Title

Root-induced changes in nutrient cycling in forests depend on exudation rates

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R.P.P. and H.J.Y. conceived the idea for all experiments; E.W. performed pilot research; H.J.Y. performed the research and analyzed the data; and R.P.P. and H.J.Y. wrote the paper.

Summary

- (1) While it is well-known that trees release carbon (C) to soils as root exudates, the factors that control the magnitude and biogeochemical impacts of this flux are poorly understood.
- (2) We quantified root exudation and microbially-mediated nutrient fluxes in the rhizosphere for four ~80 year-old tree species in a deciduous hardwood forest, Indiana, USA. We hypothesized that trees that exuded the most carbon (C) would induce the strongest rhizosphere effects (i.e., the relative difference in nutrient fluxes between rhizosphere and bulk soil). Further, we hypothesized that tree species that associate with ectomycorrhizal (ECM) fungi would exude more C than tree species that associate with arbuscular mycorrhizal (AM) fungi, resulting in a greater enhancement of nutrient cycling in ECM rhizospheres.
- (3) Mass-specific exudation rates and rhizosphere effects on C, N and P cycling were nearly two-fold greater for the two ECM tree species compared to the two AM tree species ($P < 0.05$). Moreover, across all species, exudation rates were positively correlated with multiple indices of nutrient cycling and organic matter decomposition in the rhizosphere ($P < 0.05$). Annually, we estimate that root exudation represents 2.5% of NPP in this forest, and that the exudate-induced changes in microbial N cycling may contribute ~18% of total net N mineralization.
- (4) Collectively, our results indicate that the effects of roots on nutrient cycling are consequential, particularly in forests where the C cost of mining nutrients from decomposing soil organic matter may be (e.g., ECM-dominated stands). Further, our results suggest that small C

fluxes from exudation may have disproportionate impacts on ecosystem N cycling in nutrient-limited forests.

Key words: Arbuscular mycorrhizal fungi, ectomycorrhizal fungi, plant-microbial feedbacks, priming effects, root exudation, rhizodeposition

Introduction

While it has long been known that trees allocate carbon (C) belowground to access soil resources, the extent to which tree roots accelerate nutrient cycling is largely unknown (Grayston et al., 1996; Högberg and Read, 2008; Frank and Groffman, 2009; Lambers et al., 2009). In most forests the majority of growth-limiting nutrients such as nitrogen (N) are bound in soil organic matter (SOM). Hence, allocating C to roots in order to scavenge nutrients from the soil solution is likely to provide diminishing returns over time if nutrients become locked-up in slow turnover pools as forests mature (Johnson, 2006). This has led to view that in addition to scavenging for nutrients, mature trees likely *mine* nutrients from SOM by stimulating microbes to produce extracellular enzymes through priming effects (Cheng et al., 2014). Rhizosphere priming effects have been detected in tree seedlings (Bader and Cheng 2007; Dijkstra and Cheng, 2007; Bengtson et al., 2012), in coniferous forests (Göttelicher et al., 2006; Weintraub et al., 2007; Fan et al., 2013) and in aggrading forests exposed to elevated CO₂ (Carney et al., 2007; Phillips et al., 2011; Zak et al., 2011). However, the ecosystem consequences of such effects are poorly quantified, particularly in mature forests where exudation rates and nutrient acquisition strategies may differ among co-occurring tree species.

Understanding the degree to which roots of different tree species alter nutrient availability and SOM decomposition requires a framework for classifying tree species based on their dominant traits. Phillips et al. (2013)

recently proposed a new framework to address this knowledge gap, the Mycorrhizal-Associated Nutrient Economy or “MANE” framework. The MANE framework is based on the idea that plant and microbial species (e.g., mycorrhizal fungi) that have shared a long evolutionary history possess an integrated suite of complimentary traits that contribute to predictable biogeochemical syndromes in ecosystems. For example, nearly all fine roots in temperate forests associate with either arbuscular mycorrhizal (AM) or ectomycorrhizal (ECM) fungi (Smith and Read, 2008), and forests dominated by AM-or ECM-associated trees exhibit distinct nutrient economies (Chapman et al., 2006; Phillips et al., 2013). AM-associated tree species generally have leaf litters that decompose rapidly (Cornelissen et al., 2001; Hobbie et al., 2006), resulting in the predominance of inorganic forms of nutrients that are re-acquired by plants associating with fast-growing scavenger mycorrhizal hyphae (Lambers et al., 2009). These forests tend to be characterized by elevated losses of C and nutrients (Phillips et al., 2013). In contrast, ECM trees generally have more slowly decomposing leaf litter (Cornelissen et al., 2001; Hobbie et al., 2006), and a greater proportion of nutrients in organic forms (Phillips et al., 2013) that are re-acquired by plants via ectomycorrhizal mycelium that produce extracellular enzyme to mine nutrients from SOM. A consequence of these dynamics is that ECM-dominated forests tend to cycle C and nutrients more conservatively than AM-dominated forests, and contribute to differential rates of soil C retention (Vesterdal et al., 2012; Averil et al., 2014) and N leaching losses (Midgley and Phillips, 2014).

