

Arbuscular mycorrhizal mycelial respiration in a moist tropical forest

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Summary

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- Arbuscular mycorrhizal fungi (AMF) are widespread in tropical forests and represent a major sink of photosynthate, yet their contribution to soil respiration in such ecosystems remains unknown.
- Using in-growth mesocosms we measured AMF mycelial respiration in two separate experiments: (1) an experiment in a semi-evergreen moist tropical forest, and (2) an experiment with 6-m-tall *Pseudobombax septenatum* in 4.5-m³ containers, for which we also determined the dependence of AMF mycelial respiration on the supply of carbon from the plant using girdling and root-cutting treatments.
- In the forest, AMF mycelia respired carbon at a rate of 1.4 t ha⁻¹ yr⁻¹, which accounted for 14 ± 6% of total soil respiration and 26 ± 12% of root-derived respiration. For *P. septenatum*, 40 ± 6% of root-derived respiration originated from AMF mycelia and carbon was respired < 4 h after its supply from roots.
- We conclude that arbuscular mycorrhizal mycelial respiration can be substantial in lowland tropical forests. As it is highly dependent on the recent supply of carbon from roots, a function of aboveground fixation, AMF mycelial respiration is therefore an important pathway of carbon flux from tropical forest trees to the atmosphere.

Introduction

Arbuscular mycorrhizal fungi (AMF) form symbiotic associations with almost all trees in hyper-diverse tropical forests (Alexander & Lee, 2005), which are more productive than any other terrestrial ecosystem (Melillo *et al.*, 1993). These fungi are thought to rely solely on the host plant for provision of carbon (C) and may utilize as much as 5–20% of net photosynthate (Pearson & Jakobsen, 1993), while simultaneously benefiting the host through protection against root pathogenic fungi and improved uptake of nutrients, particularly phosphorus, from strongly weathered soils (Smith & Read, 1997; Alexander & Lee, 2005). Although presently overlooked, AMF could therefore be an important component of the carbon cycle in tropical forests.

Tropical forests not only fix more C through photosynthesis than any other terrestrial ecosystem (Melillo *et al.*, 1993), they also release more through respiration, over half

of which is released from soils (Chambers *et al.*, 2004). Soils in global tropical forests release *c.* 24 Pg C yr⁻¹ into the atmosphere in soil CO₂ efflux (Raich *et al.*, 2002). Soil CO₂ efflux arises from the activity of a wide variety of below-ground organisms, but only two sources have been assessed in tropical forests: (1) ‘autotrophic respiration’, derived from roots, arbuscular mycorrhizal fungi (AMF) and the metabolism of root exudates by microorganisms inhabiting the rhizosphere, and (2) ‘heterotrophic respiration’, derived from microbial decomposition of plant detritus and soil organic matter (Hanson *et al.*, 2000). Respiration from roots and that from AMF are almost always assessed together as a single source of respiration (Hanson *et al.*, 2000; Subke *et al.*, 2006), yet their assessment as distinct sources is required given that they may respond somewhat independently to environmental change (Alberton *et al.*, 2005).

The significance of AMF as a sink of plant C and a source of soil CO₂ efflux has been revealed in studies of temperate

plant communities. The dependence of AMF respiration on current photosynthetic activity was indicated by a pulse-labelling experiment in temperate grassland where 4–6% of photo-assimilates were respired by the mycelia of AMF within 21 h of fixation (Johnson *et al.*, 2002). The quantity of plant C respired by AMF was indicated by the 16% increase in soil CO₂ efflux found when *Lolium perenne* roots were colonized by AMF (Grimoldi *et al.*, 2006) and by the finding that 8% of soil CO₂ efflux was derived from the mycelia of AMF in a barley (*Hordeum vulgare*) field (Moyano *et al.*, 2007). To date, no studies have quantified the flux of CO₂ released by AMF in a tropical forest. Additionally, very few studies have quantified seasonal patterns in root and free-living microbial sources of soil CO₂ efflux in tropical forest. Knowledge of the relative importance of these two sources of CO₂ is vital to understand the components of net ecosystem production and the forest C balance (Hanson *et al.*, 2000; Kuz'yakov, 2006; Subke *et al.*, 2006).

Here we report the results from two separate experiments designed to quantify AMF mycelial respiration in tropical forest soils in the Republic of Panama. First, we measured the contribution of AMF mycelia, roots and free-living microorganisms to soil CO₂ efflux in a semi-evergreen moist tropical forest over 9 months. Secondly, under more controlled conditions, we measured AMF mycelial and root respiration for 6-m-tall individuals of the tropical tree *Pseudobombax septenatum* grown in large (*c.* 4.5-m³) containers. For *P. septenatum*, we also determined the dependence of AMF mycelial respiration on C supplied from recent photosynthate and root nonstructural carbohydrates by measuring the reduction in CO₂ efflux after girdling trees and then after cutting around each mesocosm to sever root and AMF mycelial connections to the tree. As AMF have been shown to consume substantial portions of photosynthate in temperate studies (e.g. 5–20% in Pearson & Jakobsen, 1993; 8% in Grimoldi *et al.*, 2006) and are

considered abundant in the soils of hyper-diverse tropical forests (Alexander & Lee, 2005), we hypothesized that AMF mycelia would be a significant source of CO₂ from tropical forest soils.

Materials and Methods

In-growth mesocosm design

Mesh-walled in-growth mesocosms were used to partition sources of belowground respiration into three components according to the following size classes of in-growth: roots, AMF mycelia, and free-living soil microorganisms (Fig. 1) (Johnson *et al.*, 2001). Treatments were fine-root and mycelia in-growth (2-mm mesh; 'FR + AMF'), mycelia in-growth (35- μ m mesh; 'AMF') and soil-only controls (either rotated 35- μ m mesh or 1- μ m mesh; 'CTL'; see the 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). Mesocosms were inserted to 20 cm depth in the soil, because this is the source of the majority of microbial soil CO₂ efflux (e.g. *c.* 80% for a forest in Costa Rica; Veldkamp *et al.*, 2003) and contains the majority of fine roots (in a nearby forest Cavellier (1992) estimated that > 90% of fine roots to 100 cm depth were in the top 25 cm), which dominate root respiration (Pregitzer *et al.*, 1998; Desrochers *et al.*, 2002).

