patterns in nitrogen cycling and productivity in many terrestrial ecosystems.

Key-words: mycorrhiza, niche partitioning, nitrogen cycle, polyphenol, protein–tannin complex, rhododendron, saprotrophy

Introduction

In terrestrial ecosystems, relationships between plants and soils contribute to the regulation of plant community and biogeochemical processes (Wardle *et al*. 2004). Plants shape the soil environment and patterns of nitrogen (N) cycling through the quality of their litter, and in turn, interactions with soil microorganisms can influence plant acquisition of N, creating the potential for feedbacks between plants and soils (Ehrenfeld *et al*. 2005). For example, the acquisition of N by mycorrhizal fungi can enhance plant productivity, which perpetuates the influence of a plant on soil processes through litter inputs, and close a positive feedback loop between a plant and its soil environment. Speculation about the importance of plant–soil feedbacks leads us to two fundamental questions. First, does a plant's litter quality influence soil N dynamics to favour N acquisition by the plant's mycorrhizal symbionts? Second, do the patterns in soil N promoted by the litter chemistry of one plant hinder the ability of other plants and their symbionts to acquire N?

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These questions represent an intricate yet critical component of the N cycle that remains poorly understood.

A striking relationship emerges across biomes between the mycorrhizal association type of dominant plant species and the organic matter content of soils. Biomes characterized by ericoid mycorrhizal (ERM) and ectomycorrhizal (ECM) hosts contain organic rich soils and low N availability, while those characterized by arbuscular mycorrhizal (AM) hosts have soils with little organic content and high N availability (Read 1991). Soil characteristics across biomes could, in part, reflect the attributes of the dominant plants since relative growth rates, foliar nutrient content and litter decomposability all decrease along the gradient of AM to ECM to ERM association types (Cornelissen *et al*. 2001). If plants influence soil N dynamics through the quality of their litter, however, it remains unclear if the plants themselves are favoured by their influence on the soil environment. Furthermore, since mixtures of mycorrhizal host types often co-exist in communities within individual biomes, the question remains if similar relationships between mycorrhizal hosts, litter quality and soil N cycling occur at smaller scales within plant communities. A scenario like this provides a means for niche partitioning among mycorrhizal types within plant communities.

There is evidence that mycorrhizal fungi facilitate the partitioning of resources, such as N, which helps maintain diversity in plant communities (McKane *et al*. 2002; Reynolds *et al*. 2002). The partitioning of N among mycorrhizal types is plausible in mixed host communities, as AM, ECM and ERM roots can acquire a diversity of dissolved N forms (e.g. inorganic N and amino acids) from soil solution (Lipson & Näsholm 2001). However, N partitioning among plants is not limited to the process of N uptake. Some mycorrhizal fungi contribute to the decomposition processes that control the release and abundance of N forms, and they contrast greatly in their ability to do so. A compilation of data from pure-culture studies shows that the ability of fungal symbionts to degrade complex organic substrates follows the pattern: AM < ECM < ERM (Read & Perez-Moreno 2003 and references therein). The potential for a plant's fungal symbionts to promote the availability of N derived from organic matter, regardless of the form of N ultimately acquired by the plant, creates another dimension in the partitioning of N in plant communities. Furthermore, if a host plant supports the formation of organic matter in soils (e.g. ERM hosts), and if it associates with mycorrhizal symbionts that specialize in acquiring N from these sources (e.g. ERM fungi), it can more closely couple the cycle of N between itself and the soil environment (Northup *et al*. 1998). For ERM plants, litter chemistry and mycorrhizal saprotrophy may represent a competitive strategy to ensure N acquisition for the host plant itself while limiting N acquisition for AM and ECM plants: a plant–soil feedback hypothesis.

The influence of ERM–host–shrubs on soil properties in temperate and boreal forests is well-documented (Mallik 2003). Ericaceous shrubs produce leaf and root litter rich in polyphenolic compounds (e.g. tannins) (Gallet & Lebreton 1995; Preston 1999). These compounds can bind with organic N (i.e. protein–tannin complexes), enhancing the accumulation of organic matter and reducing decomposition and N mineralization rates in soils (see review, Kraus *et al*. 2003). Despite the influence of ericaceous shrubs on soil N cycling, and biome-level patterns between ERM hosts and soils of poor N availability it is still not clear if ERM plants can actively acquire N from the tannin complexes that accumulate in the soils they grow in. In fact, the only previous test of this hypothesis at the plant-level was negative. In a greenhouse study, ^{15}N from protein–tannin complexes was acquired at similar rates by an AM tree, an ECM tree and an ERM shrub (Bennett & Prescott 2004). To our knowledge, however, no experiments have directly tested this hypothesis with mature plants *in situ*.

Here we examine a model plant community comprised of a mixture of mycorrhizal host plants in the southern Appalachians. We test for the potential of a N-feedback in a mature forest characterized by two dominant microsites: (i) hardwood microsites with an ECM and AM hardwood overstorey and understorey, and an AM herb layer, and (ii) rhododendron microsites where understorey trees and herbs are replaced by thickets of ERM rhododendron. We propose that a N feedback in this system should have the following characteristics. First, rhododendron litter tannins should promote greater retention of N in soil organic matter (SOM) than those from hardwood trees, leading to an accumulation of complex organic N while simultaneously decreasing the abundance of inorganic N in these systems. Second, ERM roots of rhododendron should have the greatest access to the N in complex organic substrates, followed by ECM roots with moderate access and AM roots with the least access to this N source. To test this hypothesis we conducted a reciprocal-placement tracer study using ^{15}N-labeled protein–tannin complexes where the tannins were derived from the plant litter of each forest microsite. Our first objective was to quantify the retention of protein–tannin–N in SOM. We predicted that complexes derived from rhododendron litter tannins would have greater N retention than those derived from hardwood tannins. In addition, we expected that less N from rhododendron complexes would become associated with dissolved N pools, microbial biomass and mycorrhizal roots compared to the N in hardwood complexes. Our second objective was to compare ^{15}N-enrichment among mycorrhizal root types. We predicted that root enrichment would follow the pattern AM < ECM < ERM, reflecting the relative ability of each symbiont to contribute to decomposition and acquire N from protein–tannin complexes.

