

Root and arbuscular mycorrhizal mycelial interactions with soil microorganisms in lowland tropical forest

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Abstract

Tropical forests have high rates of soil carbon cycling, but little information is available on how roots, arbuscular mycorrhizal fungi (AMF), and free-living microorganisms interact and influence organic matter mineralization in these ecosystems. We used mesh ingrowth cores and isotopic tracers in phospholipid fatty acid biomarkers to investigate the effects of roots and AMF mycelia on (1) microbial community composition, microbial carbon utilization, and hydrolytic enzyme activities for large, potted tropical trees and (2) enzyme activities and litter mass loss in a lowland tropical forest. Under the tropical tree, plant-derived carbon was incorporated predominantly into bacterial groups in both rhizosphere and AMF-only soils. Gram-positive bacteria incorporated additional soil-derived carbon in rhizosphere soils, which also contained the highest microbial biomass. For hydrolytic enzymes, β -glucosidase and *N*-acetyl β -glucosaminidase activities were highest in rhizosphere soils, while phosphomonoesterase activity was highest in AMF-only soil. In the forest, leaf litter mass loss was increased by the presence of roots, but not by the presence of AMF mycelia only. Root–microbial interactions influenced organic matter cycling, with evidence for rhizosphere priming and accelerated leaf litter decomposition in the presence of roots. Although AMF mycelia alone did not stimulate organic matter mineralization, they were a conduit of carbon to other soil microorganisms.

Introduction

Tropical forests are the most productive and have the highest rates of carbon (C) turnover of all terrestrial ecosystems (Dixon *et al.*, 1994; Pan *et al.*, 2011). The soil microorganisms that decompose this large quantity of C are regarded by current ecosystem models as a spatially homogeneous and functionally redundant group (Bradford & Fierer, 2012). However, microorganisms that are closely influenced by roots and mycorrhizal fungi may differ in their community composition, function, and response to climatic change (Alberton *et al.*, 2005; Singh *et al.*, 2010). Roots and arbuscular mycorrhizal fungi (AMF) have large influences on soil microbial communities (Hawkes *et al.*, 2007; Drigo *et al.*, 2010; Hodge *et al.*, 2010) by altering the physical and chemical soil environment (Paterson, 2003; Gregory, 2006) and affecting

processes that are important in determining the ecosystem C balance (Cheng & Gershenson, 2007). These ‘rhizomicrobial’ interactions appear to be critical in determining the C balance of temperate forest (in particular through ‘rhizosphere priming’ under elevated CO₂; Phillips *et al.*, 2011), but there is little information on their potential impacts in tropical forests.

Roots influence soil microorganisms through rhizodeposition, the release of exudates and turnover of dead fine roots (Nguyen, 2003), which account for *c.* 40% of C fixed by plants (Lynch & Whipps, 1990). Root exudates, which are primarily composed of simple sugars such as glucose (Toal *et al.*, 2000), can constitute 5–10% of fixed C (Jones *et al.*, 2004). These rhizodepositions feed a diverse community of free-living microorganisms that inhabit rhizosphere soils (i.e. typically within 1 cm of the root surface) where population densities are 2–4 orders of

magnitude higher than the surrounding bulk soil (Foster, 1988). Rhizosphere-dwelling microorganisms can also benefit the plant through nitrogen (N) fixation, the chelation and solubilization of phosphorus (P) (Gregory, 2006), and the mineralization of organic nutrients during rhizosphere priming (Paterson, 2003). Rhizosphere priming is of particular importance to soil C cycling because it affects the balance of stable soil organic matter (Dijkstra & Cheng, 2007). It is thought to occur when energy rich rhizodeposits stimulate microbial decomposition of stable soil organic matter to access limiting N (Dijkstra & Cheng, 2007; Dijkstra *et al.*, 2009; Kuzyakov, 2010).

Almost all our understanding of rhizosphere effects on microbial communities and organic matter cycling comes from agro-ecosystems, grassland, and seedling studies (Marschner & Baumann, 2003; Marschner & Timonen, 2005; Paterson *et al.*, 2007; Bird *et al.*, 2011). The study of C cycling in tree rhizospheres has been highlighted as a research priority (Cheng & Gershenson, 2007), yet information remains scarce (e.g. Phillips & Fahey, 2006; Dijkstra & Cheng, 2007), particularly for tropical forest trees. Rhizosphere priming for tropical forest trees may differ mechanistically from that for temperate plants, due to differences in nutrients that limit microbial activity (P rather than N; Cleveland *et al.*, 2002) and, given the positive relationship shown between rhizosphere priming and plant production (Dijkstra *et al.*, 2006), due to the high productivity of tropical forest trees (Malhi *et al.*, 1998; Pan *et al.*, 2011).

AMF are a key component in maintaining the productivity of tropical forests (Alexander & Lee, 2005), but there are very few studies of their influence on other microbial communities or on soil processes in general, in these ecosystems. Studies in other ecosystems have shown that the external mycelium of AMF can improve plant nutrient acquisition and growth, improve soil aggregation, and suppress pathogens (Smith & Read, 2008). Although they are not saprotrophs, AMF appear able to indirectly influence organic matter mineralization to accelerate decomposition (Hodge *et al.*, 2001; Cheng *et al.*, 2012) and accelerate the loss of N from leaf litter (Tu *et al.*, 2006; Leigh *et al.*, 2009). They appear able to acquire substantial amounts of N but not C from organic matter, suggesting they acquire N as a decomposition product (Hodge & Fitter, 2010) by stimulating the activity of other soil microorganisms with saprotrophic capabilities (Hodge *et al.*, 2010). However, the underlying mechanisms by which AMF interact with other microorganisms remain unclear as both positive (Johansson *et al.*, 2004; Toljander *et al.*, 2007) and negative (Welch *et al.*, 2010; Leigh *et al.*, 2011) effects of AMF on microbial growth have been shown, with different consequences for organic matter cycling. The inconsistent evidence may be

due to differences in experimental approaches (in particular a lack of studies in natural plant–soil systems), variable responses of different microbial groups (Andrade *et al.*, 1997; Fillion *et al.*, 1999), and the extent to which availability of soil resources mediates competition between AMF and saprotrophic microorganisms.

The interaction between AMF and saprophytic fungi may be important in high-diversity lowland tropical forest, where AMF are abundant (Alexander & Lee, 2005) and rates of primary production are high (Malhi *et al.*, 1998; Pan *et al.*, 2011), especially given that large quantities of recently fixed C can be transferred to AMF from their autotrophic symbiont (Johnson *et al.*, 2002). Consequently, AMF may be a significant pathway of recently fixed C to soil and, with such a large source of energy, have a competitive advantage over other free-living microorganisms in the acquisition of soil resources.