Control mesocosms had 35- μ m mesh and were rotated 180° every week for the *P. septenatum* experiment, and had 1- μ m mesh and were static for the forest experiment. 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 as a consequence of poor drainage through mesh with a small diameter. However, preli-

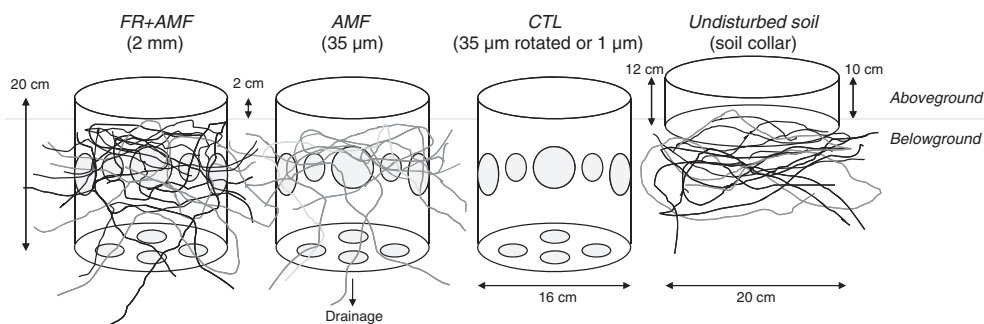


Fig. 1 In-growth mesocosms were made from PVC and nylon mesh. Treatments were 2-mm mesh (in-growth of fine roots (FR) and arbuscular mycorrhizal fungal (AMF) mycelia; 'FR + AMF') and 35- μ m mesh (in-growth of AMF mycelia; 'AMF'), and soil-only controls (no in-growth; 'CTL') were rotated 35- μ m mesh for *Pseudobombax septenatum* trees and 1- μ m mesh for the forest. Four holes (6-cm-diameter) were cut into the base and eight holes (four 6-cm-diameter and four 3-cm-diameter) were cut into the side; these holes were fitted with nylon mesh using hot silicon glue and high-strength duct tape. Undisturbed soil CO₂ efflux was measured over soil collars in the forest (undisturbed). The figure is not to scale.

minary tests indicated that the rotated design was suitable for soils in *P. septenatum* pots, but not for the forest site. For *P. septenatum*, mesocosms could be rotated freely and in a preliminary experiment there was no significant difference in soil CO₂ efflux between rotated and nonrotated 35- μ m mesh in-growth free mesocosms when measured 1 wk following rotation ($P > 0.05$, $n = 6$); whereas in the clay-loam forest soils, either mesocosms 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 mesocosms to exclude hyphae (e.g. Moyano *et al.*, 2007), which drained to field capacity (there was no significant difference in soil moisture in mesocosms compared with undisturbed forest soils during the wet season; Fig. 2), and excluded *c.* 72% of hyphal growth. In *P. septenatum*

containers, rotated 35- μ m mesh mesocosms excluded *c.* 79% of hyphal growth (compare hyphal length in CTL and AMF mesocosms for both experiments in Table 1; the hyphae present in CTL mesocosms may have included dead hyphae already in soils before the start of the experiment).

Experimental design: forest

The forest under study is mature (> 60 yr old) secondary seasonal moist tropical forest in the Republic of Panama. The site receives a mean annual rainfall of *c.* 2455 mm, with a 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 *c.* 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 (udalfs). The soils have a clay loam texture and are derived from marine sediments; total organic C, total nutrients and pH are listed in Table 2. 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 four areas for measurement of soil CO₂ efflux: three mesocosms (FR + AMF, AMF and CTL) and one soil collar (undisturbed soil) – a total of 30 mesocosms and 40 sampling areas for soil CO₂ efflux. In the forest plots we excavated 16-cm-diameter holes at mesocosm locations 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 mesocosms at a bulk density similar to that of forest soils. Soil-filled mesocosms were then reinserted into the forest at the same locations where soils were removed and any gap remaining around each mesocosm was refilled using the same soil from the appropriate depth. Soils were removed from the forest for a total of only 4 days and were kept at field moisture, but were disturbed during root removal, which therefore almost eliminated CO₂ efflux attributable to decomposition of observable dead roots. At the start of the experiment in May 2007, each mesocosm received 11.1 g (dry mass) of litter standing crop, equivalent to average litter standing crop across all plots measured in May 2007. Two weeks before measurements began in September 2007, mesocosm litter standing crop was harvested for a separate experiment and replaced with a further 7.3 g of litter standing crop, equivalent to average litter standing crop across all plots measured in September 2007.

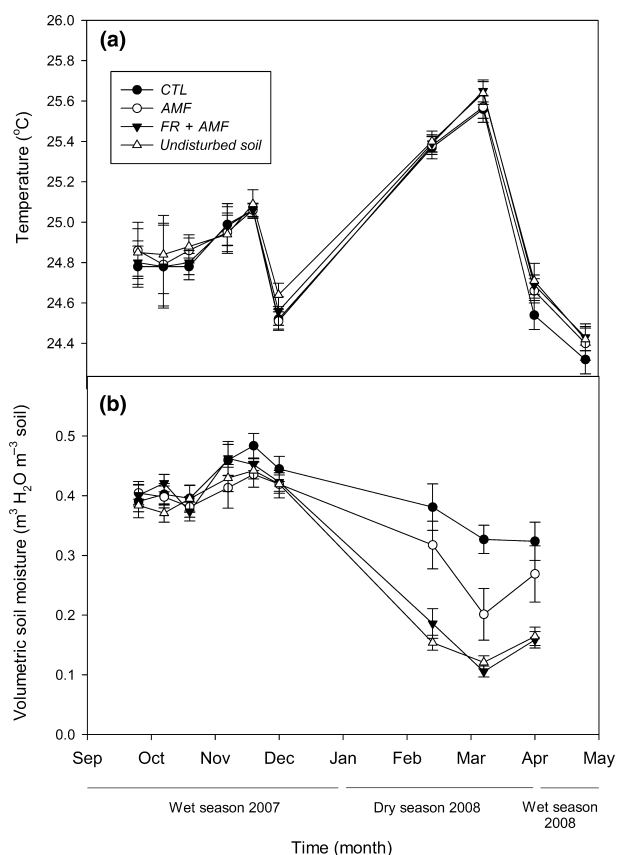


Fig. 2 Forest soil temperature (a) and soil moisture (b) for mesocosms and undisturbed soil in the tropical forest. Soil temperature was measured at 5 cm depth and volumetric soil moisture was measured at 0–6 cm depth. Data were collected immediately after soil CO₂ efflux data from September 2007 to May 2008. Missing data points resulted from equipment malfunction; soil moisture determined in June 2008 using gravimetric methods did not differ significantly between treatments (not shown in figure). Data are means \pm 1 SE ($n = 10$ for undisturbed soil and for in-growth of fine roots (FR) and arbuscular mycorrhizal fungal (AMF) mycelia ('FR + AMF'); $n = 6$ for in-growth of AMF mycelia ('AMF'); $n = 9$ for no in-growth control ('CTL')).