Methods

STUDY LOCATION

Research was conducted along the boundary of the Coweeta Hydrologic Laboratory and the Nantahala National Forest in western North Carolina, USA. Across an approximately 1 km transect at 1450 m, traversing the NW-facing slope of Big Butt, we established four blocks of paired 4 × 4 m plots in hardwood forests with and without a rhododendron (*Rhododendron maximum* L.) understorey (rhododendron and hardwood microsites, respectively). These forests are mature; all

large stems (> 38 cm) were harvested from the Coweeta basin between 1919 and 1923 (Elliot *et al*. 1999). ECM hosts *Quercus rubra* L. and *Betula alleghaniensis* Britt., and AM host *Acer rubrum* L. are dominants in the overstorey. The understorey in the hardwood microsite is characterized by AM hosts *Acer pennsylvanicum* L. and *Amelanchier arborea* (Michaux f.) Fernald, and ECM host *Castanea dentata* (Marshall) Borkh. Annual herbs, ferns and sedges (AM and non-mycorrhizal, NM) are also present in the hardwood microsite. Rhododendron is the only ERM host present in the rhododendron microsite and is abundant throughout the Coweeta basin (basal area of 0.29 m^2 ha^{-1} and 1088 stems per ha; Elliot *et al*. 1999). Soils are Incepitsols formed in the residuum of igneous and metamorphic rock. Average annual temperature is 9.4 °C with an average of 250 cm of precipitation (Swift *et al*. 1988).

PROTEIN—TANNIN COMPLEX TREATMENTS

We reciprocally added protein–tannin complexes to the two forest microsites in the form of synthesized complexes from leaf litter tannins and a common protein source. In our previous work, leaf litter mass, species composition and the protein-precipitation capacity of leaf litter differed by forest microsite (Wurzburger & Hendrick 2007). Therefore, to determine the functional differences in tannin composition between forest microsites, we extracted tannins from plant litter mixtures, rather than from individual species. We combined extracted soluble leaf litter tannins from the two microsites with either ^{15}N-enriched or non-enriched protein, resulting in four treatments: ^{15}N-protein + hardwood tannin, ^{15}N-protein + rhododendron tannin, ^{14}N-protein + hardwood tannin, and ^{14}N-protein + rhododendron tannin (the latter two are control treatments for the previous two).

To isolate tannins, we collected leaf litter from the four sampling blocks in fall, homogenized the litter of each microsite, and dried and ball-mill ground the litter into a fine powder. Tannins were extracted from the ground tissues at room temperature with 50% methanol (0.1 g tissue per mL methanol) for 1 h. This method was chosen to extract the relatively soluble hydrolysable and condensed tannin component from leaf litter containing a diversity of tannins. The supernatant was separated from tissue remnants by centrifugation and decanting. ^{15}N-enriched and non-enriched protein sources were created from the biomass of *Ustilago maydis* (Persoon) Roussel. Fungal cultures grew in 2% glucose and salt solution of modified Melin-Norkrans media (Marx 1969) with 0.5% N applied as either $(^{15}NH_4)_2SO_4$ or $(^{14}NH_4)_2SO_4$, pH 7.0, at 29 °C and shaken at 150 r.p.m. After 48 h of growth, fungal biomass was centrifuged, rinsed with deionized water, frozen and lyophilized for 24 h. Tissues were ground and extracted for protein with Tris–Glycine buffer (25 mM Tris base, 250 mM glycine, 1% SDS) (approximately 500 mg tissue per mL buffer). Samples were centrifuged at 9,000 × *g* for 40 min at 4 °C. The protein extracts were mixed with tannin extracts at a 1 : 1 ratio, acidified with drops of 1 M HCl to induce precipitation (approximately pH = 5), and stored at 4 °C for 24 h. Precipitates were centrifuged, rinsed with distilled water, frozen at –20 °C and lyophilized. The four protein–tannin complex treatments all contained 52% C, the hardwood complex contained 6.1% N, and the rhododendron complex contained 5.2% N. Atom percentage (%) enrichment (APE) of the hardwood and rhododendron complexes were as follows: non-enriched 0.39% and 0.38%; enriched 32.7% and 43.2%, respectively.

APPLICATION OF TREATMENTS

The four treatments were applied twice in each plot across the four blocks. Eight PVC rings (height 1 cm, area 0.00204 m^2) were randomly positioned in each of the eight plots, pinned into place with wire at the surface of the Oe horizon. The PVC rings allowed for guidance in the application of the tracer and subsequent soil retrieval, but did not disturb the soil or restrict the growth of roots or fungal hyphae and the movement of the tracer. In early August 2005, protein–tannin complexes were ground, homogenized, suspended in deionized water and injected with 10 mL H_2O with a syringe and needle into the top 0–5 cm of soil within the PVC ring. An additional 5 mL of deionized H_2O was used to rinse the inside of the syringe and the remaining solution applied directly to the soil surface. For all treatments N was applied in the form of protein–tannin complexes at a rate of 0.24 g N m^{-2} which is approximately half the estimated annual N input resulting from rhododendron leaf litter in the rhododendron microsite (Wurzburger & Hendrick 2007).