We used ingrowth cores to partition the growth of fine roots and AMF mycelia in two separate experiments: first, under controlled conditions for 6-m-tall tropical trees (*Pseudobombax septenatum*, family: *Bombacaceae*) in 4.6-m³ containers, and second, under field conditions in a seasonal moist lowland tropical forest in the Republic of Panama. To investigate whether fine roots and AMF influenced saprophytic microorganisms and organic matter mineralization, we measured the effects of fine roots and AMF on microbial community composition and C utilization [for *P. septenatum*; using $\delta^{13}\text{C}$ natural abundance isotopic tracers and measuring $\delta^{13}\text{C}$ in phospholipid fatty acid (PLFA) biomarkers], enzyme activity (for *P. septenatum* and the forest), and litter decomposition (for the forest). We hypothesized that: (1) due to high rates of aboveground C fixation typical of tropical forest trees, the allocation of C from roots and AMF to microorganisms would be of similar magnitude (indicated by plant labeled $\delta^{13}\text{C}$ in PLFA) and (2) as a consequence of stimulating other soil microorganisms, both roots and AMF would indirectly affect organic matter mineralization (indicated by enzyme activities, priming of soil organic matter, and litter decomposition).

Materials and methods

Ingrowth core design

Mesh-walled ingrowth cores were used to partition belowground growth into three components according to size classes of ingrowth: (1) roots, (2) AMF mycelia, and (3) free-living soil microorganisms (Fig. 1; Johnson *et al.*, 2001). Ingrowth cores (16 cm diameter and 22 cm deep) were made from PVC (Amanco, Mexichem Panamá, S.A.) with four holes (6 cm diameter) in the base and eight holes (four 6 cm diameter and four 3 cm diameter) in the

side; these holes were fitted with nylon mesh (Normesh Limited, UK) using silicon glue and high-strength duct tape. Treatments were fine root and mycelia ingrowth (2-mm mesh; 'FR + AMF'), mycelia ingrowth (35- μ m mesh; 'AMF'), and soil-only controls (either rotated 35 μ m mesh or unrotated 1 μ m mesh; 'CTL'; see following paragraph for explanation of why two different designs were used). Mesh sizes were chosen according to typical size classes of soil microorganisms (< 1 μ m), AMF hyphae (2–20 μ m), and fine roots (< 2 mm; Friese & Allen, 1991; Coleman & Crossley, 2003). Cores were inserted to 20 cm depth in the soil, because this is the source of the majority of microbial soil respiration (e.g. about 80% for a forest in Costa Rica; Veldkamp *et al.*, 2003) and contains the majority of fine roots [in a nearby forest Cavelier (1992) estimated that > 90% fine roots to 100 cm depth were in the top 25 cm], which dominate root respiration (Pregitzer *et al.*, 1998; Desrochers *et al.*, 2002).

Soil-only controls had a 35- μ m mesh and were rotated 180° every week for *P. septenatum*, and had a 1- μ m mesh and were static for the forest. We planned to use the rotated design for both experiments (Johnson *et al.*, 2001), because this guarantees that hyphae are effectively excluded and minimizes problems associated with water-logging of soils due to poor drainage through mesh with a small diameter. However, the rotated design proved to be suitable for soils in *P. septenatum* pots, but not for the forest site. For *P. septenatum*, cores could be rotated freely, whereas in the clay loam forest soils, cores either could not be rotated or, when they could, rotation resulted in major disturbance to surrounding soils. Therefore, in the forest we used 1- μ m mesh cores to exclude hyphae (e.g. Moyano *et al.*, 2007), which drained to field capacity (there was no significant difference in soil moisture in cores compared with undisturbed forest soils during the wet season; Nottingham *et al.*, 2010) and excluded c. 72% of hyphal growth. In *P. septenatum* containers, rotated 35- μ m mesh cores excluded 79% of hyphal growth (compare hyphal length density in CTL and AMF cores for both experiments in Supporting Information, Table S1; the hyphae present in CTL cores may have included dead hyphae already in soils prior to the start of the experiment).

Experimental design: *P. septenatum*

Six plants of *P. septenatum* (*Bombacaceae*) were planted in large containers (1.8 m diameter, 1.8 m tall) in 2004. In October 2007, the trees measured 6.0 ± 0.4 m tall with diameter at 1.3 m of 14.7 ± 1.0 cm. *Pseudobombax septenatum* is a fast-growing light-demanding tropical tree that occurs commonly in secondary lowland tropical forest. It is fully deciduous, losing its leaves at the start of

the dry season in January and regrowing them prior to the following wet season; all trees were in full leaf throughout the experiment. The trees were situated at the forest edge in Gamboa, Panama.

Containers were filled with soil collected several years previously from 0 to 20 cm depth in a nearby plantation that was formerly under mature forest. Soil for the cores was collected from 0 to 20 cm depth from a different nearby plantation forest. The latter plantation forest site was primary forest prior to 1952, pasture between 1952–1953 and 1997–1998 (*C4* grasses *Hyparrhenia rufa* and *Ischaemum indicum*) and was afforested in 1997–1998 (see Wilsey *et al.*, 2002 for details of this site). Soil was sampled in December 2006, sieved (< 5 mm), stored at field moisture for a few days, and then mixed with sand in a 70 : 30 soil-to-sand ratio; the sand had minimal organic C and CaCO₃ and had a similar pH to soils (sand pH 6.0 and soil pH 5.5). Total organic nutrients in container and core soils were similar (Table S1), and a preliminary analysis of spores in both soils showed a relatively high abundance of spores from the genus *Glomus*. The resulting soil : sand mix had a $\delta^{13}\text{C}$ value with a *C4*-vegetation signal of -19.62‰ (SE = 0.1; $n = 6$ control cores; SE = 1 standard error).

Cores were inserted into the *P. septenatum* containers midway between the tree trunk and container edge in May 2007 immediately following leaf flush at the onset of the rainy season. One replicate of each of the three treatments (CTL, AMF, and FR + AMF) was installed in each container, resulting in a total of three cores in each container ($n = 6$ trees).

In November 2007, bulk soils (0–10 cm) were sampled from all cores, and, for FR + AMF cores only, 'rhizosphere soil' was sampled (soil adhering to fine roots after shaking). These soils were analyzed immediately for microbial C, mineral N, resin-extractable phosphate, and enzyme activities; samples were stored frozen (-35 °C) prior to extraction of PLFA. For rhizosphere soil, we only measured enzyme activities and PLFA, due to low quantity of sampled soil. Due to limited resources, we did not measure PLFA for FR + AMF cores.