Table 1 Bulk density and abundance of fine roots (FR) and arbuscular mycorrhizal fungi (AMF) for mesocosm soil and undisturbed forest soil measured at the end of each experiment

	Forest			<i>Pseudobombax septenatum</i>		
	Bulk density (g cm ⁻³)	Fine-root biomass (g kg ⁻¹)	AMF hyphal length (m g ⁻¹)	Bulk density (g cm ⁻³)	Fine-root biomass (g kg ⁻¹)	AMF hyphal length (m g ⁻¹)
CTL	0.54 ± 0.07 ^a	0.00 ± 0.00 ^a	0.15 ± 0.02 ^a	0.91 ± 0.02	0.00 ± 0.00 ^a	0.28 ± 0.04 ^a
AMF	0.57 ± 0.06 ^a	0.00 ± 0.00 ^a	0.54 ± 0.07 ^b	0.92 ± 0.02	0.00 ± 0.00 ^a	1.32 ± 0.21 ^b
FR + AMF	0.63 ± 0.05 ^a	3.93 ± 0.52 ^b	0.46 ± 0.04 ^b	0.90 ± 0.02	1.35 ± 0.04 ^b	1.47 ± 0.30 ^b
Undisturbed soil	0.76 ± 0.05 ^b	3.80 ± 0.47 ^b				

Significant differences between treatments according to split-plot ANOVA are indicated by different lowercase letters (where $P < 0.05$). Data are means and error bars are 1 SE (for *P. septenatum*, $n = 6$; for the forest: control (CTL), $n = 9$; AMF, $n = 6$; FR + AMF, $n = 10$; undisturbed soil, $n = 10$; CTL and AMF mesocosms with significant root in-growth are not reported).

Experimental design: *Pseudobombax septenatum*

Six plants of *Pseudobombax septenatum* (Bombacaceae) were planted in large containers (1.8 m diameter and 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 with the first rains in April/May; all trees were in full leaf throughout the experiment.

Containers were filled with soil collected from 0 to 20 cm depth in a nearby orchard in 2004. Mesocosm soil for the *P. septenatum* experiment were different from the container soil because we originally intended to use differences in $\delta^{13}\text{C}$ signatures of C3 plants and C4 soils (Ineson *et al.*, 1996) to partition sources of soil CO₂ efflux in further detail. However, the technique did not work because the $\delta^{13}\text{C}$ signatures of the C3 and C4 sources were not sufficiently different. Soil for the mesocosms was collected from 0 to 20 cm depth from a recently reforested pasture (see Wilsey *et al.*, 2002 for details of this site) in December 2006. It was sieved (5 mm) and stored at field moisture for a few days and then mixed with sand (70 : 30, soil:sand); the sand had minimal organic C and CaCO₃ and had a similar pH to that of soil (sand pH 6 and soil pH 5.5). Total organic nutrients in container and mesocosm soils were similar (Table 2) and a preliminary analysis of spores

in both soils showed a relatively high abundance of spores from the genus *Glomus*.

Mesocosms 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. Two replicates of each of the three mesocosm treatments (2 × CTL, 2 × AMF and 2 × FR + AMF) were installed in each container, resulting in a total of six mesocosms in each container. Soil CO₂ efflux data were averaged for each pair of mesocosms with a container, with the resulting average value representing a single experimental replication to give $n = 6$ (containers) per treatment (or $n = 3$ when including the girdling effect; see methods section 'soil CO₂ efflux'). During intensive measurements (*c.* 6 months following mesocosm installation; 3 d before and 12 d after a girdling treatment), containers were covered with large tarpaulin sheets at night and during daytime rain showers to prevent water-logging but were watered with *c.* 50 l of water each day per container at the end of measurements, water being applied equally to mesocosm and surrounding soils using a hose. Tarpaulin sheets were removed at least 2 h before measurements to allow soil surface CO₂ gradients to equilibrate.

Fine-root biomass and AMF hyphal length

Fine-root biomass (for all mesocosm soils) and AMF hyphal length (for a representative subsample of mesocosm soils) were determined for all mesocosms at the end of each exper-

Table 2 Total carbon (C), nitrogen (N) and phosphorus (P), and pH in *Pseudobombax septenatum* and forest soils

	C (g kg ⁻¹)	N (g kg ⁻¹)	P (mg kg ⁻¹)	C:N	C:P	N:P	pH
<i>P. septenatum</i> mesocosms	12.5	1.2	167	10	75	7	5.7
<i>P. septenatum</i> containers	12.7	1.1	256	11	50	4	5.7
Forest	40.1	3.5	278	11	144	13	6.3

All soils were sampled from 0 to 10 cm depth. Soils in *P. septenatum* mesocosms were a soil:sand mixture (70 : 30) and the values reported are for the mixture; the mesocosm soil was different from the soil in the containers (see Materials and Methods section for details). Data are single analyses of pooled subsamples.

iment. For *P. septenatum*, two out of 12 AMF mesocosms contained fine roots and for the forest, four AMF and one CTL mesocosm contained fine roots; all these mesocosms were eliminated from subsequent analyses. Hyphae were extracted using an aqueous extraction and membrane filter technique and stained with trypan blue, and hyphal length was determined using the grid line intersect method where AMF hyphae were distinguished from non-AMF hyphae by the presence of nonregular septa, dichotomous branching, irregular wall thickness and/or connection to chlamydospores (Rillig *et al.*, 2002).