SOIL SAMPLING AND EXTRACTIONS

Soils were harvested 11 weeks (late October) and 13 months (early September) after tracer application; harvest times are hereafter referred to as 3 months and 1 year. We chose late-summer and fall for sample collection because it is a period of high fine root production in these forests (Davis *et al*. 2004), which means that N acquired by roots would be more likely to remain associated with those growing tissues than be allocated elsewhere in the plant. Soils were sampled with a stainless-steel cylinder of the same diameter as the PVC ring to a depth of 10 cm. Soil cores were placed on ice, transported to the laboratory and immediately processed. Soil cores were sieved (4 mm) and further sorted by hand to separate roots from soil. Approximately 10–20 g fresh mass of root-free soil was extracted with 80 mL 0.5 M K_2SO_4 for measures of dissolved N. An additional 5–10 g fresh mass of soil was extracted with 40 mL K_2SO_4 and 0.5 mL $CHCl_3$ for extraction of labile microbial N (LMN) (Fierer & Schimel 2003). Briefly, samples (and appropriate controls) were shaken at 150 rpm at room temperature for 3 h, and gravity filtered with Whatman No. 1 filters (Whatman, Maidstone, UK) and extracts were bubbled with house air to remove any remaining $CHCl_3$. From the first set of extractions, inorganic N (NH_4^+ and NO_3^+) was measured using a continuous flow colorimetric assay (Technicon AutoAnalyzer). Total dissolved N (TDN) was determined via persulfate digestion (Cabrera & Beare 1993) from the first sets of extracts, while microbial TDN (TDNm) was determined from the second set of extracts. Dissolved organic N (DON) was calculated through subtraction of DIN from TDN (DON = TDN – DIN), and LMN determined from subtraction of TDN from TDNm (LMN = TDNm – TDN). Remaining soils were dried at 60 °C, weighed to determine moisture content, ground and measured for % carbon (C) and N and N isotopes.

The ^{15}N content of soil N pools (DIN, TDN and TDNm) were analysed from the 3-month sampling only. N forms were diffused into pairs of glass filter disks (Whatman GF/D, previously baked in muffle furnace at 500 °C for 4 h), acidified with 35 µL 2 M H_2SO_4 and wrapped in Teflon tape (Stark & Hart 1996). For DIN extracts, KCl was added (to reach 1.5 M) to raise the ionic strength of the solution, along with MgO and Devarda's alloy (–100 mesh, Sigma-Aldrich, Milwaukee, WI). For persulfate digests, 270 µL 10 M NaOH was added to raise the pH > 13. Blank samples were used to account for any background contamination of N for all soil extractions and N diffusions. Filter packets were dried for at least 48 h in a desiccator with concentrated H_2SO_4, then wrapped in silver capsules (Costech Analytical Technologies Inc., Valencia, CA) and analysed for total N and ^{15}N.

MYCORRHIZAL ROOT SAMPLING

Mycorrhizal roots (≤0.5 mm diameter) from each soil core were sorted from soil and organic matter and categorized by mycorrhizal type. Roots were sorted by AM, ECM and ERM root categories based upon anatomical characters of the host plant roots and mycorrhizal type under a dissecting microscope. The hardwood microsite contained ECM and AM hosts and uncommon NM hosts (*Carex* spp.), whereas the rhododendron microsite contained ERM, ECM and AM hosts. ECM roots (0.2–0.3 mm diameter) were distinguished from other roots by their root branching patterns and the presence of fungal mantles. ERM hair roots of *R. maximum* roots were distinguished based upon their root diameter (0.1 mm), a subsample was verified under magnification to contain hyphal coils within cortical root cells. AM roots of *Acer* spp. and herbs (0.3–0.5 mm) were easily distinguished from other mycorrhizal roots, and subsamples were inspected for arbuscules (Wurzburger & Bledsoe 2001). All mycorrhizal types exhibited substantial fungal colonization. Since *Carex* spp. were rare, their roots were combined with AM root samples. All roots were washed in deionized H_2O, brushed to remove adhering organic matter, soaked in 0.5 mM $CaCl_2$ to remove apoplastic or externally bound ^{15}N (Näsholm *et al.* 1998) and then rinsed again in H_2O. Roots were dried, weighed, ground to a fine powder and analysed for % N and ^{15}N.

^{15}N ANALYSIS AND CALCULATIONS

C, N and ^{15}N were analysed from ground soil and mycorrhizal roots and glass filters with an isotope ratio mass spectrometer at the Stable Isotope Facility at the Odum School of Ecology, University of Georgia. For all enriched samples, APE was calculated with eqn 1, with pairs of enriched and non-enriched samples where S indicates the enriched sample and C is the control sample.

$$\mathrm{APE} = [(^{15}\mathrm{N\ atoms/total\ N\ atoms})_S - (^{15}\mathrm{N\ atoms/total\ N\ atoms})_C] \times 100. \quad \text{eqn 1}$$

Percent recovery of ^{15}N involved multiplying the APE of a pool by the total N of the pool, and then accounting for the portion of undetected ^{14}N associated with the applied treatments from their original atom %. The APE of DIN, TDN and TDNm were determined directly, while the APE of DON and LMN were calculated from eqns 2 and 3, where AP is the atom % of ^{15}N and C is the concentration of N.

$$\mathrm{APE_{DON}} = \frac{(\mathrm{AP_{TDN}} * \mathrm{C_{TDN}}) - (\mathrm{AP_{DIN}} * \mathrm{C_{DIN}})}{\mathrm{C_{DON}}} \quad \text{eqn 2}$$

$$\mathrm{APE_{LMN}} = \frac{(\mathrm{AP_{TDNm}} * \mathrm{C_{TDNm}}) - (\mathrm{AP_{TDN}} * \mathrm{C_{TDN}})}{\mathrm{C_{LMN}}} \quad \text{eqn 3}$$

STATISTICAL ANALYSES

All data were tested for normality and transformed when necessary. We used split-plot or split–split-plot ANOVAs using SAS software (SAS Institute, Cary, NC) to analyse data. Block ($n = 4$) was a random effect and incorporated into each model. Fixed effects included time ($n = 2$), microsite ($n = 2$), complex type ($n = 2$) and mycorrhizal root type ($n = 3$). Specifically, a split-plot repeated measures design was used for soil N pools (% N, DIN, DON and LMN) to examine the effects of time and microsite on N pool size. A split–split-plot repeated measures design was used to analyse root biomass and root % N by microsite (whole-plot factor) and mycorrhizal root type (split-plot factor). Total ^{15}N soil and total root ^{15}N recovery were analysed with a split-plot ANOVA by microsite (whole-plot factor) and complex type (split-plot factor). To analyse root ^{15}N-enrichment (APE) we used a split-plot design, with complex type as the whole plot factor and mycorrhizal root type the split-plot factor. ^{15}N recovery was also analysed with a split-plot ANOVA by microsite (whole plot factor) and LMN and mycorrhizal roots (split-plot factor). For all analyses, we performed a *post hoc* means separation technique to determine significant differences ($\alpha < 0.05$) among all factor combinations.