Experimental design: forest

The forest under study was mature (> 60 years old) secondary seasonal moist lowland tropical forest in the Republic of Panama. The site receives a mean annual rainfall of 2455 mm, with a strong dry season from January to April (in 2008, 6.5% of annual rainfall fell during these 4 months), and has an average monthly temperature of 27 °C; based on measurements from nearby Barro Colorado Island where the monthly means varied by < 1 °C during the year (Windsor, 1990). For a detailed

description of forest composition, the reader is referred to site '15' in Pyke *et al.* (2001), which is located just a few kilometers from our study site. Although the soils at our site have not been classified in detail (e.g. US Soil Taxonomy), preliminary data indicate that they are Alfisols (Oxyaquic Hapludalfs). The soils have a clay loam texture and are derived from marine sediments; total organic C, total nutrients, and pH are listed in Table S1 (Turner & Engelbrecht, 2011). A preliminary analysis of spores from these soils showed a relatively high abundance of spores from the genus *Glomus*, which is abundant in nearby forest in Panama (Husband *et al.*, 2002).

Ten plots were randomly located in a 10-ha area of forest. Each plot measured 1 m² and contained three cores: (1) FR + AMF, (2) AMF, and (3) CTL, a total of 30 cores. In the forest plots, we excavated holes at core locations by forcing an 18-cm-diameter tube into the soil, and we collected soils at depths 0–5, 5–10, 10–15, and 15–20 cm. Each soil section was kept separate, and all visible root material was removed by hand. Each 5-cm soil profile, still moist, was replaced inside cores at a similar bulk density to forest soils. Soil-filled cores were then re-inserted into the forest at the same locations where soils were removed, and any gap remaining around each core was refilled using the same soil from the appropriate depth. Soils were removed from the forest for a total of only 4 days and kept at field moisture. At the start of the experiment in May 2007, mixed-species leaf litter was collected from each of the ten forest plots, cut into *c.* 5-cm² pieces and well mixed. One week following collection, a subsample of this litter (11.1 g dry mass) was added to each core, equivalent to average litter standing crop across all plots measured at the same time, on 24 May 2007. Leaf litter was cut into 5-cm² pieces to ensure that all treatments and controls received the same quantity of homogenized mixed-species leaf litter. Although cutting of litter likely accelerated rates of decomposition compared with undisturbed litter, this did not affect our experimental aim to determine relative differences between treatments and controls.

Three months later, on 17 September, soils were sampled from cores (0–3 cm) and analyzed for enzyme activities. We collected surface soils from root ingrowth cores only in the forest instead of 'rhizosphere' soils adhering to fine roots, because destructive sampling was not possible in this case but recognizing that roots grow in surface soils at this forest site (Cavelier, 1992). We did not measure 'rhizosphere' enzyme activities in the forest soil cores because they were not destructively sampled until 1 year later as part of another experiment. At the same time as soil sampling, leaf litter was collected from inside each core, washed, air-dried for 7 days, and weighed. New litterfall was not excluded from the cores, and litter was

harvested only if it lay exactly within the core area. Thus, we compared change in litter standing crop, which was the net result of decomposition and litterfall.

Soil nutrients, enzymes, and microbial biomass

Soil inorganic N (in NO₃⁻ and NH₄⁺) and microbial C were determined by K₂SO₄ extraction. Microbial C was determined as the difference between chloroform-fumigated and unfumigated soil samples following a 24-h fumigation period (Vance *et al.*, 1987) and corrected for efficiency of the extraction procedure with *k*-factor of 0.45 (Wu *et al.*, 1990). Total C and N in the extracts were determined by automated combustion and gas chromatography (GC) on a TOC-V_{CHN} analyzer (Shimadzu, Columbia, MD). Readily exchangeable phosphate (extractable P) and microbial P were determined by extraction with anion-exchange membranes and hexanol fumigation based on the method described by Kouno *et al.* (1995) and described in Turner & Romero (2010). Phosphate was recovered from anion-exchange membranes by shaking for 1 h in 50 mL of 0.25 M H₂SO₄, with detection in the acid solution by automated molybdate colorimetry using a Lachat Quickchem 8500 (Hach Ltd, Loveland, CO). Extractable P was determined by P recovered from unfumigated samples, and microbial P was calculated as the difference between the fumigated and unfumigated samples.

Five enzymes involved in C and nutrient cycling were measured using microplate fluorimetric assays with 200 μM methylumbelliferone (MU)-linked substrates as described in Turner & Romero (2010): β-glucosidase (degradation of β-bonds in simple sugars), cellobiohydrolase (degradation of cellulose), *N*-acetyl β-glucosaminidase (degradation of *N*-glycosidic bonds), phosphomonoesterase (degradation of monoester-linked simple organic phosphates), and xylanase (degradation of hemicellulose). A further enzyme, phenol oxidase (degradation of phenolic compounds), was measured using 5 mM *L*-dihydroxyphenylalanine (*L*-DOPA) as substrate as described in Nottingham *et al.* (2012). All enzymes were measured in the forest experiment, but only β-glucosidase, *N*-acetyl β-glucosaminidase, and phosphomonoesterase in the *P. septenatum* experiment. Following sampling, soils were stored at 3 °C and assayed within 3 days, which does not appear to greatly alter observed activities in tropical forest soils (Turner & Romero, 2010).

Phospholipid fatty acids

PLFA were extracted from freeze-dried soils using the method described in Nottingham *et al.* (2009). PLFA fingerprints were identified and quantified by GC, and the

$\delta^{13}\text{C}$ values of PLFA were determined using gas chromatography–combustion–isotope ratio mass spectrometry (GC-C-IRMS). PLFA biomarkers were grouped as Gram-positive bacterial (15:0, i15:0, a15:0, i16:0, i17:0, a17:0, 7Me17:0), Gram-negative bacterial (16:1 ω 5, 16:1 ω 7, 17:1 ω 8, 7,9cy17:0, 18:1 ω 7, 7,8cy19:0, 19:1), fungal (18:2 ω 6, 18:1 ω 9), and nonspecific saturated (14:0, 16:0, 18:0; Frostegård & Bååth, 1993; Zelles, 1999).

Statistics and calculations

We used the natural abundance difference in $\delta^{13}\text{C}$ values of C4 soils and C3 plants (e.g. Fu & Cheng, 2002) to partition the mass of C in PLFA into ‘soil-derived’ and ‘plant-derived’ components. The mass of soil-derived C within individual PLFAs in AMF ingrowth or rhizosphere soil was provided by total PLFA-C concentration from no ingrowth control soils. The percentage of plant-derived C in PLFA in AMF ingrowth and rhizosphere soils was calculated according to:

$$\%C_{\text{plant-derived}} = \left[\frac{(\delta_C - \delta_T)}{(\delta_C - \delta_L)} \right] \times 100 \quad (1)$$