Soil CO₂ efflux

Soil CO₂ efflux was measured using a Li-8100 soil CO₂ flux system (Li-Cor, Lincoln, NE, USA). Undisturbed soil CO₂ efflux in the forest was measured over the 20-cm-diameter, 12-cm-deep PVC soil collars, which were inserted to 2 cm depth at least 1 month before measurements began and remained fixed throughout the experiment (Fig. 1). To enable measurements of mesocosm CO₂ efflux, a soil collar was made from PVC, 13 cm deep, that fitted tightly to the rim of each mesocosm at the base (16 cm diameter) and to the Li-8100 soil chamber at the top (20 cm diameter). The Li-8100 was calibrated to account for the additional volume. Immediately following all soil CO₂ efflux measurements, soil temperature and volumetric soil moisture were measured at 5 and 0–6 cm depths, respectively. Volumetric soil moisture was measured using a thetaprobe (Delta-T Devices, Cambridge, UK), which was calibrated to soil types following the procedure described by the manufacturer.

For the forest site, CO₂ efflux was measured between 07:00 and 13:00 h for all treatments and controls in all 10 plots every 2 wk from 17 September 2007 to 17 June 2008 and at least 12 h after any rainfall event. No measurements were made for 3 months immediately following mesocosm installation to allow any increased soil CO₂ efflux following soil aeration to subside and to allow time for in-growth of fine roots and mycelia; Cavalier (1989) reported that it took 5 months (including the whole of the dry season) for 9-cm-diameter 'ingrowth cores' to reach maximum root biomass (< 2-mm-diameter roots) in a nearby forest.

For *P. septenatum*, mesocosm CO₂ efflux was measured between 07:00 and 12:00 h each day for 2 wk when the trees were in full leaf (24 October to 12 November 2007); *c.* 6 months following installation of mesocosms. On the day of girdling (27 October), soil CO₂ efflux data were collected in the morning, three trees were girdled around midday, and a further set of CO₂ efflux data were collected in the late afternoon. Trees were stratified according to the average FR + AMF mesocosm CO₂ efflux measured in the morning and randomly allocated to girdled or nongirdled. Two weeks following girdling, one of each mesocosm treatment was removed from each container, thus cutting all

root and AMF mycelial connections. Mesocosm CO₂ efflux was measured immediately before removal and then 10 min, 120 min, 240 min and 24 h afterwards. We measured CO₂ efflux in paired cut and uncut mesocosms before and 24 h after removal of cut mesocosms.

Calculations and statistics

The percentage contributions of fine roots (root), AMF mycelia (mycelial) and free-living microorganisms (microbial) to the CO₂ efflux (mg C m⁻² h⁻¹) from root-ingrowth (FR + AMF_{efflux}) mesocosms were calculated as follows:

$$\text{Roots}(\%) = (\text{FR} + \text{AMF}_{\text{efflux}} - \text{AMF}_{\text{efflux}}) / \text{FR} + \text{AMF}_{\text{efflux}} \times 100 \quad \text{Eqn 1}$$

$$\text{Mycelial}(\%) = (\text{AMF}_{\text{efflux}} - \text{CTL}_{\text{efflux}}) / \text{FR} + \text{AMF}_{\text{efflux}} \times 100 \quad \text{Eqn 2}$$

$$\text{Microbial}(\%) = \text{CTL}_{\text{efflux}} / \text{FR} + \text{AMF}_{\text{efflux}} \times 100 \quad \text{Eqn 3}$$

For forest soils, FR + AMF_{efflux} is CO₂ efflux measured for FR + AMF mesocosms, AMF_{efflux} is CO₂ efflux measured for AMF mesocosms and CTL_{efflux} is CO₂ efflux measured for CTL mesocosms (see Heinemeyer *et al.*, 2007 for a similar approach). Soil CO₂ efflux measured for soil collars (undisturbed soil_{efflux}) was multiplied by the results from Eqns 1–3 to estimate the absolute contributions of fine roots, AMF mycelia and free-living microorganisms. Thus, of total soil CO₂ efflux (undisturbed soil_{efflux}), 'roots' is the portion derived from fine roots and rhizosphere-dwelling microorganisms (Eqn 1), 'AMF' is the portion derived from AMF mycelia and hyphosphere-dwelling microorganisms (Eqn 2), and 'microbial' is the portion derived from free-living soil microorganisms (Eqn 3). For *P. septenatum*, Eqns 1 and 2 only were used to determine the contribution of AMF mycelia to 'root-derived CO₂ efflux'. Root-derived CO₂ efflux (root + mycelial) is the respiration by roots and any organism using C recently derived from roots (i.e. mycorrhizal fungi and rhizosphere microorganisms; Kuzyakov, 2006). For each sampling date and for each plot (for the forest) or tree (for *P. septenatum*), we calculated respiration components and calculated averages and standard errors according to the variation in calculated components among all plots/trees.

Variations in CO₂ efflux, soil temperature and soil moisture were compared between undisturbed soil (in forest), FR + AMF, AMF and CTL treatments and partitioned root, mycelial and microbial components using split-plot repeated measures ANOVA (*n* = 10 per treatment for the forest). For *P. septenatum* the analysis was initially completed with 'girdle effect' as an additional fixed main plot factor (*n* = 3). However, girdled and nongirdled mesocosms were finally pooled because there was no girdling effect

for soil CO₂ efflux (therefore $n = 6$ per treatment for *P. septenatum*). Dependent variables were CO₂ efflux, soil temperature and soil moisture and were individually analyzed with mesocosm treatment and time as the fixed main plot factors, which were nested with either individual tree (*P. septenatum*) or plot (forest). For *P. septenatum*, root-cutting effects on FR + AMF and AMF mesocosm CO₂ efflux were analyzed using the same method with time as the fixed main plot factor, which was nested within individual tree. Analyses were performed with all data included (September 2007 to June 2008) and then separately for late wet season 2007 (September–December 2007), dry season 2008 (January–April 2008) and early wet season 2008 (May–June 2008). Treatment effects on fine-root biomass and AMF hyphal length were analyzed using split-plot ANOVA, with fine-root biomass or AMF hyphal length as the fixed factor, which was nested within either individual tree (*P. septenatum*) or plot (forest). We investigated the relationships between CO₂ efflux and soil temperature and soil moisture for each mesocosm by respectively fitting exponential and log-normal models using SIGMAPLOT (version 10; Systat Inc., Chicago, IL, USA). Pair-wise comparisons were performed using Tukey post-hoc analyses and significant interactions were determined at $P \leq 0.05$. Before analysis, data were tested for normality using Ryan–Joiner tests and nonnormal data were log-transformed. All data are presented as mean \pm 1 SE. All statistical analyses were performed using MINITAB (version 15; Minitab Inc., State College, PA, USA).