Results

SOIL POOLS AND CONCENTRATIONS

In the rhododendron microsite, mean values of soil % N were greater (but not significant) than in the hardwood microsite (Table 1). Soil extracted DIN and DON pools were not different between microsites, but LMN was nearly twofold greater in the rhododendron microsite than in the hardwood microsite ($F_{1,12} = 14.63$, $P = 0.002$) (Table 1). Total mycorrhizal root biomass was also nearly twofold greater in the rhododendron than in the hardwood microsite. In the rhododendron microsite, root biomass was significantly different among mycorrhizal root types ($F_{2,15} = 16.36$, $P = 0.0002$); both ERM and ECM root biomass was greater than AM root biomass. In the hardwood microsite, there was no difference in the biomass of ECM and AM roots. Mycorrhizal root % N differed by root type in both microsites. In the hardwood microsite, ECM % N was greater than AM ($F_{1,9} = 5.80$, $P = 0.04$), and in the rhododendron microsite, ECM and AM root % N was greater than that of ERM ($F_{2,15} = 19.86$, $P < 0.0001$) (Table 1).

^{15}N RECOVERY IN SOILS

We found support for our first prediction that the application of the rhododendron protein–tannin complex would lead to greater N retention in SOM than would the hardwood complex over the course of the study. Forty four percent more ^{15}N was retained in SOM from rhododendron complexes than from hardwood complexes in both microsites ($F_{1,15} = 22.32$, $P < 0.001$) (Fig. 1). The amount of ^{15}N recovered in SOM did not differ between the 3-month and 1-year sampling. Although SOM retention values were greater in the hardwood than in the rhododendron microsite, the difference was not significant.

We expected that the retention of ^{15}N in SOM would be inversely related to recovery of ^{15}N in other labile and biologically active components of soil N at the 3-month sampling. We predicted that ^{15}N recovery among dissolved N pools, microbial biomass and mycorrhizal roots would be greater for the hardwood than the rhododendron complex, reflecting the difference in decomposability of the substrates. In support of this hypothesis, in the hardwood microsite, ^{15}N recovery from hardwood complexes was at least twice as great as that from the rhododendron complex for DIN, LMN and DON. For example, ^{15}N recovery in DIN was related to complex type ($F_{1,9} = 7.51$, $P = 0.02$). Microsite was also a

Table 1. Soil N pools, mycorrhizal root biomass and % N in hardwood and rhododendron forest microsites. Values are means and (standard errors) from $n = 4$ blocks of paired sampling plots, combined from the 3-month and 1-year sampling dates. Letters denote significant differences ($\alpha < 0.05$) between forest microsites for soil N variables, and among mycorrhizal root types and microsites for root biomass and root % N. Soil N pools are dissolved inorganic N (DIN), dissolved organic N (DON), and labile microbial N (LMN), and mycorrhizal types include arbuscular mycorrhiza (AM), ectomycorrhiza (ECM) and ericoid mycorrhiza (ERM)

	Hardwood	Rhododendron
Soil N		
Total N (%)	0.55 (0.05) a	0.73 (0.09) a
DIN (mg kg^{-1})	13.6 (+0.94, –0.88) a	16.8 (+2.0, –1.8) a
DON (mg kg^{-1})	52.9 (+2.5, –2.4) a	54.0 (+3.0, –2.9) a
LMN (mg kg^{-1})	44.7 (3.4) b	85.8 (11) a
Mycorrhizal root biomass (g m^{-2})		
AM	24.0 (+6.6, –5.1) a	13.7 (+3.7, –2.9) b
ECM	33.6 (+9.7, –7.5) a	51.4 (+9.9, –8.3) a
ERM		42.4 (+8.9, –7.4) a
Mycorrhizal root N (%)		
AM	1.40 (+0.059, –0.058) a	1.53 (+0.041, –0.041) a
ECM	1.56 (+0.064, –0.063) b	1.63 (+0.060, –0.059) a
ERM		1.34 (+0.033, –0.033) b

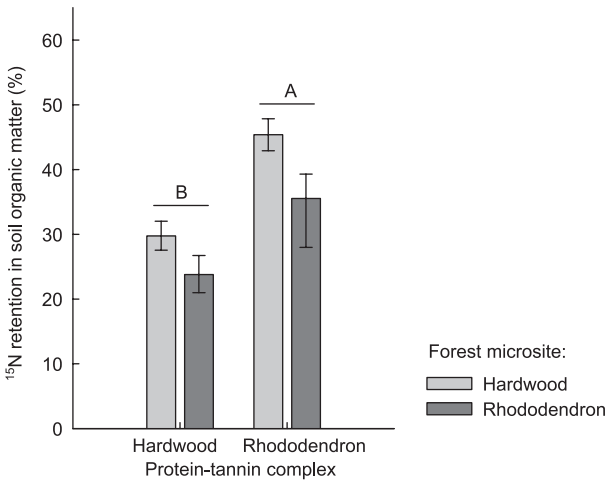

Fig. 1. Percentage retention of ^{15}N in soil organic matter from hardwood and rhododendron protein–tannin complexes applied to soils in hardwood and rhododendron forest microsites. Mean values ± standard errors from $n = 4$ blocks of paired sampling plots combined from the 3-month and 1-year sampling dates. Significant differences ($\alpha < 0.05$) between complex types are indicated by different capital letters.

significant factor with ^{15}N recovery in DIN ($F_{1,9} = 11.47$, $P = 0.01$), and we observed greater recovery in the hardwood than in the rhododendron microsite (Fig. 2a). Recovery of ^{15}N in LMN followed a similar pattern to DIN, (a complex type by microsite interaction; $F_{1,6} = 8.48$, $P = 0.03$) with greater ^{15}N recovery from hardwood than rhododendron complexes, but only in the hardwood microsite (Fig. 2b). The recovery of ^{15}N in the DON pool was not significantly related to either forest microsite or complex type. However, mean values of ^{15}N recovery in DON were greatest with the hardwood complex in the hardwood microsite, mirroring the trend with DIN and LMN (Fig. 2c). ^{15}N recovery in total mycorrhizal root biomass was related to forest microsite ($F_{1,6} = 12.98$, $P = 0.01$), and we observed nearly 300% greater root recovery in the rhododendron microsite than in the hardwood microsite (Fig. 2d).