where δ_C is the $\delta^{13}\text{C}$ value of a specific PLFA from control soils, δ_T is the $\delta^{13}\text{C}$ value for the same PLFA from AMF ingrowth or rhizosphere soils, and δ_L is the $\delta^{13}\text{C}$ value of the plant-derived C input (e.g. Waldrop & Firestone, 2004). The $\delta^{13}\text{C}$ value for the plant-derived C input was determined by averaging $\delta^{13}\text{C}$ values for homogenized leaf and fine root samples for each tree: fine roots $\delta^{13}\text{C} = -27.8\text{‰}$ (SE 0.27); leaf $\delta^{13}\text{C} = -29.32\text{‰}$ (SE 0.22); thus ‘plant-derived’ $\delta^{13}\text{C}$ input to soil = -28.56‰ (SE = 0.61). The $\delta^{13}\text{C}$ values of plant leaves, organic acids, and storage carbohydrates are similar for well-watered plants (Ghashghaie *et al.*, 2001), so leaf $\delta^{13}\text{C}$ values are a reasonable approximation of root-derived inputs resulting from exudation of low-molecular-weight sugars and organic acids. Thus, we assumed an equal input of plant-derived C to soil from root turnover and exudation. For each individual PLFA, we calculated standard errors using the IsoError model (Phillips & Gregg, 2001), which accounts for the variability within $\delta^{13}\text{C}$ end-members used in mass balance calculations (C4 $\delta^{13}\text{C}$: control soil PLFA and C3 $\delta^{13}\text{C}$: leaves and fine roots).

Treatment effects on all ingrowth core parameters were analyzed using split-plot ANOVA, with ‘tree’ or ‘forest plot’ as the random nested factor and respective soil parameters as the fixed factor. Treatment effects on microbial community composition (PLFA concentration) were examined using principle components analysis to construct new variables from multivariate, normally distributed

(log-transformed) PLFA data. Prior to analysis, data were tested for normality using a Ryan–Joiner test and non-normal data were log-transformed. For all data, pair-wise comparisons were performed using Tukey *post hoc* analyses with significant interactions determined at $P \leq 0.05$. All statistical analyses were performed using MINITAB (version 15; Minitab Inc., PA).

Results

In the *P. septenatum* experiment, there were effects of AMF and roots on microbial community composition according to PLFA, which were summarized by multivariate analyses (Fig. 1) and treatment effects on the concentration of individual PLFA (Fig. 2). In the multivariate analyses, there was a clear separation along the x -axis according to treatment, with the largest differences between rhizosphere soils and soil-only controls (explaining 66% of variation) and along the y -axis (explaining 24% of variation; Fig. 1). Total PLFA abundance followed the trend AMF < soil-only < rhizosphere, with significantly higher concentrations in rhizosphere soils compared with AMF cores ($P = 0.04$), which was largely due to difference in total bacteria ($P = 0.02$) and, more specifically, Gram-positive bacteria ($P < 0.01$; Fig. 2). For individual PLFAs, there were higher PLFA concentrations for Gram-positive bacteria (i15:0, a15:0, i16:0) and saturated (16:0) biomarkers for rhizosphere soils compared with both soil-only and AMF cores ($P < 0.05$; data not shown).

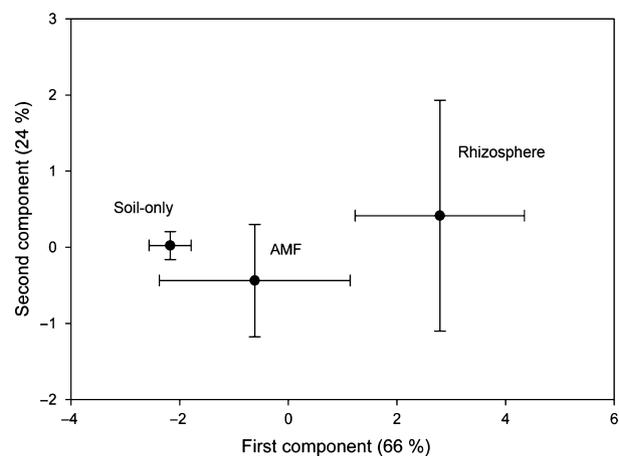


Fig. 1. Microbial community composition in AMF soils, rhizosphere soils, and soil-only controls for the *Pseudobombax septenatum* experiment. The results are from a principal components analysis of PLFA concentration and show the distribution of PLFA according to treatments. PLFA biomarkers were grouped as Gram-positive bacterial, Gram-negative bacterial, fungal, and nonspecific saturated according to Frostegård & Bååth (1993) and Zelles (1999).

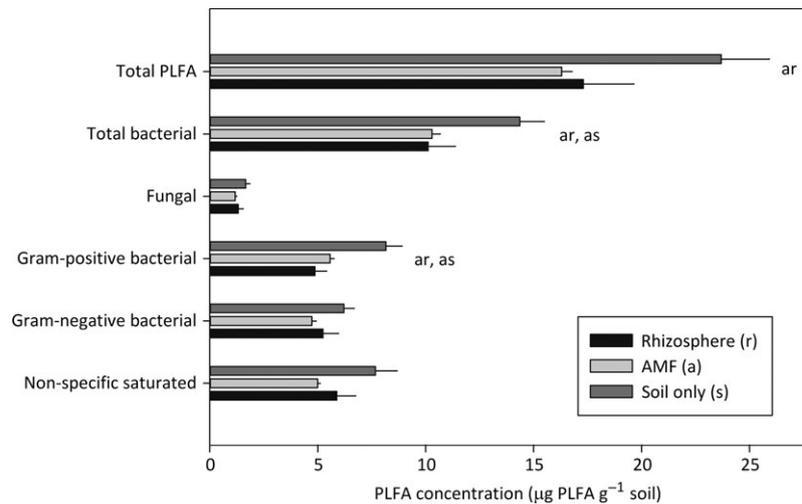


Fig. 2. Concentrations of PLFA carbon within soil microbial groups for the *Pseudobombax septenatum* experiment. The assignment of PLFA to microbial groups is described within the methods. Soils were sampled from soil-only and AMF cores, and the rhizosphere (root-adhered soil). Significant differences determined by Tukey HSD ($P \leq 0.05$) between treatment pairs are highlighted by letters (e.g. as = significant difference between AMF and soil-only treatments). Data are means \pm 1 standard error of six replicates per treatment.

The proximity of soil to roots and the presence of AMF led to changes in both soil-derived and plant-derived C in microbial groups (Fig. 3). The incorporation of soil-derived C into microbial groups was higher for total PLFA ($P = 0.05$), total bacterial ($P < 0.05$), and Gram-positive bacterial ($P < 0.01$) groups in rhizosphere compared with AMF soil (Fig. 3a). Plant-derived C was incorporated into all groups except for nonspecific saturated PLFA, with highest incorporation for rhizosphere soil, and the highest incorporation into Gram-positive bacterial groups for AMF and rhizosphere soils (Fig. 3b). For rhizosphere soils compared with soil-only controls, soil-derived C was higher by 17% in total PLFA, 13% in total bacteria, and 29% in Gram-positive bacteria. In contrast, for AMF soils compared with soil-only controls, soil-derived C was lower by 20% in total PLFA, 19% in total bacteria, 17% in Gram-positive bacteria, 21% in Gram-negative bacteria, and 41% in fungal groups (thus resulting in significantly higher soil-derived C within microbial groups in rhizosphere soils compared with AMF soils; Fig. 3).