Results

In-growth mesocosms and the soil abiotic environment

Mesocosms generally excluded fine roots and AMF where appropriate (Table 1). Soil temperature did not differ significantly among mesocosm treatments and undisturbed soils over the entire year in the forest ($P > 0.05$; or when only dry or wet season data were included $P > 0.05$; Fig. 2a) or in *P. septenatum* soils ($P > 0.05$; average soil temperature over 2 wk was $29 \pm 1^\circ\text{C}$ for soils in all mesocosms). Bulk density did not differ significantly among mesocosm treatments in either experiment ($P > 0.05$ for all comparisons), but was significantly lower in forest mesocosms compared with undisturbed soil ($P = 0.05$; Table 1).

Moisture in forest soils did not differ significantly among treatments over the entire year ($P > 0.05$; Fig. 2b). However, during the dry season (January–April 2008), soil moisture was significantly lower in FR + AMF mesocosms and undisturbed soil when compared with AMF mesocosms ($P < 0.05$ for both comparisons) and soil-only controls ($P < 0.01$ for both comparisons). Dry season soil moisture was not significantly different between FR + AMF meso-

cosms and undisturbed soil ($P > 0.05$) or between AMF and CTL mesocosms ($P > 0.05$). Soil moisture in *P. septenatum* mesocosms decreased in the order CTL > AMF > FR + AMF, with a significant difference between CTL and FR + AMF ($P < 0.05$); average values of volumetric soil moisture in mesocosms measured over 2 wk were 0.311 ± 0.018 , 0.304 ± 0.017 , and $0.266 \pm 0.026 \text{ m}^3 \text{ H}_2\text{O m}^{-3} \text{ soil}$, respectively.

The physical structure of soils was affected by mesocosms during the dry season in the forest only. Small cracks appeared between soils and the inner circumference of all mesocosm treatments during the dry season, which disappeared soon after the first rains of the wet season. Cracking severity was scored on a scale of 1–5 for all mesocosms on 1 March 2008 and there was no significant difference in cracking severity among mesocosm treatments ($P > 0.05$).

Tropical forest soil CO₂ efflux

For the tropical forest site, soil CO₂ efflux varied significantly over the year, from $115 \pm 9 \text{ mg C m}^{-2} \text{ h}^{-1}$ in March 2008 during the dry season to $248 \pm 11 \text{ mg C m}^{-2} \text{ h}^{-1}$ in June 2008 in the early wet season ($P < 0.001$; see undisturbed soil in Fig. 3). Soil and mesocosm CO₂ efflux decreased in the order FR + AMF > undisturbed soil > AMF > CTL. The CO₂ efflux from FR + AMF mesocosms followed the same pattern as undisturbed soil CO₂ efflux, except during the dry season, when CO₂ efflux was much higher from FR + AMF mesocosms compared with undisturbed soils (Fig. 3a).

Mycelial CO₂ efflux tended to increase during the year but there was no significant seasonal variation (Fig. 3b; $P > 0.05$); average values were $1 \pm 8 \text{ mg C m}^{-2} \text{ h}^{-1}$ during the late wet season of 2007, $19 \pm 9 \text{ mg C m}^{-2} \text{ h}^{-1}$ during the dry season of 2008 and $32 \pm 15 \text{ mg C m}^{-2} \text{ h}^{-1}$ during the early wet season of 2008. During the dry and early wet seasons of 2008, mycelia contributed $c. 14 \pm 6\%$ and $14 \pm 6\%$ of FR + AMF mesocosm CO₂ efflux or $23 \pm 10\%$ and $29 \pm 13\%$ of 'root-derived' (root + mycelial) CO₂ efflux, respectively.

Root CO₂ efflux did not vary significantly throughout the year, with an average of $64 \pm 8 \text{ mg C m}^{-2} \text{ h}^{-1}$, constituting $c. 58 \pm 7$, 63 ± 9 and $77 \pm 10\%$ of FR + AMF mesocosm CO₂ efflux during the late wet season of 2007, the dry season of 2008 and the early wet season of 2008, respectively ($P > 0.05$; Fig. 3b).

Microbial CO₂ efflux varied significantly throughout the year ($P < 0.001$), from $105 \pm 4 \text{ mg C m}^{-2} \text{ h}^{-1}$ during the late wet season in 2007, to $51 \pm 4 \text{ mg C m}^{-2} \text{ h}^{-1}$ during the dry season of 2008, to $107 \pm 4 \text{ mg C m}^{-2} \text{ h}^{-1}$ during the early wet season of 2008. Microbial CO₂ efflux constituted 64 ± 2 , 38 ± 3 and $46 \pm 2\%$ of FR + AMF mesocosm CO₂ efflux during the late wet season of 2007, the dry season of 2008 and the early wet season of 2008, respectively.