Given differences in nutrient economy between AM- and ECM-dominated forests, we hypothesized that additional belowground processes, such as root exudation and rhizosphere priming, represent critical adaptations to these unique biogeochemical syndromes. Root exudation – the release of soluble C compounds from roots to soils – has long been presumed to stimulate soil

microbial activity and nutrient availability (Smith, 1976; Grayston et al., 1996). Recently, both empirical (Kuzyakov et al., 2010; Drake et al., 2013) and theoretical (Wutzler and Reichstein, 2013; Cheng *et al.* 2014) studies have indicated that elevated rates of exudation may enhance nutrient release by accelerating SOM decomposition via rhizosphere priming effects. Consequently, we hypothesized that ECM trees would exude more C from roots than AM trees given that most soil nutrients in ECM-dominated soils exist in organic forms (Phillips et al., 2013), and therefore are unavailable to trees in the absence of microbial priming. Previous investigations indicate that ECM trees may exude more C than AM trees (Smith, 1976; Grayston et al., 1996; Phillips and Fahey, 2005), and that ECM roots may have greater effects on soil biogeochemistry than AM roots (Phillips and Fahey, 2006). However, no studies to our knowledge have measured both processes simultaneously in mature forests, or scaled these results to estimate the ecosystem-impacts of root-induced changes in nutrient fluxes.

In this study, we quantified root exudation and microbially-mediated nutrient fluxes in the rhizosphere of mature AM and ECM trees in a deciduous hardwood forest, Indiana, USA. We asked the question: to what extent do species differences in root exudation influence C and nutrient cycling in the rhizosphere, and to what degree can C fluxes from AM and ECM roots influence ecosystem-scale nutrient cycling. Such differences are likely to be consequential for ecosystem C balance in forests in the wake of global change, as tree species that can mine nutrients from SOM may delay progressive nutrient limitation whereas trees with scavenging strategies may show productivity declines over time (Drake et al., 2011). Overall, our study directly links C inputs released from roots to soil microbial activities, as a means of understanding the biogeochemical consequences of root-microbe interactions at the ecosystem-scale.

Materials and methods

Site description

The research was conducted at Indiana University's Griffy Woods (GW) Research and Teaching Preserve, an ~80-yr-old forest in south central Indiana. The site contains a rich assemblage of both AM and ECM tree species. Dominant AM tree species include sugar maple (*Acer saccharum* Marsh), tulip poplar (*Liriodendron tulipifera*), white ash (*Fraxinus americana* L.), black walnut (*Juglans nigra*), and sassafras (*Sassafras albidum*), while dominant ECM trees include northern red oak (*Quercus rubra* L.), black oak (*Quercus velutina* Lam.), American beech (*Fagus grandifolia* Ehrh.), shagbark hickory (*Carya ovata* P. Mill.), white oak (*Quercus alba* L.) and bitternut hickory (*Carya cordiformis* Wangenh.). The climate is humid continental, with mean annual precipitation of 1200 mm and mean annual temperature of 11.6°C. Soils at GW are silty-loams derived from sandstone, shale and, to a lesser extent, limestone (primarily from the Berks-Weikert Complex).

We measured exudate fluxes for white oak (ECM), American beech (ECM), sugar maple (AM), and tulip poplar (AM) replicate (n=3), 10m x 10m monodominant plots where >80% of the basal area was composed of the target tree species (on average, 3-4 trees per plot). Additionally, we collected rhizosphere and bulk soils from the plots within one week of the exudation measurements, to measure the degree to which each species' roots influenced indices of C and nutrient cycling (i.e., rhizosphere effects) related to the exudation patterns. All plots were located in similar landscape positions (e.g., slope, aspect) to avoid topographic effects.

Exudation measurements

Exudates were collected in June, July, August and October of 2013 from intact fine roots using a modified culture-based cuvette system developed

especially for field-based exudate collections (Phillips et al., 2008). Terminal fine roots of target species were carefully unearthed from the upper 10 cm of soil mineral horizon by hand. In order to ensure that roots were from the targeted species, all root systems were traced back to a parent tree, or identified based on characteristics (e.g., diameter and morphology) known to be unique to the targeted species. Soil particles adhering to fine roots were removed by gentle washing, and forceps were used to dislodge SOM aggregates. After a short equilibration period, the intact root system (i.e., roots still attached to the tree) was placed into a 30 ml glass cuvette, and the remaining volume was filled with sterile glass beads. A C- and N-free salt solution (0.1 mM KH_2PO_4 , 0.2 mM K_2SO_4 , 0.2 mM MgSO_4 , 0.3 mM CaCl_2) was added to the cuvette to buffer the roots, and the entire root cuvette system was sealed with Parafilm. After 24 h, exudates were collected by flushing the cuvette three times with fresh solution. The trap solutions were filtered through sterile 0.22 μm syringe filters within 2-5 h after collection, and stored at -20°C until analysis. Total non-particulate organic C accumulated in the trap solutions in each cuvette was analyzed on a TOC-TN analyzer (TOC-VCPH, Shimadzu, Japan).