The incorporation of soil-derived C in individual PLFAs differed significantly between treatments. In rhizosphere soil, soil-derived C incorporation was higher for Gram-positive (i15:0, a15:0, i16:0) and nonspecific saturated (16:0) biomarkers and lower for Gram-negative (7,9cy-17:0, 19:1) biomarkers compared with soil-only controls ($P < 0.01$). In AMF soil, soil-derived C incorporation was lower for Gram-negative (7,9cy-17:0, 18:1 ω 7, 19:1) and fungal (18:1 ω 9) biomarkers compared with soil-only controls ($P < 0.01$; Fig. 4a). There was plant-derived C incorporation in rhizosphere soil for

Gram-positive (i15:0, i16:0, 7Me-17:0, i17:0), Gram-negative (16:1 ω 7, 7,9cy-17:0, 18:1 ω 7, 19:1), and fungal (18:2 ω 6, 18:1 ω 9) PLFA biomarkers compared with soil-only controls ($P < 0.001$); and in AMF soil for Gram-positive (i15:0, i16:0, 7Me-17:0, i17:0, a17:0), Gram-negative (16:1 ω 7, 7,9cy-17:0, 18:1 ω 7, 19:1), and fungal (18:1 ω 9) PLFA biomarkers compared with soil-only controls ($P < 0.001$; Fig. 4b).

There were no consistent patterns in overall enzyme activities between treatments; each enzyme showed specific effects (Fig. 5). Phosphomonoesterase activity was higher in AMF cores when compared to all soil types (for all comparisons, $P < 0.01$). *N*-acetyl β -glucosaminidase activity was highest in rhizosphere soil, significantly higher than soil-only cores ($P < 0.05$). β -glucosidase activity was progressively higher in the order: soil-only < AMF < FR + AMF < rhizosphere, with significantly higher activity in FR + AMF cores and rhizosphere soil compared with soil-only controls ($P < 0.001$).

Fine roots and AMF also influenced soil mineral nutrient concentrations, with higher NO_3^- in FR + AMF cores ($P < 0.001$) and a trend for lower resin-extractable P in FR + AMF ($P = 0.07$) cores compared with soil-only controls (Table 1). Microbial C as determined by fumigation-extraction and microbial P showed no significant differences between treatments (Table 1).

In the forest, the litter standing crop after 3 months of decomposition followed the trend: FR + AMF < AMF < soil-only and was significantly lower for FR + AMF compared with soil-only cores ($P = 0.01$; Table 1). There were no significant treatment effects on enzyme activities, although we did note a consistent trend of higher

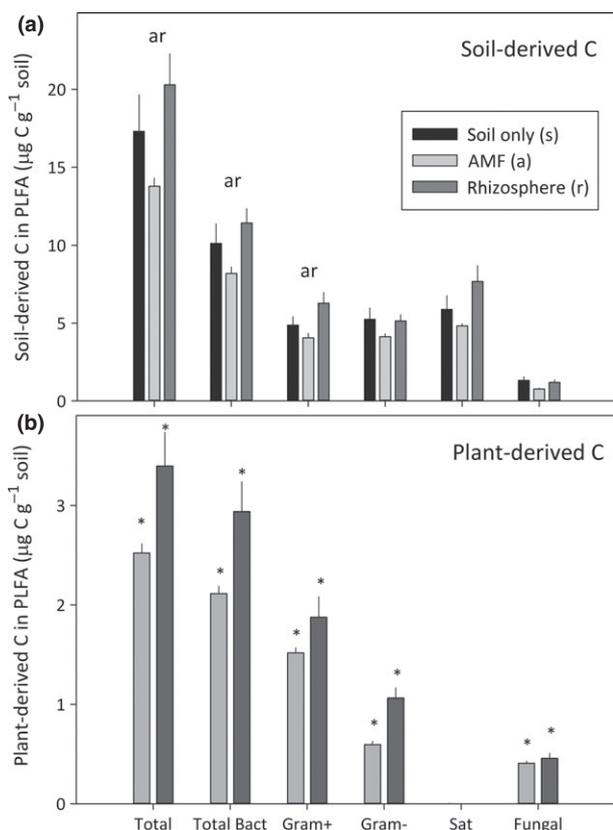


Fig. 3. Concentration of (a) soil-derived carbon and (b) plant-derived carbon within microbial groups for the *Pseudobombax septenatum* experiment. Measurements were performed following 7 months of root and AMF mycelial ingrowth. The assignment of PLFA to microbial groups is described within the methods. Microbial groups are: *Total*, *total bact* (total bacterial), *Gram+* (Gram-positive bacterial), *Gram-* (Gram-negative bacterial), *Sat* (nonspecific saturated), and *fungal*. Significant differences determined by Tukey HSD ($P \leq 0.05$) are highlighted (a) by letters between treatment pairs (e.g. sa = significant difference between soil-only and AMF treatments) and (b) by asterisks between treatments and soil-only. Data are means \pm 1 standard error of six replicates per treatment.

activities for all seven measured enzymes for FR + AMF and AMF cores compared with soil-only controls (14 comparisons; Table 1).

Discussion

Allocation of carbon from *P. septenatum* roots and AMF to soil microorganisms

The flux of C from *P. septenatum* to soil microorganisms was stimulated by both the presence of roots and AMF-only in soil (Fig. 3), thus supporting our first hypothesis that AMF are a significant pathway of C to soils for the tropical forest tree under study. Our objective was not to determine absolute C flow from plant to soil, but to

determine the relative importance of C flow from fine roots compared with AMF mycelia. Our results show that AMF mycelia alone were almost as influential as roots in supplying plant-derived C to the wider microbial community (Figs 3 and 4).

The significant C flow from roots and AMF to soil microbial communities was likely attributable to high plant C fixation, which may be typical of many tropical forest trees growing in full sunlight. Throughout our experiment, *P. septenatum* trees were in full sunlight and had high rates of diurnal C fixation; midday photosynthetic rates were $13.59 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ (SE 2.28; average of 11 leaves per tree, $n = 6$ trees; L. Cernusak, unpublished data), which is comparable to rates reported for rain forest trees (e.g. Malhi *et al.*, 1998 reported peak daytime rates of $24\text{--}28 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$). In addition, *P. septenatum* trees had high rates of xylem sap velocity (M. Tobin, unpublished data) and high rates of root-derived respiration (Nottingham *et al.*, 2010). Further experiments are required to measure the influence of roots and AMF on C flow to soil microorganisms in intact forest, which we did not measure in our forest experiment.

Our second hypothesis was that, due to this large deposition of plant-derived C, roots and AMF would indirectly affect organic matter mineralization by other soil microorganisms. This hypothesis was supported but our findings are nuanced, with complex influences indicated by changes in the microbial community composition, enzyme activities, priming of soil organic matter, and litter decomposition. We discuss this evidence below, separately considering root and AMF influences on these indices of organic matter cycling.

