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

Huajun Yin [a, b], Emily Wheeler [b], Richard P. Phillips [b, *]

[a] Key Laboratory of Mountain Ecological Restoration and Bioresource Utilization & Ecological Restoration and Biodiversity Conservation, Chengdu Institute of Biology, Chinese Academy of Sciences, No 9 Section 4, Renmin Nan Road, Chengdu, 610041, China
[b] Department of Biology, 1001 E. Third St, Indiana University, Bloomington, IN, 47403, USA

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ABSTRACT

(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 greatest (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.

1. 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, 2006; 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 (Göttlicher et al., 2006; Weintraub et al., 2007; Fan et al., 2013) and in aggrading forests exposed to elevated CO_2 (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

* Corresponding author. Tel.: +1 812 856 0593.
E-mail addresses: rpp6@indiana.edu, rhizosphere@gmail.com (R.P. Phillips).

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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 many plant and microbial species (e.g., mycorrhizal fungi) that share 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; Averill 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, 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.

2. Materials and methods

2.1. 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* L.), white ash (*Fraxinus americana* L.), black walnut (*Juglans nigra* L.), and sassafras (*Sassafras albidum* (Nutt.) Nees), 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 ($n = 3$), 10 m × 10 m 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.

2.2. 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 (mg C g^{-1} root day^{-1}) were calculated by dividing the total amount of C flushed by