^{15}N-ENRICHMENT OF MYCORRHIZAL ROOTS

We predicted that root ^{15}N-enrichment would be greater in soils receiving hardwood vs. rhododendron complexes, reflecting the difference in bioavailability between the substrates. We also predicted that enrichment levels among mycorrhizal root types would follow the pattern: AM < ECM < ERM, reflecting the saprotrophic abilities of the fungal symbionts. In the hardwood microsite, ECM and AM root enrichment was greater with hardwood than with rhododendron complexes ($F_{1,3} = 14.33$, $P = 0.03$). Although ECM roots were generally more enriched than AM roots, the difference was not significant (Fig. 3a). In the rhododendron microsite, root enrichment varied among mycorrhizal root type ($F_{2,27} = 5.79$, $P = 0.01$); ERM roots were twice as enriched than AM roots when treated with the hardwood complex, and ERM roots were twice as enriched than both ECM and AM roots when treated with the rhododendron complex (Fig. 3b). Time was a significant factor in root enrichment in the rhododendron microsite ($F_{1,3} = 23.05$, $P = 0.02$), as enrichment declined by nearly one half between the 3-month to the 1-year sampling. However, the pattern of enrichment among mycorrhizal root types remained consistent for both sampling periods.

Discussion

Our results provide evidence that an ERM plant can enhance soil N retention through the composition of its litter tannins, supporting the first part of our feedback hypothesis. In our previous work, tannin extracts from rhododendron foliage, leaf litter and roots were more astringent than those from hardwood species (Wurzburger & Hendrick 2007). Therefore, not only do rhododendron litter tannin extracts have a strong tendency to complex with organic N, but the resulting

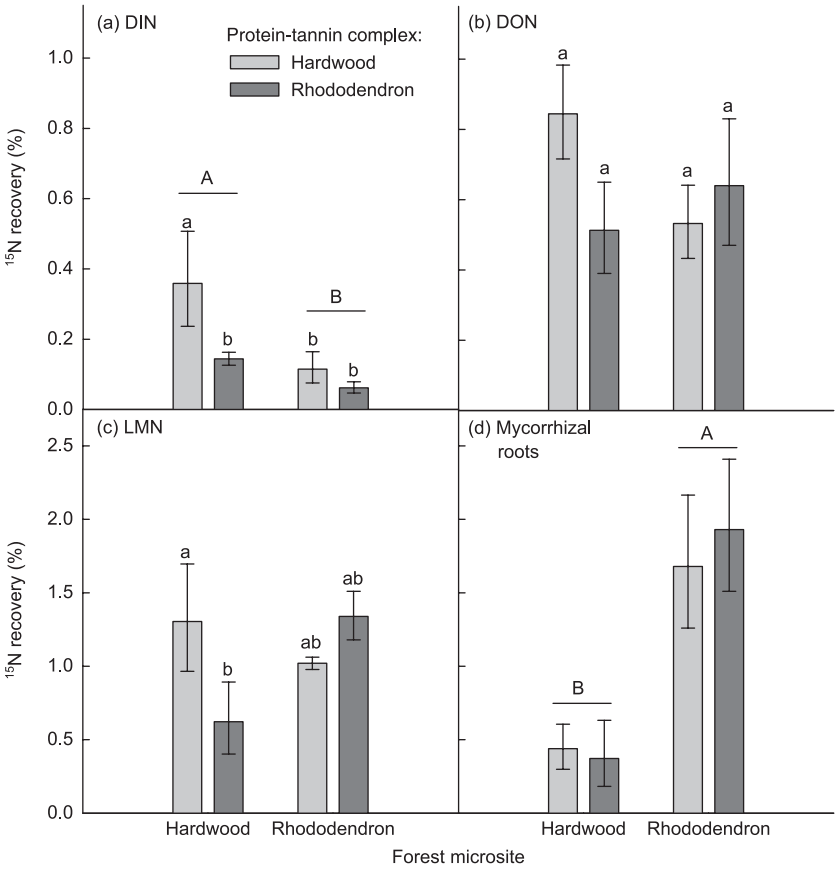

Fig. 2. Recovery of ^{15}N in soil N pools after hardwood and rhododendron protein–tannin complexes were applied to soils in hardwood and rhododendron forest microsites. Soil pools include: (a) dissolved inorganic N (DIN), (b) dissolved organic N (DON), (c) labile microbial N (LMN) and (d) mycorrhizal root N. Values are means ± standard errors from $n = 4$ blocks of paired sampling plots from the 3-month sampling. Significant differences ($\alpha < 0.05$) within an individual N pool between complex types denoted by different lowercase letters and between microsites denoted by different uppercase letters.