For each tree species, we collected exudates from two cuvettes containing roots and one cuvette without roots as a non-rooted control. This resulted in a total of six samples (and three controls) per species during each sampling date. Control cuvettes (beads only) were used to account for C contamination resulting from non-exudate sources for each plot. Exudation rates were calculated as the mass of C (mg) flushed from each root system (minus the average C concentration in control cuvettes) over the 24 h incubation period. Mass-specific rates of root exudation ($\text{mg C g}^{-1} \text{ root day}^{-1}$) were calculated by dividing the total amount of C flushed by the total fine biomass ($< 1\text{mm}$ diameter) within each cuvette. Plot-level exudation rates for each species were calculated by multiplying the average mass-specific exudation rate (mg C g^{-1}

root day⁻¹) by the average fine root biomass (g root m⁻²) in each plot to a depth of 15cm.

Soil sampling

Soils were sampled in the upper 15 cm of the soil with a 5 cm diameter stainless steel core in June, July, August and October of 2013. We chose these dates to correspond with the exudation collections (e.g., all soil samples were collected within one week of a given exudation measurement). Five replicate soil cores within each plot were collected to ensure that fine roots would have a sufficient mass of adhering rhizosphere soil. The collected soil cores were mixed to obtain one composite fresh sample for each plot. Soils were transported immediately to the laboratory following field sampling, and fine roots with adhering soil were separated from non-adhering soil within 6 h of collection. Soil adhering to fine roots was dislodged using fine forceps; this fraction was operationally classified as rhizosphere soil (*sensu* Phillips and Fahey, 2006), while that not adhering was defined as bulk soil. All soils were sieved through a 2 mm mesh. Each soil sample was divided into two subsamples. One subsample was stored in a -80°C freezer for extracellular enzyme activity assays. The second subsample was processed within 24-48 h of collection to determine soil organic matter (SOM), pH, extractable total N (ETN), extractable organic C (EOC), soil microbial biomass C (MBC) and N (MBN), and net N and C mineralization rates (details below). After separation from soil, all the fine roots were carefully washed and then dried at 100°C for 48 h to measure fine root biomass (FRB).

2.4 Laboratory analyses

Net N mineralization rates were measured using a 15-d aerobic laboratory incubation at 23°C by quantifying the change in 2 M KCl extractable pools of NH₄⁺ and NO₃⁻. Two 5 g replicates of sieved soil were placed into 15 mL

centrifuge tubes. One sample was extracted immediately with 10 mL of 2 M KCl, shaken for 1 h, centrifuged at 3000 rpm and filtered with Whatman no. 1 filter paper. The other sample was incubated for 15 days in the dark prior to extraction. Incubated samples were covered with pierced Parafilm and dampened Kimwipes to maintain soil moisture while allowing for gas exchange. Extracts were frozen prior to analysis. $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ concentrations were measured colorimetrically by flow injection on a Lachat Quik Chem 8000 Flow Injection Analyzer (Lachat Instruments, Loveland, CO, USA). For each sample, extractable $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ concentrations were scaled to mg N g soil^{-1} using extract volume, sample mass, and moisture content. Net mineralization rates (N_{min}) were calculated as the change in inorganic N (NH_4^+ and NO_3^-) before and after the 15-d incubation.

Soil C mineralization rates (C_{min}) were measured in the lab using short-term incubations. Five grams of soil for each sample was placed in a septum-sealed glass jar and incubated at $\sim 23^\circ\text{C}$. Three headspace samples (1 mL) were taken at 2-h intervals and injected into a portable photosynthesis system (Li-6200, LI-COR Inc, Lincoln, NE). Soil C mineralization rate was calculated as the change in headspace CO_2 concentration measured over the incubation period ($\mu\text{g C-CO}_2 \text{ g}^{-1} \text{ soil h}^{-1}$). For both C and N mineralization assays, soils were not pre-incubated to minimize the extent to which labile C – a critical attribute of rhizosphere soil – would become depleted prior to the assay.

Soil extractable total N (ETN) and extractable organic C (EOC) were measured in K_2SO_4 extracts using a TOC analyzer (Shimadzu TOC-V_{CPN}, Shimadzu Scientific Instruments, USA). Soil microbial biomass C (MBC) and N (MBN) concentrations were determined with the chloroform fumigation extraction method (Vance et al., 1987). The MBC and MBN were calculated from the differences between the total extractable C and N in the fumigated and unfumigated samples with efficiency factors (K_{ec} and K_{en}) of 0.45 and 0.54,

respectively (Vance et al., 1987). Extractable organic N concentration (N_{org}) was calculated as the difference between ETN and inorganic N (N_{inorg}).