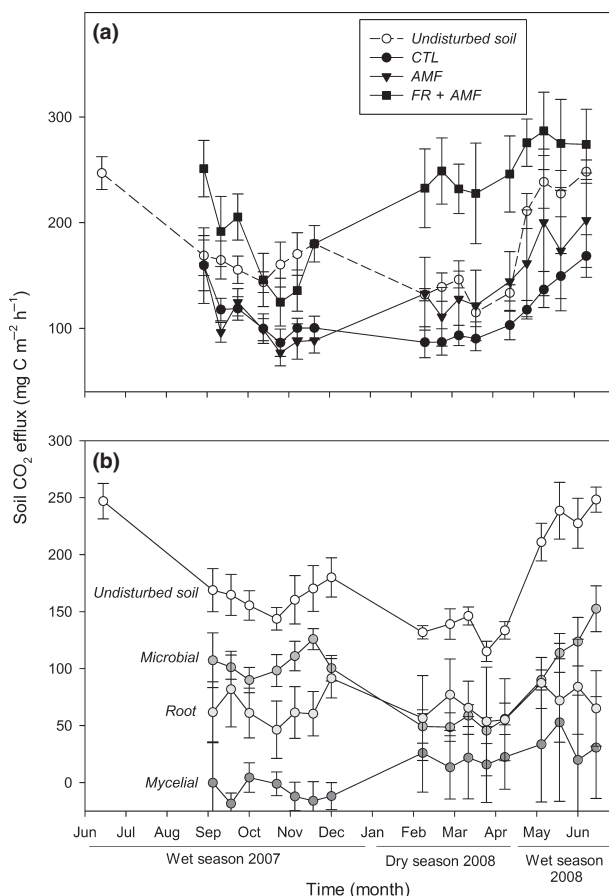


Fig. 3 Tropical forest soil CO₂ efflux partitioned into microbial, arbuscular mycorrhizal fungi (AMF) and fine-root (FR) components. (a) Soil CO₂ efflux from undisturbed soils measured over 1 yr from June 2007 to June 2008 and from control (CTL), AMF and FR + AMF mesocosms measured over 9 months from September 2007 to June 2008. (b) Partitioned components of soil CO₂ efflux over 9 months from September 2007 to June 2008. The 'microbial', 'mycelial' and 'root' components of undisturbed soil CO₂ efflux were calculated using values for mesocosm CO₂ efflux in (a) and Eqns 1–3. In (b) undisturbed soil = fine root + AMF + microbial. Of the respiration components shown in (b), undisturbed soil and microbial varied significantly over time according to repeated measures ANOVA (where $P < 0.05$). Data are means and error bars are 1 SE ($n = 10$ for undisturbed soil and FR + AMF/root; $n = 6$ for AMF/mycelial; $n = 9$ for CTL/microbial).

Mycelial, root, and microbial sources of CO₂ efflux were significantly different from each other over the year ($P < 0.001$), but when data were grouped according to dry and wet seasons there was no significant difference between root and microbial CO₂ effluxes during the dry season ($P > 0.05$).

Soil CO₂ efflux was not significantly affected by soil temperature for any of the treatments, either overall or for the dry or wet season alone. There was no significant effect of soil moisture on soil CO₂ efflux for any treatment, although it is notable that the highest rates of soil CO₂ efflux occurred at the beginning of the wet season (see May/June; Fig. 3).

Pseudobombax septenatum fine-root and AMF CO₂ efflux

For *P. septenatum*, soil CO₂ efflux decreased in the order FR + AMF > AMF > CTL, with significant differences among treatments ($P < 0.001$). Average soil CO₂ efflux measured above FR + AMF, AMF and CTL mesocosms was 611 ± 28 , 389 ± 24 and 239 ± 11 mg C m⁻² h⁻¹, respectively (Fig. 4a). Using Eqns 1 and 2 we calculated that root CO₂ efflux was 222 ± 19 mg C m⁻² h⁻¹ and mycelial CO₂ efflux was 147 ± 17 mg C m⁻² h⁻¹. Thus, root-derived (root + mycelial) CO₂ efflux was $40 \pm 6\%$ from mycelia and $60 \pm 6\%$ from roots.

Girdling had no significant effect on CO₂ efflux or soil moisture; there were no significant differences in CO₂ efflux or soil moisture for FR + AMF, AMF, or CTL mesocosms between girdled and nongirdled trees ($P > 0.05$ for all comparisons; data not shown). Root-cutting, on the other hand, caused a large and very significant reduction (*c.* 75%) in CO₂ efflux in < 10 min for both fine roots and mycelia ($P < 0.001$; Fig. 4b). We estimate that *c.* 28% and 44% of this reduction, for FR + AMF and AMF mesocosms, respectively, was attributable to the loss of CO₂ diffusing from the soil in the large containers (the quantity of CO₂ diffusing from soil in the large containers was estimated by the reduction in CO₂ efflux following removal of CTL mesocosms; 173 ± 9 mg C m⁻² h⁻¹). We calculate that severing roots and hyphae reduced respiration to negligible levels after 4 h for roots and after 2 h for mycelia (the point at which FR + AMF and AMF = CTL in Fig. 4b).

There were no significant relationships between soil CO₂ efflux and either soil temperature or soil moisture for all mesocosms and for both girdled and nongirdled trees in the *P. septenatum* experiment (for all cases $R^2 < 0.01$, $P > 0.05$; data not shown).

Discussion

AMF mycelia as a source of CO₂ efflux from tropical soils

The extraradical mycelia of AMF contributed *c.* $14 \pm 6\%$ of soil CO₂ efflux in the tropical forest during dry and wet seasons in 2008, which was equivalent to $26 \pm 12\%$ of annual 'autotrophic' or 'root-derived' CO₂ efflux. The slight trend of increased AMF mycelial respiration during the year was attributed to the gradual colonization of mesocosms by AMF hyphae. At the end of the experiment there was no significant difference in hyphal length between AMF and FR + AMF mesocosms or in fine-root biomass between FR + AMF mesocosms and undisturbed soils (Table 1). We therefore suggest that measurements made during the later stages of the forest experiment accurately represent AMF mycelial respiration in undisturbed forest soils.

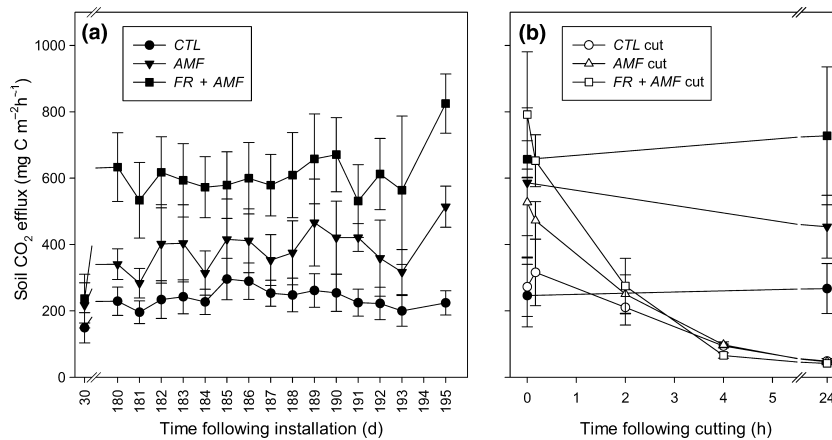


Fig. 4 Soil CO₂ efflux measured above mesocosms (control (CTL), arbuscular mycorrhizal fungi (AMF) and fine roots (FR) + AMF) installed within *Pseudobombax septenatum* containers: (a) following installation (at day 0), and (b) following cutting of fine-root and hyphal connections to trees and removal from containers (at hour 0). In view of the lack of effect of girdling (performed on day 183) on CO₂ efflux, data are pooled for girdled and nongirdled trees. Data are means and error bars are 1 SE ($n = 6$).