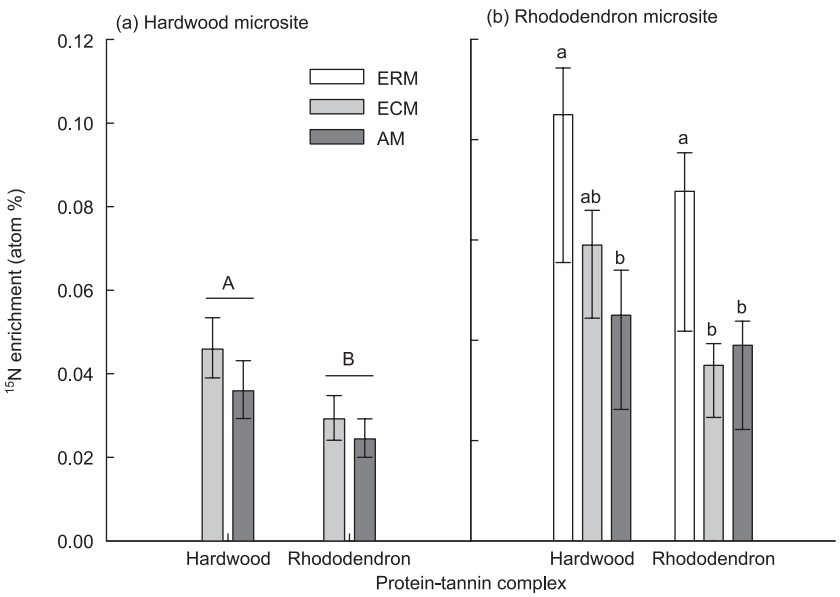

Fig. 3. Mycorrhizal root ^{15}N-enrichment (atom %) after hardwood and rhododendron protein–tannin complexes were applied to soils in: (a) hardwood and (b) rhododendron forest microsites. Mycorrhiza types include arbuscular mycorrhiza (AM), ectomycorrhiza (ECM) and ericoid mycorrhiza (ERM). Values are means ± standard errors from $n = 4$ blocks of paired sampling plots combined from the 3-month and 1-year sampling dates. Significant differences ($\alpha < 0.05$) in enrichment among mycorrhizal root types are denoted by different lowercase letters, and significant differences ($\alpha < 0.05$) in enrichment between complex types are denoted by different uppercase letters.

complexes are well-retained in organic matter. The recovery of ^{15}N in dissolved pools, microbial biomass and roots bolster our conclusions about ^{15}N retention in SOM and reveal striking patterns in microbial and root functioning. When hardwood complexes were applied in the hardwood microsite, we observed a greater recovery of ^{15}N in dissolved N pools and microbial biomass than was observed with rhododendron complexes, supporting our assertion that the N in hardwood complexes is more bioavailable for soil microorganisms than is the N in rhododendron complexes.

Our most dramatic result was the approximately 300% greater recovery of ^{15}N in roots from the rhododendron microsite vs. the hardwood microsite. Because root biomass was 87% greater in the rhododendron than in the hardwood

microsite, the remaining disparity in recovery is explained by the composition of mycorrhizal root types and variations in enrichment among these root types. ERM roots were only present in the rhododendron microsite, where they accounted for approximately 40% of root biomass and were generally more enriched in ^{15}N than were AM and ECM roots. These results support a critical part of the feedback hypothesis, and suggest that ERM roots of rhododendron have a greater ability to capture the N complexed by its own litter tannins than do co-occurring AM and ECM roots of hardwood trees and herbs. Despite the incomplete recovery of the applied ^{15}N within the experimental soil volumes in our study, root recovery was not correlated with ^{15}N retention in SOM ($r^2 = 0.004$, $P = 0.81$), nor with ^{15}N recovery in dissolved and microbial N pools ($r^2 = 0.04$, $P = 0.47$). Therefore, patterns in root recovery are not an artefact of tracer losses over the experimental period, and more likely depict functional differences among mycorrhizal roots in the acquisition of N.

The results of our work contrast those of a previous study where no difference was detected in ^{15}N-enrichment among ERM, ECM and AM plants incubated with protein–tannin complexes (Bennett & Prescott 2004). The discrepancy between this study and ours might lie in the relatively short-term (20 days) incubation, or the growth of plants in isolation of one another. Since our study was conducted in the field, it is possible that functional differences among mycorrhizal root types are more apparent when the roots of various species co-exist and compete for N sources.

Although our results of root enrichment support an N feedback hypothesis for rhododendron, the precise mechanism for greater N capture by ERM roots is not clear. Greater ^{15}N-enrichment of ERM roots among root types complements the long-standing hypothesis that ERM fungi are highly saprotrophic compared with AM and ECM fungi, allowing their hosts to readily acquire N from organic substrates (Read & Perez-Moreno 2003). However, an alternate hypothesis is that the level of ^{15}N-enrichment in ERM roots observed in our study is simply a reflection of their high specific root length (see Wurzburger & Hendrick 2007), increasing the opportunity for the interception and capture of mobile forms of N. We propose that both mechanisms may be critical and at work in this system. The idea of enhanced saprotrophy by ERM fungal symbionts is supported by greater differentiation in enrichment among mycorrhizal types when they encounter a more recalcitrant substrate. Specifically, ERM roots were more enriched in ^{15}N than were AM roots when exposed to the (more bioavailable) hardwood complexes, and ERM roots were more enriched than both AM and ECM roots when exposed to the (less bioavailable) rhododendron complexes. This pattern suggests that ERM roots actively contribute to the degradation of substrates rather than passively intercepting mobilized N. In support of this idea, the activity of phenol-oxidizing enzymes are elevated in rhododendron soils (Wurzburger & Hendrick 2007), and ERM roots of rhododendron associate with a number of taxonomically diverse fungi (N. Wurzburger, unpublished data). While the contributions these root fungi make to soil saprotrophy is unknown, cultured ascomycetes contain a diversity of laccase gene sequences (N. Wurzburger, unpublished data).

We propose that saprotrophy may not always be an economical investment for mycorrhizal roots and fungi if it is not paired with an effective strategy for nutrient capture. For example, when mycorrhizal fungi produce extracellular enzymes, they can liberate N from complex organic substrates, but the external processing of these substrates does not preclude other microorganisms and co-occurring mycorrhizal roots from acquiring the newly-available forms of N. ERM roots may enhance their ability to capture the N released after enzymatic catabolism with their high specific root length providing a greater root surface area and interception of nutrients per unit biomass compared to most ECM and AM roots. However, a majority of saprotrophic activity and nutrient interception may occur in the extraradical portion of the fungal mycelium, and the combined lengths from roots and hyphae contribute to a plant's soil exploration. ERM roots appear to have substantially less mycelial biomass than do ECM and AM roots (Read 1984), but there is little documentation to substantiate this conclusion. Further investigations are needed to tease apart the complementary contributions of saprotrophy and root (and hyphal) length in the acquisition of N from complex organic substrates in the ERM, ECM and AM symbioses.