We measured the potential activity of five extracellular enzymes that degrade a range of substrates that are common constituents of SOM. These included acid phosphatase (herein abbreviated as AP, which releases inorganic phosphate from organic matter), β -1,4-glucosidase (herein abbreviated as BG, which hydrolyzes cellobiose into glucose), β -1,4-*N*-acetylglucosaminidase (hereafter NAG, which breaks down chitin), and peroxidase and polyphenol oxidase (herein abbreviated as PER and PPO, respectively, which degrade lignin). Given that PER and PPO degrade relatively stable components of SOM, we considered these enzyme activities as proxies for SOM decomposition and potential indicators of priming effects. Enzyme assays were based on a modification of previous methods (Saiya-Cork et al., 2002). Briefly, all the assays were run by mixing 1.5 g of soil with 100 mL of 50 mM of substrate, pH 5.0. The suspensions were continuously stirred and twenty-four 200 μ L aliquots of the suspension were transferred to 96-well microplates. Microplates were incubated in the dark at 23°C for 2 h (NAG and AP), 5 h (BG) and 4 h (PER and PPO). NAG, BG and AP activities were measured fluorometrically (excitation, 365 nm; emission, 450 nm) using substrates linked to a fluorescent tag (4-methylumbelliferone), while PER and PPO activities were measured colorometrically using L-dihydroxyphenylalanine as the substrate.

Soil subsamples for each assay were expressed on dry mass equivalent basis after oven-drying subsamples to constant mass at 105 °C. Soil pH was measured using a bench top electrode pH meter. For each sample, 5 grams of dry weight-equivalent soil was placed in a 50 mL centrifuge tube and 40 mL of 0.01 M $CaCl_2$ solution was added to the tubes. The suspensions were shaken for 30 minutes and vortexed immediately prior to analysis.

Calculations and statistics

We estimated annual exudation at GW ($\text{g C m}^{-2} \text{ y}^{-1}$) by multiplying the average exudation rate of all four species by the average standing crop of fine roots at the site based on measurements collected from eight plots over four sampling dates. We then multiplied daily exudation rates by the number of days in the growing season in 2013 (200 days) derived from measurements of canopy phenology in a nearby forest with similar tree species (the Morgan Monroe State Forest; Brzostek *et al.*, 2014). To account for the relative abundance of trees at GW, we used a weighted average based on the average exudation rate for AM and ECM trees, and the relative abundance of AM (57% of the basal area) and ECM trees (43% of the basal area) in 30 randomly located 15m x 15m plots at GW. Net primary production at GW for 2013 was calculated as the sum of 1) wood and coarse-root production (basal area increment and allometry), 2) root production (ingrowth cores), 3) litter production (litter baskets) and 4) exudation (as described above).

Rhizosphere effects (RE), the percentage difference of a given response variable between paired rhizosphere and bulk soil samples (i.e., $[\text{rhizosphere process rate} - \text{bulk soil process rate}] / \text{bulk soil process rate}$), were calculated in order to quantify the effects of roots and rhizosphere processes relative to bulk soil processes (Phillips and Fahey, 2006). To estimate the ecosystem consequences of exudate inputs, we multiplied the rhizosphere effects on C and N cycling by the percentage of rhizosphere soil volume in the upper 15cm of soil (where most of the fine roots reside). Rhizosphere volume in upper surface soils was calculated by assuming that fine roots were cylinders, and that exudates diffuse on average 1 mm from the root surface (Darrah, 1991; Jones, 1998; Herman *et al.*, 2006). To calculate fine root volume, we measured fine root biomass in AM and ECM-dominated forest plots at GW (average of four collections over the 2013 growing season), and converted these values based on literature values of specific root length and fine root

diameter for AM and ECM tree species found at GW (Table 2; Comas et al., 2009; McCormick et al., 2013).

Statistical analyses were performed in SPSS version 18.0 (SPSS Inc., Chicago, IL). All response variables were averaged within each plot for a given tree species and sampling date. Repeated measures ANOVA was used to examine the effects of tree species, mycorrhizal association, sampling date, and their interactions on root exudation rate. One-way analysis of variance (ANOVA) was performed to test the significant differences between tree species or mycorrhizal associations for a given response variable and sampling date. We used linear regression to examine the relationship between exudation rate and rhizosphere effects on extracellular enzyme activity. The statistical tests were considered significant at the $P < 0.05$ level.

Results

Exudation difference among tree species

Over the four sampling dates, mass-specific exudation rates ranged from 0.18 to 0.49 mg C g⁻¹ root day⁻¹ and were strongly influenced by tree species and mycorrhizal associations ($P=0.003$ and $P<0.001$ respectively). Mass-specific exudation for beech and oak were consistently greater than for tulip and maple, but significant differences among tree species were only found in July and August of 2013 (Fig. 1). When grouped by mycorrhizal association, the average mass-specific exudation rate (mg C g⁻¹ root day⁻¹) was two times greater in ECM species compared to AM species (Fig. 1).

When these point-in-time measurements were scaled to estimate growing season fluxes, exudation rates for beech- and oak-dominated plots were nearly three-fold greater (26 g C m⁻² year⁻¹) than those of maple- and tulip-dominated plots (8 g C m⁻² year⁻¹), as a result of both greater fine root biomass and mass-specific rates. Based upon the distribution of AM and ECM

trees at GW, we estimate that $\sim 17 \text{ g C m}^{-2} \text{ year}^{-1}$ were released as soluble root exudates in GW, a C flux approximately 2.5% of NPP in this forest ($679 \text{ g C m}^{-2} \text{ y}^{-1}$).