There are very few published estimates of mycorrhizal mycelial respiration in natural ecosystems with which to compare our estimates in this tropical forest. Using mesh in-growth cores in a temperate agricultural system, Moyano *et al.* (2007) estimated an average annual AMF mycelial respiration of 15 mg C m⁻² h⁻¹, while in a temperate coniferous forest Heinemeyer *et al.* (2007) estimated an average annual ectomycorrhizal (EM) mycelial respiration of 26 mg C m⁻² h⁻¹. The similarity between our estimate of 26 ± 12 mg C m⁻² h⁻¹ for lowland tropical forest (averaged over the dry and early wet seasons in 2008) and that of Heinemeyer *et al.* (2007) is surprising given the large differences in soils, vegetation and mycorrhizal type (e.g. greater mycelial respiration may be expected for EM fungi because they have a mycelial network an order of magnitude greater than that of AMF; Smith & Read, 1997).

Our finding that AMF mycelia are a significant source of soil CO₂ efflux at the forest site was further supported by measurements for *P. septenatum* under more controlled conditions. In this case, there were greater absolute CO₂ efflux values (mycelia respired 147 ± 17 mg C m⁻² h⁻¹; Fig. 4) and higher contributions of mycelia to root-derived CO₂ efflux (40 ± 6% compared with 26 ± 12% for the forest during the wet season).

The higher AMF mycelial respiration for *P. septenatum* compared with the forest probably reflects the higher incident radiation for *P. septenatum*, which is a fast-growing light-demanding species. Light intensity has a major influence on the respiration of AMF mycelia by altering rates of photosynthetic activity and allocation of plant C to the fungi (Heinemeyer *et al.*, 2006). The pot-grown *P. septenatum* plants were well illuminated, which will have allowed high rates of C fixation and high growth rates, provided that there were sufficient nutrients; the high rates of AMF mycelial respiration for *P. septenatum* may thus reflect high allocation of plant C to AMF in order to acquire those nutrients. Differences in the allocation of plant C to AMF according to plant nutrient demand could also result in root

colonization by different mycorrhizal species with different rates of respiration (e.g. according to their parasitic or mutualistic traits; Johnson *et al.*, 1997), which can be addressed in future studies that use molecular tools to identify mycorrhizal communities.

In both experiments our estimates of AMF mycelial respiration may be subject to several minor sources of error. First, our experimental design did not account for respiration by any soil macrofauna and saprophytic fungi that were excluded from control mesocosms. However, for *P. septenatum*, the rapid decrease in AMF mycelial respiration to negligible levels after severing all in-growth indicates that the vast majority of respiration was indeed from AMF mycelia alone (Fig. 4b). Secondly, the lower bulk density of mesocosm soils could have caused preferential in-growth of roots and hyphae, although the absence of a significant difference in fine roots between forest mesocosms and undisturbed forest soils ($P > 0.05$; Table 1) suggests that this was negligible. Thirdly, higher soil moisture in AMF compared with CTL mesocosms during the dry season in the forest (Fig. 2b) may have resulted in higher AMF mycelial in-growth and overestimation of mycelial respiration, but our data show no difference in hyphal length between AMF and CTL mesocosms at the end of the experiment (Table 1). Fourthly, we may have overestimated fine-root and AMF mycelial respiration, because roots and hyphae inside mesocosms were potentially younger and therefore had higher metabolic rates than roots and hyphae in undisturbed soils. However, fine roots and hyphae turn over relatively rapidly (e.g. AMF hyphae can turn over every 5–6 d; Staddon *et al.*, 2003; and fine roots in a lowland tropical forest can turn over 0.4–0.7 times per year; Silver *et al.*, 2005), so it is likely that this artefact would be greatly diminished towards the end of the experiment.

Fifthly, while a significant portion of our 'root' CO₂ efflux estimate may be from rhizosphere-dwelling microorganisms, which contributed 50–60% of root-derived CO₂ efflux in a study using *Lolium perenne* seedlings (Kuzyakov,

2002), it is not clear whether a portion of 'mycelial respiration' is from hyphosphere-dwelling microorganisms; no study has reported significant respiration originating from these microorganisms. The presence of mycorrhizal hyphae can lead to either a small increase or a small decrease in the abundance of other microorganisms (Johansson *et al.*, 2004), which suggests that hyphosphere-dwelling microorganisms contribute a negligible net flux of CO₂ from soils. Sixthly, growth of hyphae within control mesocosms potentially resulted in an underestimation of AMF mycelial CO₂ efflux by as much as 21–28% (*c.* 72 and 79% of hyphae were excluded from CTL mesocosms in the forest and *P. septenatum* containers, respectively; Table 1). Lastly, we quantified AMF respiration from the extraradical mycelia only, yet AMF biomass inside roots is significant and may comprise up to 20% of root weight (Smith & Read, 1997), which suggests that the entire contribution to root-derived CO₂ efflux by AMF is greater than our estimated contribution by mycelia.

The dependence of AMF mycelial CO₂ efflux on plant C supply

Remarkably, girdling of *P. septenatum* had no significant effect on respiration of fine roots or mycelia, which is surprising given that the majority of FR + AMF mesocosm CO₂ efflux was derived from fine roots and mycelia, which utilize plant-derived C. *Pseudobombax septenatum* has a well-defined separation of xylem and phloem tissue and we are confident that phloem tissue was completely severed by girdling. By contrast, girdling of *Pinus sylvestris* caused a 37% reduction in root CO₂ efflux over the first 5 d (Högberg *et al.*, 2001) and isotopic labeling studies have measured time lags of just 1–4 d between photosynthetic C fixation and release of that same C within soil CO₂ efflux (Ekblad & Högberg, 2001). We hypothesize that, in the short term, respiration of roots and mycelia was maintained by root carbohydrate reserves; this interpretation is supported by the rapid drop to virtually zero of root and AMF mycelial CO₂ efflux following root and mycelia cutting when microcosms were removed from the surrounding soil, and the later excavation of a large tap root (*c.* 0.04 m³). The same mechanism was suggested following no response of girdling on soil CO₂ efflux for a plantation of *Eucalyptus grandis* × *E. urophylla* clones in Brazil (Binkley *et al.*, 2006).