We observed a consistent pattern in root enrichment among the mycorrhizal root types between the 3-month and 1-year sampling despite the fact that all values of root enrichment declined over time. A general decline in enrichment could be attributed to fine root turnover; although fine root production peaks in late summer in these forests (Davis *et al*. 2004), the relative differences in root production and turnover among co-occurring ERM, ECM and AM host plants remain unknown. Furthermore, we do not know the precise location of ^{15}N in the mycorrhizal root (root vs. fungal tissues); we suggest, however, that both would contribute to the N budget of the host. A decline in root enrichment over time may also reflect allocation of ^{15}N to other plant tissues, diluting the original concentration of ^{15}N in roots. We expect that ^{15}N was distributed throughout plant tissues; however, we applied ^{15}N at a low dosage (240 mg N m^{-2}) to small areas (0.00204 m^2) of the forest floor. With this level of application, it is unlikely that we could detect the tracer over background levels of ^{15}N in the aboveground tissues of these mature shrubs and trees. In a previous study, root and leaf ^{15}N-enrichment values were positively correlated in trees a year after tracer application (Zak *et al*. 2007), providing evidence that root enrichment is a useful proxy for whole plant acquisition of ^{15}N.

Despite the hypothesized importance of tannins in N cycling (Hättenschwiler & Vitousek 2000) we know little about the abundance and degradation of protein–tannin–N in terrestrial ecosystems. No attempts to quantify the abundance of protein–tannin complexes in soils have been successful (see review, Rillig *et al*. 2007). Evidence from tracer addition experiments suggests that complexes are stable and largely retained in SOM over a 1 year period. Over 90% of recovered ^{15}N was distributed in organic matter in our study. Similarly, in a study

in forest soils of Oregon, over 95% of recovered ^{15}N from protein–tannin complexes was distributed in organic matter (Holub & Lajtha 2004). The high retention of ^{15}N in organic matter and the relatively low recovery of ^{15}N in microbial biomass led the authors to speculate that plants compete poorly for protein–tannin–N. However, roots were excluded from the experimental soil volume, preventing mycorrhizal fungi and other root-associated microorganisms from contributing to decomposition of the substrate. With our 'open-core' system, abiotic and biotic processes were allowed to occur naturally, a trade-off being the average 40% ^{15}N recovery due to losses (e.g. leaching and movement of leaf litter). Despite this, total recovery of ^{15}N differed more between complex types than it did between microsites and the 3-month and 1-year sampling. These results give us confidence that the fraction of ^{15}N retained in the SOM pool is an indicator of the relative stability of each complex type. Furthermore, our results suggest that mycorrhizal fungi and root associated microorganisms contribute to the breakdown of protein–tannin complexes, and that plants can indeed compete for this N source.

In the southern Appalachians, rhododendron has expanded its coverage across the landscape, altering forest community structure and reducing understorey diversity (Baker & Van Lear 1998; Lambers & Clark 2003). Decreased light availability in rhododendron thickets may contribute to the influence of rhododendron on plant community structure (Lei *et al*. 2006), as could the altered patterns of N cycling in these forest microsites (Wurzburger & Hendrick 2007). Here, we provide mechanistic support for an intricate N feedback between rhododendron litter chemistry, soil N dynamics and mycorrhizal fungi. Not only is the N complexed by rhododendron tannins well-retained in SOM, but the ERM roots of rhododendron appear to have better access to this N source than do the roots of other co-occurring mycorrhizal types. It remains unknown how important protein–tannin complexes are as a source of N for forest plants and if the feedback process detailed in this study contributes significantly to plant productivity and community dynamics. However, two additional characteristics support the idea of an operative N feedback in this forest community. First, ERM plants generally have lower productivity and longer lived foliage than do ECM and AM plants (Cornelissen *et al*. 2001), meaning that N acquisition may be disproportionately more important for rhododendron than for hardwood species. Second, although protein–tannin complexes decompose slowly, standing stocks of this N source are likely to be significantly greater in soils under rhododendron than depicted by the levels of ^{15}N addition in this study. This study highlights the need to broaden our consideration for N partitioning in plant communities to include the saprotrophy of mycorrhizal symbionts as a potential mechanism to acquire N, and complex organic substrates as a potential source of N. Feedback processes between plant litter chemistry and mycorrhizal roots and fungi may be highly complex and occur at fine spatial scales, but they have the potential to drive patterns in N cycling and productivity in terrestrial ecosystems.

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References

Baker, T.T. & Van Lear, D.H. (1998) Relations between density of rhododendron thickets and diversity of riparian forests. *Forest Ecology and Management*, **109**, 21–32.

Bennett, J.N. & Prescott, C.E. (2004) Organic and inorganic nitrogen nutrition of western red cedar, western hemlock and salal in mineral-N limited cedar-hemlock forests. *Oecologia*, **141**, 468–476.

Cabrera, M.L. & Beare, M.H. (1993) Alkaline persulfate oxidation for determining total nitrogen in microbial biomass extracts. *Soil Science Society of America Journal*, **57**, 1007–1012.

Cornelissen, J.H.C., Aerts, R., Cerabolini, B., Werger, M.J.A. & van der Heijden, M.G.A. (2001) Carbon cycling traits of plant species are linked with mycorrhizal strategy. *Oecologia*, **129**, 611–619.

Davis, J.P., Haines, B., Coleman, D. & Hendrick, R. (2004) Fine root dynamics along an elevational gradient in the southern Appalachian Mountains, USA. *Forest Ecology and Management*, **187**, 19–34.

Ehrenfeld, J.G., Ravit, B. & Elgersma, K. (2005) Feedback in the plant–soil system. *Annual Reviews of Environment and Resources*, **30**, 75–115.

Elliott, K.J., Vose, J.M., Swank, W.T. & Bolstad, P.V. (1999) Long-term patterns in vegetation-site relationships in a southern Appalachian forest. *Journal of the Torrey Botanical Society*, **126**, 320–334.