Root effects for *P. septenatum*: influence of rhizodeposition on microbial activity and community composition

The presence of fine roots affected several soil parameters specifically related to C mineralization in both experiments, which suggested a strong influence of rhizodeposition on microbial communities in these soils. Focusing first on the rhizosphere of *P. septenatum*, higher abundance of PLFA (notably Gram-positive bacteria biomarkers) and increased activities of β -glucosidase (hydrolysis of β -bonds in simple sugar dimers) and *N*-acetyl β -glucosaminidase (hydrolysis of chitooligosaccharides into amino sugars) strongly suggested the influence of rhizodeposition, C input through fine root turnover and exudations, in influencing the microbial community composition and activity.

Similar rhizosphere effects on microbial activity have been reported in studies performed in temperate forests. In a temperate forest plantation of mixed deciduous

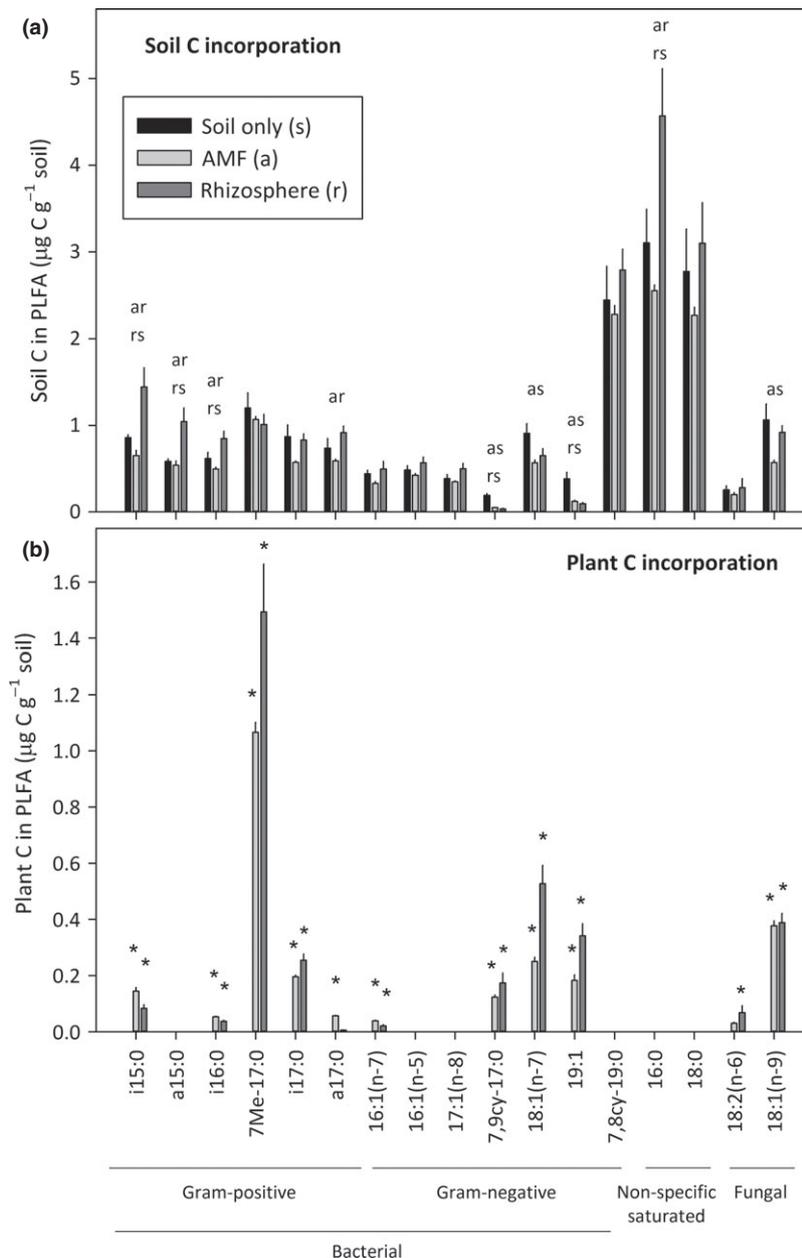


Fig. 4. The concentration of (a) soil-derived carbon and (b) plant-derived carbon within individual PLFA biomarkers for the *Pseudobombax septenatum* experiment. Measurements were performed following 7 months of root and AMF mycelial ingrowth. Significant differences determined by Tukey HSD ($P \leq 0.05$) are highlighted (a) by letters between treatment pairs (e.g. as = significant difference between AMF and soil-only treatments) and (b) by asterisks between treatments and soil-only. Data are means \pm 1 standard error of six replicates per treatment.

species, Phillips & Fahey (2006) measured higher microbial biomass, rates of C and N mineralization, and phosphatase activity in root-adhered compared with bulk soil. In a north European beech forest, Kaiser *et al.* (2010) measured a large reduction in enzymatic activity (protease and cellulase) following a girdling treatment to halt the flux of belowground root-derived C. Of the limited studies performed in lowland tropical forest, Toberman *et al.* (2011) reported higher microbial biomass and mass-specific respiration rates in root-adhered compared with bulk soil.

We found a large shift in microbial community composition in rhizosphere soil (Fig. 1) and, in particular, an increased abundance of Gram-positive bacterial biomarkers (Figs 2–4). A consistent finding in studies of various plant rhizospheres is an increased abundance of bacteria (Steer & Harris, 2000; Fierer *et al.*, 2007; Paterson *et al.*, 2007; Bird *et al.*, 2011). A meta-analysis of microbial communities in the rhizosphere of different plants found increased abundance of Gram-positive bacteria and *Proteobacteria* (Hawkes *et al.*, 2007). Together with our study, these findings suggest that rhizodeposition leads to

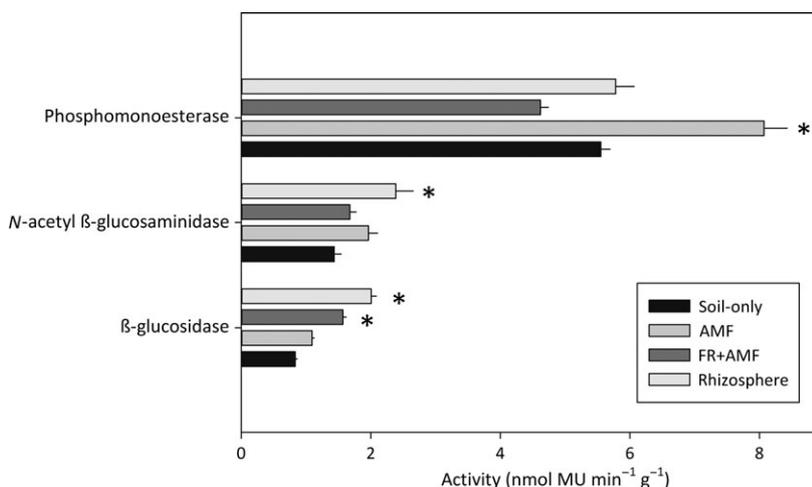


Fig. 5. Soil enzyme activities for the *Pseudobombax septenatum* experiment. Activities are for bulk soil from soil-only, AMF, and FR + AMF cores and for rhizosphere soil from FR + AMF cores. Significant differences determined by Tukey HSD ($P \leq 0.05$) between treatments and soil-only controls are indicated by asterisks. Data are means \pm 1 standard error of six replicates per treatment.

stimulation of similar bacterial communities across different plant functional types.