Soil physico-chemical properties

We detected multiple differences in soil physical and chemical properties in our plots (Table 1). Soil pH for AM plots (4.56 on average) was significantly greater than for ECM plots (3.92 on average). Additionally, we found a greater MBC:MBN ratio in ECM plots (ranging from 4.12 to 4.89) than in AM plots (ranging from 3.59 to 3.92), as well as greater ETN and $\text{N}_{\text{org}}:\text{N}_{\text{inorg}}$. Differences in soil properties between rhizosphere and bulk soils were also apparent in all trees. ETN and the ratios of MBC:MBN and $\text{N}_{\text{org}}:\text{N}_{\text{inorg}}$ were generally enriched in the rhizosphere, but significant differences between soil fractions were only observed for ETN in beech (Table 1). For both tulip and maple, pH in the rhizosphere was greater than the bulk soil across all sampling dates. In contrast, the average pH in beech and oak soils was slightly lower in the rhizosphere relative to the bulk soil, suggesting a trend toward rhizosphere acidification for ECM species and a rhizosphere neutralization trend for AM species.

Rhizosphere effects in ECM and AM trees

In general, ECM trees had significantly greater rhizosphere effects on microbial activity, nutrient cycling and SOM decomposition than AM tree species (Fig. 2). In ECM plots, C mineralization and BG (both indices of labile C turnover) were 67% and 40% greater in the rhizosphere (relative to bulk soil), yet only 43% and 27% enhanced in the rhizosphere (relative to bulk soil) in AM plots. Similarly, rates of N cycling (N mineralization and NAG) were 60% and 33% greater in the rhizosphere of ECM trees, but only 35% and 6% greater in the rhizosphere of AM trees. Phosphatase activities were also

enhanced in ECM rhizospheres to a greater extent (47%) than in AM rhizospheres (19%). Rhizosphere effects on PPO, a potential indicator of priming, were greater in the ECM rhizospheres (33%) than in the AM rhizospheres (6%). Rhizosphere PER, another possible indicator of priming, was significantly enhanced in the rhizosphere (relative to bulk soil) in all soils, but the magnitude of the rhizosphere effect was not significantly different between the mycorrhizal types ($P > 0.05$). Across all variables in Figure 2, rhizosphere effects in ECM soils were more than twice as large as those in AM soils ($P = 0.006$).

Given differences between ECM and AM roots in the extent of the volume of their rhizospheres (58% and 18%, respectively), and the relative abundance of ECM and AM trees in this forest (43% and 57% AM, respectively), we calculated the percent contribution of root-induced microbial activity to ecosystem fluxes. We estimate that 21% of the labile C mineralized in this forest can be attributed to root exudation (Table 2). Using the same scaling approach, we estimate that 18% of the N mineralized in this forest can be attributed to rhizosphere microbes fueled by root exudation (Table 2). To the extent that PPO activity represents a conservative proxy for phenol/lignin decomposition, and that the rhizosphere enhancement of PPO is caused by priming effects), we estimate that ~1% of the ecosystem decomposition of phenol/lignin decomposition results from root activity (data not shown).

Exudation and rhizosphere effects

Pairing measurements of exudation and rhizosphere effects from the same plots and on the same approximate dates, we found that the magnitude of exudation rates was positively correlated with rhizosphere microbial activities (Fig. 3). Exudation rates were positively correlated with rhizosphere effects on nearly all indices of C, N and P cycling. For example, exudation was correlated with the rhizosphere enhancement of BG ($R^2 = 0.34$; $P=0.009$), C

mineralization ($R^2 = 0.43$; $P=0.007$), NAG ($R^2 = 0.55$; $P=0.001$), N mineralization ($R^2 = 0.59$; $P=0.004$), AP ($R^2 = 0.72$; $P<0.001$), and PPO activity ($R^2 = 0.69$; $P<0.001$) across all species and sampling dates. Exudation rates were not significantly correlated with rates of PER, or other biogeochemical transformation rates such as net nitrification (data not shown).

Discussion

Root-microbe interactions play a central role in coupling C and nutrient cycles (Cheng et al., 2014), and knowledge of how trees and their root-associated microbes influence ecosystem processes is critical for predicting the biogeochemical consequences of shifts in forest composition. Here we show that fine roots of ECM tree species release nearly three-fold more exudates to soil than the roots of AM trees on an annual basis, and that elevated exudation rates in ECM soils may be responsible for the enhanced rates of C, N and P cycling in these soils. Further, our scaled estimates suggest that even modest fluxes of labile C from root exudation may have large effects on ecosystem C, N and P cycling, particularly in stands dominated by ECM tree species. Using a numerical model that combines rhizosphere effect sizes with fine root morphology, we estimate that microbial activity fueled by exudation contributes ~20% of the C and N mineralized in this forest. While these estimates rely on several assumptions (discussed below), our results indicate that root exudates are clearly an important driver of nutrient cycling in forests, particularly in stands where the dominant trees use root-derived C to accelerate rhizosphere mineralization and priming.