Fierer, N. & Schimel, J.P. (2003) A proposed mechanism for the pulse in carbon dioxide production commonly observed following the rapid rewetting of a dry soil. *Soil Science Society of America Journal*, **67**, 798–805.

Gallet, C. & Lebreton, P. (1995) Evolution of phenolic patterns in plants and associated litters and humus of a mountain forest ecosystem. *Soil Biology and Biochemistry*, **27**, 157–165.

Hättenschwiler, S. & Vitousek, P.M. (2000) The role of polyphenols in terrestrial ecosystem nutrient cycling. *Trends in Ecology and Evolution*, **15**, 238–243.

Holub, S.M. & Lajtha, K. (2004) The fate and retention of organic and inorganic 15N-nitrogen in an old-growth forest soil in western Oregon. *Ecosystems*, **7**, 368–380.

Kraus, T.E.C., Dahlgren, R.A. & Zasoski, R.J. (2003) Tannins in nutrient dynamics of forest ecosystems – a review. *Plant and Soil*, **256**, 41–66.

Lambers, J.H.R. & Clark, J.S. (2003) Effects of dispersal, shrubs, and density-dependent mortality on seed and seedling distributions in temperate forests. *Canadian Journal of Forest Research*, **33**, 783–795.

Lei, T.T., Nilsen, E.T. & Semones, S.W. (2006) Light environment under *Rhododendron maximum* thickets and estimated carbon gain of regenerating forest tree seedlings. *Plant Ecology*, **184**, 145–156.

Lipson, D. & Näsholm, T. (2001) The unexpected versatility of plants: organic nitrogen use and availability in terrestrial ecosystems. *Oecologia*, **128**, 305–316.

Mallik, A.U. (2003) Conifer regeneration problems in boreal and temperate forests with ericaceous understories: role of disturbance, seedbed limitation, and keystone species change. *Critical Reviews in Plant Sciences*, **22**, 341–366.

Marx, D.H. (1969) The influence of ectotrophic mycorrhizal fungi on the resistance of pine roots to pathogenic infection. I Antagonsim of mycorrhizal fungi to root pathogenic fungi and soil bacteria. *Phytopathology*, **59**, 153–163.

McKane, R.B., Johnson, R.C., Shaver, G.R., Nadelhoffer, K.J., Rastetter, E.B., Fry, B., Giblin, A.E., Kielland, K., Kwiatkowski, B.L., Laundre, J.A. & Murray, G. (2002) Resource-based niches provide a basis for plant species diversity and dominance in arctic tundra. *Nature*, **415**, 68–71.

Näsholm, T., Ekblad, A., Nordin, A., Giesler, R., Högberg, M.N. & Högberg, P. (1998) Boreal forest plants take up organic nitrogen. *Nature*, **392**, 914–916.

Northup, R.R., Dahlgren, R.A. & McColl, J.G. (1998) Polyphenols as regulators of plant–litter–soil interactions in northern California's pygmy forest: a positive feedback? *Biogeochemistry*, **42**, 189–220.

Preston, C.M. (1999) Condensed tannins of salal (*Gaultheria shallon* Pursh): a contribution factor to seedling 'growth check' on northern Vancouver island?

Plant Polyphenols 2, Chemistry, Biology, Pharmacology, Ecology (eds G.G. Gross, R.W. Hemingway, T. Yoshida, S.J.Branham), pp. 825–841. Kluwer Academic, New York.

Read, D.J. & Perez-Moreno, J. (2003) Mycorrhizas and nutrient cycling in ecosystems – a journey towards relevance? *New Phytologist*, **157**, 475–492.

Read, D.J. (1984) The structure and function of the vegetative mycelium of mycorrhizal roots. *The Ecology and Physiology of the Fungal Mycelium British Mycological Society Symposium* (eds D.H. Jennings & A.D.M Rayner), vol. 8, pp. 215–240. Cambridge University Press, Cambridge.

Read, D.J. (1991) Mycorrhizas in ecosystems. *Experientia*, **47**, 376–391.

Reynolds, H.L., Packer, A., Bever, J.D. & Clay, K. (2002) Grassroots ecology: plant–microbe–soil interactions as drivers of plant community structure and dynamics. *Ecology*, **84**, 2281–2291.

Rillig, M.C., Caldwell, B.A., Wosten, H.A.B. & Sollins, P. (2007) Role of proteins in soil carbon and nitrogen storage: controls on persistence. *Biogeochemistry*, **85**, 25–44.

Stark, J.M. & Hart, S.C. (1996) Diffusion technique for preparing salt solutions, Kjeldahl digests, and persulfate digests for Nitrogen-15 analysis. *Soil Science Society of America Journal*, **60**, 1848–1855.

Swift, L.W., Cunningham, G.B., & Douglass, J.E. (1988) Climate and hydrology. *Ecological Studies, Vol. 66: Forest Hydrology and Ecology at Coweeta* (eds. W.T. Swank & D.A. Crossley), pp. 35–55. Springer-Verlag, New York.

Wardle, D.A., Bardgett, R.D., Klironomos, J.N., Setälä, H., van der Putten, W.H. & Wall, D.H. (2004) Ecological linkages between aboveground and belowground biota. *Science*, **304**, 1629–1633.

Wurzburger, N. & Bledsoe, C.S. (2001) Comparison of ericoid and ectomycorrhizal colonization and ectomycorrhizal morphotypes in mixed conifer and pygmy forests on the northern California coast. *Canadian Journal of Botany*, **79**, 1202–1210.

Wurzburger, N. & Hendrick, R.L. (2007) Rhododendron thickets alter N cycling and soil extracellular enzyme activities in southern Appalachian hardwood forests. *Pedobiologia*, **50**, 563–576.

Zak, D.R., Holmes, W.E. & Pregitzer, K.S. (2007) Atmospheric CO_2 and O_3 alter the flow of ^{15}N in developing forest ecosystems. *Ecology*, **88**: 2630–2639.

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