Root effects in the forest: influence of roots on litter decomposition

In the forest, the decreased litter mass in the presence of roots could be due to both direct nutrient acquisition by foraging roots (Hertel *et al.*, 2003; Sayer *et al.*, 2006) or stimulated rhizomicrobial activity (Subke *et al.*, 2004; Kuzyakov *et al.*, 2007). The lack of effect on litter mass loss in our 35- μ m mesh ingrowth cores, which likely included both AMF and saprotrophic fungi, indicated the direct influence of roots (rather than fungi) on litter mass loss. It is well known that roots in lowland tropical forests proliferate in organic horizons where present (Hertel *et al.*, 2003), and there is evidence for increased root foraging under increased litter inputs (Sayer *et al.*, 2006), which could in part explain our observation of increased litter mass loss in the presence of roots. An additional explanation is that litter decomposition was stimulated by rhizomicrobial activity, which is supported by our observation of stimulated microbial biomass and enzyme activity in the presence of roots for the *P. septenatum* experiment. The positive effect of rhizomicrobial activity on litter decomposition has been shown in a controlled study, in which litter decomposition was stimulated by the addition of artificial root exudates to soil (Kuzyakov *et al.*, 2007), and in a Spruce forest where the presence of roots increased the rate of litter-derived respiration (Subke *et al.*, 2004). Although the sensitivity of treatment effects in our litter decomposition study was likely compromised by the lack of new litterfall exclusion (future studies

should control for this), our findings do suggest a positive influence of rhizomicrobial activity on litter mass loss in a lowland tropical forest.

Root effects for *P. septenatum*: rhizomicrobial utilization of plant and soil carbon

It is well understood that bacterial microbial groups, which are typically copiotrophic (growing in high organic substrate conditions; Fierer *et al.*, 2007), are the first to metabolize easily available organic inputs to soil (Paterson *et al.*, 2007), which is consistent with our measurement of the highest concentration of plant-derived C (assumed to largely consist of labile root inputs) in bacterial biomarkers (Fig. 3b). Distinct groups of rhizosphere-inhabiting Gram-negative (18:1 ω 7) and Gram-positive (7Me-17:0) bacterial groups appeared to be active in utilizing plant-derived C (Fig. 4b). It is likely that a large proportion of low-molecular-weight plant-derived C inputs were rapidly metabolized and respired as CO₂, because rates of *P. septenatum* root-derived soil CO₂ efflux were high (222 ± 19 mg C m⁻² h⁻¹ from fine roots and 147 ± 17 mg C m⁻² h⁻¹ from AMF mycelia; Nottingham *et al.*, 2010), and in seedling studies, 50–60% of root-derived respiration was derived from rhizomicrobial respiration (Kuzyakov, 2002).

The significantly higher incorporation of soil-derived organic C in rhizosphere PLFA biomarkers when compared to soil-only controls is an indication of priming (Kuzyakov, 2010). Priming cannot be directly inferred because we did not measure changes in soil-derived CO₂ efflux in the presence of roots. However, rhizosphere priming was indirectly indicated by the microbial C

Table 1. Soil microbial C, nutrients, and enzyme activities for soil-only, AMF and fine root (FR + AMF) cores, and undisturbed soil (forest experiment only). Litter standing crop measured on September 17, 2007, after 3 months of decomposition within soil-only, AMF, and FR + AMF cores is shown for the forest (litter standing crop was 578 g m⁻² on 24 May)

	Soil only	AMF	FR + AMF
<i>P. septenatum</i>			
AMF hyphal length density (m g ⁻¹)	0.28 (0.04) ^a	1.32 (0.21) ^b	1.47 (0.30) ^b
Microbial C (mg kg ⁻¹)	132 (35)	87 (22)	161 (33)
NH ₄ ⁺ - N (mg kg ⁻¹)	0.62 (0.14)	0.17 (0.05)	0.19 (0.12)
NO ₃ ⁻ - N (mg kg ⁻¹)	0.76 (0.20) ^a	1.64 (0.30) ^{ab}	2.04 (0.43) ^b
Resin-extractable P (mg kg ⁻¹)	3.0 (0.1)	2.7 (0.1)	2.6 (0.2)
Microbial P (mg kg ⁻¹)	8.8 (0.5)	8.0 (0.3)	8.3 (0.4)
Forest			
AMF hyphal length density (m g ⁻¹)	0.15 (0.02) ^a	0.54 (0.07) ^b	0.46 (0.04) ^b
Litter standing crop (g m ⁻²)	293 (22) ^b	238 (26) ^{ab}	212 (24) ^a
β-glucosidase (nmol MU min ⁻¹ g ⁻¹)	2.74 (0.65)	2.86 (0.37)	3.23 (0.45)
Cellobiohydrolase (nmol MU min ⁻¹ g ⁻¹)	0.57 (0.16)	0.68 (0.14)	0.71 (0.15)
N-acetyl β-glucosaminidase (nmol MU min ⁻¹ g ⁻¹)	4.14 (0.81)	5.06 (0.87)	4.81 (0.69)
Phosphomonoesterase (nmol MU min ⁻¹ g ⁻¹)	21.54 (2.50)	24.40 (2.35)	25.84 (2.63)
Phenol oxidase (mg h ⁻¹ g ⁻¹)	0.17 (0.02)	0.19 (0.02)	0.18 (0.03)
Sulfatase (nmol MU min ⁻¹ g ⁻¹)	0.28 (0.05)	0.37 (0.05)	0.32 (0.05)
Xylanase (nmol MU min ⁻¹ g ⁻¹)	1.58 (0.21)	1.70 (0.13)	1.82 (0.19)

AMF, arbuscular mycorrhizal fungi.