Annual exudation in forests

Our estimated rate of exudation (2.5% of NPP) falls well within the range of those reported previously in temperate forests. Using mass balance, Fahey

et al. (2005) estimated rhizosphere C flux - the sum of root exudation and C flux to mycorrhizal fungi - as 14% of NPP in a northern hardwood forest dominated by ECM trees. Given that more than half of rhizosphere C flux can be used to support ECM mycelium (Högberg et al., 2008), the annual exudate flux reported in the Fahey et al. (2005) study may be closer to 5-6% of NPP. Drake et al. (2011), using a mass balance approach, estimated rhizosphere C flux to be 6% of NPP in an ECM-dominated plantation in the southeastern US. In a companion study carried out in the same forest, cuvette-based measurements of exudation indicated that approximately half of the rhizosphere C flux, or 2.5% of NPP, was derived from root exudation (Phillips et al., 2011). Finally, using an optimal allocation, cost-benefit model, Brzostek et al. (*In revision*) estimated that ~4% of NPP is needed to support N acquisition in central hardwood forests. Assuming that at least half of this C cost is needed to directly support mycorrhizal fungi, it seems plausible that the remaining C allocated to roots for nutrient acquisition would be released to soil as exudates (~2% of NPP).

Differences in exudation rates among forests may also relate to site-specific factors such as nutrient availability, rooting densities, and mycorrhizal associations of the dominant trees. For example, exudation rates may be greater in forests where the majority of the soil N is contained in SOM (e.g., northern hardwood forests) relative to forests where inorganic N forms in soil predominate (e.g., central hardwood forests). Many of the tree species that produce low quality litters associate with ECM fungi, and forests dominated by ECM trees have been reported to have wide soil C:N (Averill et al., 2014) and a greater fraction of N in organic forms relative to AM-dominated stands (Phillips et al., 2013). Hence, greater exudation rates may reflect a greater cost to trees of acquiring nutrients. It has often been presumed that ECM trees exude more C than AM trees, though few if any studies have investigated exudation rates from multiple tree species within a

given mycorrhizal group (Smith, 1976; Phillips and Fahey, 2005). In this study, we found that mass-specific exudation rates of ECM trees exceeded those of AM species nearly three-fold. While some of this difference may reflect the growth rates of ECM and AM trees (e.g., the relative basal area increment of ECM trees was ~25% greater than of AM trees; data not shown), factors other than growth must contribute to the large differences in exudation. In the Brzostek et al. study (*In Revision*), the cost of N uptake was nearly two-fold greater in stands dominated by ECM stands relative to AM stands owing to the greater availability of N in the AM-dominated stands.

Our results also suggest that the timing of exudation measurements can contribute to variable estimates among ecosystems. Exudation rates exhibited seasonal variation coincident with patterns of soil temperature, with the highest exudation rates (and soil temperatures) occurring in July and August and with low rates in June and October (Fig. S1). While soil temperature is known to influence belowground C fluxes (Urbanski et al., 2007), girdling and pulse labeling studies indicate that exudate fluxes are also driven by source-sink relationships (Phillips et al., 2008; Kaiser et al., 2010). Hence, collecting exudates during only one (Smith 1976) or two (Brzostek *et al.*, 2013) time periods during the growing season may result in artificially low (or high) estimates.

Magnitude of rhizosphere effects

We hypothesized that the magnitude of rhizosphere effects on soil biogeochemical processes would be greater in ECM plots than in AM plots as a result of higher exudate inputs and the greater percentage of nutrients locked up in SOM. Exudation of labile C is believed to provide an energy subsidy to rhizosphere microbes, which subsequently release extracellular enzymes to release nutrients from SOM (Kuzyakov, 2010). In addition, differences in the capacity of AM vs ECM fungi to synthesize extracellular

enzymes may explain some of the variation in the magnitude of rhizosphere effects. ECM fungi are known synthesize many different hydrolytic enzymes and oxidative extracellular enzymes to degrade SOM (Brzostek et al., 2013). In contrast, AM fungi only produce a narrow range of hydrolytic enzymes and few oxidative enzymes (Veresoglou et al., 2012). As a result, ECM species have greater ability to access organic N sources that are inaccessible to AM species (Averill et al., 2014). The results from our study support our initial hypothesis, as greater labile C inputs to soils in ECM-dominated plots likely induced greater rhizosphere effects.

It has been suggested that rhizosphere priming effects are biogeochemical consequences of root-derived C fluxes to soil (Cheng et al., 2014). Our results support this finding, and suggest that there are important linkages between the magnitude of root C fluxes and the proportion nutrients bound up in SOM. This may explain variation in the magnitude of rhizosphere priming effects reported in other studies. Recent exudation addition experiments indicate that rhizosphere priming may be greatest in soils that have a high ratio of labile-to-stable soil C owing to the presence of a larger microbial biomass (de Graaff et al., 2013), or in soils with a N economy dominated by organic N (Drake et al., 2013). Consistent with this, greater exudation and rhizosphere priming effects have been invoked to explain the differential response of AM and ECM trees to CO₂ enrichment. At the Oak Ridge FACE experiment, the effects of elevated CO₂ on AM sweetgum trees did not persist, as the ability of trees to mine nutrients from SOM likely diminished over time (Iversen et al., 2012). In contrast, enhanced levels of forest productivity under CO₂ enrichment were sustained at the Duke FACE site, where ECM loblolly pine trees were able up-regulate exudation in order to mine nutrients from SOM (Phillips et al., 2011). In both cases, the priming influenced the accumulation and degradation of C stored in the SOM, indicating that this process effectively results in the transfer of C and nutrients from soil to

biomass pools. The degree to which such shifts affect long-term ecosystem C storage in forests, and subsequent feedbacks to global climate, warrants further study (Heiman and Reichstein, 2008).

The contrasting magnitude of rhizosphere priming effects and root contributions to N cycling between AM and ECM trees suggests that exudation may be an evolved mechanism that trees employ for responding to diverse nutrient economies. This is supported by recent theoretical and empirical work that considers soils as “banks” for N, releasing N when plant demand is high and retaining it when plant demand is low (Perveen et al., 2014). In our study, ECM tree species appear to mineralize SOM decomposition and N mineralization in the rhizosphere, a process which would couple rhizosphere C fluxes with nutrient return (i.e., greater need to exude and prime, to get nutrients out of SOM) due to low nutrient availability. In contrast, AM litters generally have faster decomposition rates leading to higher mineral nutrients (Cornelissen et al., 2001; Hobbie et al., 2006), presumably resulting in a reduced need for plants to mine nutrients from SOM. Thus, enhanced exudation may be an evolutionarily stable strategy for increasing nutrient availability if trees invest more energy-rich C in fueling microbial activity (i.e., a greater C cost) in exchange for the greater nutrient return of SOM-degrading microbes access (Cheng et al., 2014). Such a strategy would be particularly viable in stands where co-occurring tree species have both scavenging and mining nutrient acquisition strategies (Smith and Read, 2008; Lambers et al., 2009; Dijkstra et al., 2013).

Measurement and scaling considerations

Our exudation measurements focused exclusively total exudate fluxes, without considering how variable exudate stoichiometries might influence rhizosphere effects. Theory and experiments predict that exuding a small amount of N may actually enhance rhizosphere N cycling, as there is a N cost

associated with making extracellular enzymes (Drake et al., 2013). While trees are known to release a small amount of amino acids as exudates (Smith, 1976), the acids are more likely to be re-absorbed by the roots than acquired by rhizosphere microbes (Jones *et al.*, 2004). Experimental approaches, such as addition of simulated exudates varying in C-to-N ratios to AM and ECM soils, could help address the role of exudate stoichiometry on rhizosphere effects.

Annual estimates of exudation reported in this study may also be conservative given that we only accounted for roots in the upper 15cm of soil, and did not account for exudates released outside of the growing season. Exudate fluxes are often driven by source-sink dynamics related to recently-assimilated C. However, basal exudation, which is driven by concentration gradients (of low molecular mass organics) that exist between root cells and the root apoplast (Jones et al., 2004) is also known to occur. Whether basal exudation rates vary by tree species and mycorrhizal association, and have consequences for nutrient cycling, warrants further study. Similarly, our estimates of rhizosphere effects are almost certainly conservative given that we did not include the activities fine roots growing below 15cm depth, and rhizosphere effects were quantified using root-free soil incubations. Much of the C exuded by roots is assimilated by rhizosphere microbes within hours (Hogberg et al., 2008; Shahzad et al., 2012), and so a substantial fraction of the labile C that exists in the rhizosphere is likely depleted shortly after soil collection - a process that should reduce differences in microbial C and N cycling between rhizosphere and bulk soils.

Our estimates of the contribution of exudates to ecosystem C and N cycling were based on several assumptions that require further testing. First, rhizosphere soil volume was estimated using a diffusion distance of 1 mm from the root surface, a value that is smaller than the more commonly used value of 2mm (Darrah, 1991; Jones, 1998; Herman et al., 2006), but one that yielded an estimate of rhizosphere volume (35%) comparable to other forests

with similar tree species (20-30%; Phillips and Fahey, 2006). Second, we did not consider that different types of exudates may differ in their diffusion distances (Jones, 1998), and that AM and ECM trees may differ in the types of compounds they exude. Third, while priming effects were estimated in this study using rhizosphere effects on oxidative enzyme activities, recent studies suggest that oxidative enzymes may represent good proxies for gross N mineralization but not necessarily for SOM priming (Bengston et al., 2012; Zhu et al., 2014). Finally, given recent evidence that positive priming effects, or the net accumulation of C, may also result from faster turnover of microbial biomass or differences in microbial C-use efficiency (Cheng et al., 2014), more work is needed to investigate the consequences of enhanced exudation on net C balance in soil. The development of new experimental approaches for addressing these questions should lead to improved estimates of the role of roots in driving nutrient cycling in forests.

Collectively, our results provide robust evidence that tree species and mycorrhizal associations can differentially drive the magnitude of root impacts on nutrient availability through labile C inputs to soils. The variations in root exudation fluxes and concomitant rhizosphere effects between ECM and AM trees may be consequence of evolutionary processes, and could have important implications for ecosystem C storage in temperate forests. As such, shifts in forest species composition resulting from forest management, land use or global environmental change could have substantial biogeochemical consequences on ecosystem C-nutrient coupling via root-microbe-soil interactions.