Significant differences between treatments are indicated by different superscript letters (where $P \leq 0.05$). Values are means \pm 1 standard error ($n = 6$ for *Pseudobombax septenatum*, $n = 10$ for the forest).

balance and the higher concentration of soil-derived C in PLFA in rhizosphere soil compared with soil-only controls (higher by 17% or 3.0 $\mu\text{g C g}^{-1}$ soil). The increase in soil-derived C in PLFA in rhizosphere soil was similar to the additional incorporation of plant-derived C (3.4 $\mu\text{g C g}^{-1}$ soil; Fig. 3), which suggests similar influences on the microbial C balance by rhizosphere priming and rhizodeposition within the 6-month period of this experiment; although our study provides no information on the microbial turnover rate of C from these two sources. The additional incorporation of soil-derived C in rhizosphere microorganisms was highest in Gram-positive bacterial biomarkers (i15:0, a15:0, i16:0; Fig. 4a). The consistent finding of stimulated soil C utilization in plant rhizospheres by Gram-positive bacteria measured in different systems and on different time scales (Bird *et al.*, 2011; Fig. 4a) suggests that these microbial groups may be important in determining long-term soil C dynamics in the rhizosphere. However, given that a variety of microbial groups have been suggested to regulate priming in soil incubation experiments (Gram-negative bacteria: Waldrop & Firestone, 2004; Nottingham *et al.*, 2009; Fungi: Fontaine *et al.*, 2011; Shahzad *et al.*, 2012), the question of whether priming results from the activity of specific groups of microorganisms (rhizosphere or free-living) remains open. If specific microorganisms do metabolize and accumulate primed soil C, it is likely that various microbial groups are involved during successional

stages of decomposition (Kuzyakov, 2010) and redistribution of both plant-derived and soil-derived C occurs across trophic levels (Fitter *et al.*, 2005).

Priming effects have been hypothesized to occur in response to microbial nutrient limitation and, in particular, due to the acquisition of N from organic matter (Blagodatskaya & Kuzyakov, 2008; Dijkstra *et al.*, 2009). The increased activity of the N-degrading enzyme N-acetyl β-glucosaminidase in rhizosphere soils (Fig. 5) similarly indicated that rhizosphere priming may have occurred due to microbial N acquisition (Dijkstra *et al.*, 2009) and is consistent with a study of rhizosphere effects in *Avena* (wild oat), which similarly showed increased bacterial growth and N-acetyl β-glucosaminidase activity (DeAngelis *et al.*, 2008). A recent study has shown that N acquisition is critical in regulating priming even in strongly weathered soils where P availability is the dominant limitation on microbial activity (Sullivan & Hart, 2013; Nottingham AT, Turner BL, Stott A & Tanner EVJ, in review), which may similarly apply to the rhizosphere of tropical trees. The increased activity of N-acetyl β-glucosaminidase, which has been shown to correlate with ergosterol concentration and fungal biomass, may also indicate increased turnover of fungal residues (Burns & Dick, 2002). We found no evidence for higher fungal biomass in rhizosphere soils (Fig. 1), although this may be due to the insensitivity of PLFA as an AMF biomarker.

AMF effects: influence of AMF mycelia on nutrient acquisition and mineralization

The presence of AMF mycelia alone in soils increased activity of phosphomonoesterase for *P. septenatum* (Fig. 5), but not in the forest (Table 1). The absence of treatment effects on phosphomonoesterase in the forest may have been due to the presence of leaf litter for all treatments and controls, which led to high enzyme production during litter degradation (phosphomonoesterase activity was three times higher in the forest compared with *P. septenatum* soils). Thus, treatment effects were clearer in *P. septenatum* soils because the only organic matter input was due to the presence of either roots or AMF mycelia. It is well known that AMF can improve plant uptake of phosphate ions directly (Smith & Read, 2008), and some studies show evidence that AMF hydrolyze phosphomonoesters by producing extracellular phosphatases (Joner & Jakobsen, 1995; Koide & Kabir, 2000). Phosphomonoesterases hydrolyze phosphomonoesters biochemically, cleaving phosphomonoester bonds to leave the residual organic compound intact (Quiquampoix & Mousain, 2005), and therefore may have little impact on organic matter turnover. Indeed, we have no evidence that AMF affected rates of decomposition in the forest (Table 1). Phosphate hydrolysis by the extraradical mycelium of *Glomus intraradices* was demonstrated in axenic conditions by Koide & Kabir (2000), and stimulated phosphomonoesterase activity in the presence of AMF in pot studies was demonstrated by Phillips & Fahey (2006) and George *et al.* (2006) who measured stimulated activity of phosphomonoesterase in tree-rhizosphere soils when inoculated with AMF. We measured significant phosphomonoesterase activity in field soils with AMF but no roots, demonstrating that the external hyphae either directly produced phosphatases or stimulated phosphatase production by other microorganisms.

AMF effects: influence of AMF on microbial communities and carbon cycling

As obligate symbionts, AMF have no saprotrophic qualities, but they have been implicated in accelerating organic matter mineralization by indirectly affecting microorganisms inhabiting the 'hyposphere' (where bacteria thrive close to the hyphae on exuded and secreted hyphal C; Toljander *et al.*, 2007). In our study, AMF were clearly significant in supplying plant-derived C to the wider microbial community (Fig. 3b), which supports a recent study by Herman *et al.* (2012), who measured increased C flow into diverse microbial groups in the presence of AMF. However, the presence of AMF simultaneously suppressed activity of soil-C-decomposing microorganisms

(Fig. 3a) and had no effect on total microbial biomass, in contrast to the overall stimulation of microorganisms in rhizosphere soils (Fig. 2). This finding contrasts with a study that measured enhanced bacterial growth (=increased microbial biomass) in the presence of AMF exudates *in vitro* (Toljander *et al.*, 2007), but supports experiments that measured enhanced bacterial growth when AMF were excluded from soils (Burke *et al.*, 2002; Welc *et al.*, 2010) and reduced AMF growth when a bacteria inoculum was added to soil (Leigh *et al.*, 2011). Phosphorus competition was suggested by Leigh *et al.* (2011) to explain reduced AMF growth in the presence of bacteria. Similarly, competition for phosphate may have constrained microbial abundance in the presence of AMF in our study, which is supported by the abundance of water and N in our experiments (pots were watered daily; Table S1 for N values) and the large positive influence of AMF on phosphomonoesterase activity.

Conclusion

Our study demonstrates that tropical forest soil microbial communities and organic matter cycling are influenced by the presence of roots and AMF mycelia. For a tropical tree species, the supply of plant-derived C to the wider microbial community was stimulated by the presence of roots and, almost to the same extent, by AMF mycelia alone. Organic matter mineralization was influenced by the presence of roots (litter mass loss in a lowland tropical forest and indirect evidence for priming of soil C for a tropical tree species), at least in part by stimulating rhizosphere-inhabiting microorganisms. In contrast, we have no evidence that organic matter mineralization was influenced by the presence of AMF mycelia alone, although they did increase phosphomonoesterase activity. Our findings, which support studies performed in temperate systems, prompt further study of how different organisms influence organic matter cycling in tropical forest soils and demonstrate the need to consider root and mycorrhizal interactions when studying soil microbial communities in these ecosystems.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Total carbon, nitrogen and phosphorus, and pH in *P. septenatum* and forest soils.