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Table 1 Soil chemistry in rhizosphere and bulk soils for four tree species in Griffy Woods (GW), Indiana. Values are means and ± 1 SE for replicate tree species plots across the four sampling dates. Different lowercase letters in the same column indicate significant differences among tree species at $P < 0.05$ for a given response variable. * indicate significant differences at $P < 0.05$ between rhizosphere and bulk soil for a given tree species and soil variable. RS =

rhizosphere soil; BS = bulk soil; ETN = extractable total N; N_{org}: N_{inorg} = extractable organic N: inorganic N.

Tree species	pH		ETN ($\mu\text{g N g}^{-1}$ soil)		MBC: MBN		N _{org} : N _{inorg}	
	RS	BS	RS	BS	RS	BS	RS	BS
Tulip	5.01	4.92	12.31	13.17	3.86	3.59	4.83	4.29
	(0.27) ^a	(0.22) ^a	(1.68) ^b	(1.91) ^a	(0.52) ^b	(0.32) ^c	(0.99) ^b	(0.63) ^b
Maple	4.19	4.10	17.83	15.43	3.92	3.90	5.73	5.92
	(0.18) ^{ab}	(0.19) ^b	(2.05) ^a	(2.60) ^a	(0.45) ^b	(0.29) ^{bc}	(1.42) ^b	(1.05) ^{ab}
Oak	3.96	4.07	21.38	15.92	4.41	4.12	8.92	6.43
	(0.12) ^b	(0.11) ^b	(2.91) ^a	(3.40) ^a	(0.69) ^{ab}	(0.43) ^{ab}	(2.15) ^a	(1.80) ^{ab}
Beech	3.79	3.86	18.47	12.75	4.89	4.41	10.31	9.07
	(0.06) ^b	(0.06) ^b	(1.76) ^a	(1.93) ^{a*}	(0.73) ^a	(0.32) ^a	(2.06) ^a	(2.21) ^a

Table 2. Rhizosphere effects (RE) and their contributions to C and N cycling at Griffy Woods (GW), Indiana. Rhizosphere volume (Rhizo. Vol.) was calculated by multiplying the mean fine root length (FRL) and diameter of AM and ECM trees at GW by the exudate diffusion distance (1mm from the root surface). RE_{Cmin} and RE_{Nmin} are the rhizosphere effect on net C and N mineralization rates (n=4 dates). Root contributions to C and N cycling at the ecosystem scale are the products of the rhizosphere effects on C and N mineralization, and the volume of rhizosphere soil. “Ecosystem” estimates are community-weighted means based on the relative abundance of AM (57%) and ECM (43%) trees at GW.

Tree species	FRL (km m⁻²)^a	Rhizo. volume (%)	RE_{Cmin} (%)	Root contrib. to ecosys C cycling (%)	RE_{Nmin} (%)	Root contrib. to ecosys. N cycling (%)
AM trees	6	8	34	43	34	6
ECM trees	22	58	56	67	56	32
<i>Ecosystem</i>	<i>13</i>	<i>35</i>	<i>43</i>	<i>21</i>	<i>43</i>	<i>18</i>

^aMean fine root length for AM and ECM tree species present at GW was estimated from Comas et al., 2009 and McCormick et al., 2013

FIGURE LEGENDS

Figure 1.

Seasonal variation in mass-specific exudation rate ($\text{mg C g}^{-1} \text{ root day}^{-1}$) for four tree species at the Griffy Woods in 2013. Values are means ± 1 SE and different lowercase letters indicate significant differences ($P < 0.05$) among tree species at a given sampling date. AM and ECM values represent the average values between tulip and maple species, and oak and beech species, respectively, across four sampling dates. Significant differences between AM and ECM species are noted by asterisks ($***P < 0.001$) in the top right corner.

Figure 2.

Rhizosphere effects for C, N and P cycling for AM and ECM tree species at Griffey Woods. Values are means (± 1 SE) of four sampling dates for C mineralization ($C_{\min}$), β -1,4-glucosidase (BG), net N mineralization rate ($N_{\min}$), β -1,4-*N*-acetylglucosaminidase (NAG), acid phosphatase (AP), polyphenol oxidase (PPO), and peroxidase (PER). A and E represent the average values of all seven variables for the two AM species (tulip and maple) and two ECM species (oak and beech). Lowercase letters indicate significant differences ($P < 0.05$) among tree species for a given variable.

Figure 3.

Relationship between plot level exudation and rhizosphere effects across four tree species and sampling dates. Values are means (± 1 SE) for replicate plots of each tree species ($n = 3$) for **a)** β -1,4-glucosidase (BG), **b)** C mineralization ($C_{\min}$), **c)** β -1,4-*N*-acetylglucosaminidase (NAG), **d)** net N mineralization ($N_{\min}$), **e)** acid phosphatase activity (AP), and **f)** polyphenol oxidase (PPO). Open circles and triangles are for the ECM trees beech and oak, respectively,

while filled diamonds and squares are for AM trees tulip and maple, respectively.

Figure 1.

Figure 2.

Figure 3.