The very rapid decrease in respiration by mycelia severed from roots is consistent with the findings of Johnson *et al.* (2002), who detected a ¹³C label in mycelial respiration 9–14 h after plant uptake, and with those of Söderström & Read (1987), who measured significant reduction of EM mycelial respiration within hours of severing hyphae in a laboratory study. The negligible rates of CO₂ efflux once hyphae were cut also showed that microbial decomposition of dead hyphae, which have been shown to turn over rap-

idly (Staddon *et al.*, 2003), did not contribute a significant source of CO₂. Our findings are consistent with growth and maintenance respiratory losses being the major source of AMF mycelial CO₂ efflux, in contrast to slow decomposition of hyphal residues, probably as a result of the abundance of recalcitrant compounds such as chitin (Rillig, 2004) that make a negligible contribution to CO₂ efflux.

Seasonal dynamics in components of tropical forest soil CO₂ efflux

In addition to demonstrating the considerable contribution of AMF mycelia to soil CO₂ efflux, our data also provide new insight into how fine roots and microorganisms affect seasonal variation in tropical forest soil CO₂ efflux. Fine-root CO₂ efflux at the forest site did not vary significantly throughout the year and maintained an average rate of 64 ± 8 mg C m⁻² h⁻¹ (note that this estimate includes an unknown portion of respiration by rhizosphere-dwelling microorganisms). This suggests that fine-root CO₂ efflux was not limited by water during the dry season despite significant drying of soils (Fig. 2b) and significant reductions in soil water potential (see 'pipeline' plots in Santiago *et al.*, 2004; which are just a few km from our site), a hypothesis supported by a 7-yr throughfall exclusion experiment in an Amazonian forest (Brando *et al.*, 2008). The near-surface roots probably have a sufficient supply of water during the dry season from their connections with deeper roots of the same trees. We showed that the major seasonal shift in soil CO₂ efflux at the forest site (from 115 ± 9 mg C m⁻² h⁻¹ during the dry season to 248 ± 11 mg C m⁻² h⁻¹ at the start of the wet season) was attributable to significant variation in microbial CO₂ efflux (free-living microbial rather than rhizomicrobial; Fig. 3b). The major seasonal constraints on microbial activity are litterfall and precipitation (Vasconcelos *et al.*, 2004; Valentini *et al.*, 2008), so we propose that the seasonality of soil CO₂ efflux in our semi-evergreen moist tropical forest was a consequence of the decomposition of litter leachate following rainfall, seasonal priming of soil C stimulated by input of labile litter C (Sayer *et al.*, 2007), and rewetting effects (Jarvis *et al.*, 2007).

Our estimates of seasonal variation in root and microbial activity depend largely on the assumption that any effects of mesocosms on the soil abiotic environment were consistent across all treatments at all times (CTL, AMF and FR + AMF), which may not be entirely the case in the dry season. This 'dry season effect' of higher CO₂ efflux from FR + AMF mesocosms compared with undisturbed soils (Fig. 3a) was probably caused by cracking of the soil in the mesocosms, which was consistent across all mesocosm treatments. The only effect that was not consistent across all mesocosms was higher soil moisture in the control and AMF mesocosms during the dry season (Fig. 2b), which

may have resulted in slight underestimation of root respiration and overestimation of microbial and AMF mycelial respiration.

It was also assumed that CO₂ production from roots and mycelia beneath control mesocosms was negligible, an assumption supported by studies showing that 90% of fine roots were in the top 25 cm of soil in nearby forest (Cavelier, 1992); estimates of microbial CO₂ efflux may have been overestimated if this assumption was not met. We conclude that our estimates of root, AMF mycelial and microbial components of CO₂ efflux (Fig. 3b) may be subject to minor errors during the dry season because of differences in mesocosm soil moisture (Fig. 2b) but are accurate during the wet season, when most annual CO₂ efflux occurred and when mesocosm effects were small and likely to have been consistent across all treatments.

Mycorrhizal mycelial respiration in moist tropical forests and environmental change

The large release of CO₂ from AMF in tropical forest soils to the atmosphere has implications for ecosystem carbon storage under future climate scenarios. Mycorrhizal fungi have a physiology and chemistry very different to those of plant roots (Smith & Read, 1997) and therefore may respond independently to changes in climate and plant production. A meta-analysis of laboratory and field CO₂ enrichment studies using temperate plants has shown that plants consistently allocate more C to mycorrhizal fungi under elevated CO₂ and nutrient-limiting conditions (Alberton *et al.*, 2005). Therefore, in a world with elevated concentrations of atmospheric CO₂, there may be significant increases in the allocation of C to AMF in tropical forests, which generally grow on nutrient-poor soils (Vitousek & Sanford, 1986), and which already appear to be increasing in productivity, probably in response to raised concentrations of atmospheric CO₂ (Phillips *et al.*, 2008). The fate of plant C allocated to mycorrhizal fungi could have a substantial impact on the future C balance of tropical forests, whether stabilized in soils or, as we have shown, released as a major component of the CO₂ efflux from soils.

Conclusions

We have shown that the extraradical mycelia of AMF are a major source of CO₂ efflux from soils in a semi-evergreen moist tropical forest, releasing $1.4 \pm 0.6 \text{ t C ha}^{-1} \text{ yr}^{-1}$. Our study provides the first evidence in a tropical system that AMF mycelia are an important pathway of C flux from tropical forest trees to the atmosphere, by rapidly returning plant-derived C to the atmosphere (Fig. 4b) through their high rates of respiration (Figs 3, 4). Mycorrhizal fungi are therefore an important component of the C cycle in tropical forests and require more attention. Further information

is now required on mycorrhizal respiration in different tropical forests where nutrient limitation of plant growth and community composition of mycorrhiza may vary (e.g. Husband *et al.*, 2002 cf. Lovelock *et al.*, 2003), and on how increased concentrations of atmospheric CO₂ will affect the allocation of C from plants to mycorrhizal fungi in tropical forests.

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References

- Alberton O, Kuyper TW, Gorissen A. 2005. Taking mycocentrism seriously: mycorrhizal fungal and plant responses to elevated CO₂. *New Phytologist* 167: 859–868.
- Alexander IJ, Lee SS. 2005. Mycorrhizas and ecosystem processes in tropical rain forest: implications for diversity. In: Burslem DFRP, Pinard MA, Hartley SE, eds. *Biotic interactions in the tropics: their role in the maintenance of species diversity*. Cambridge, UK: Cambridge University Press, 165–203.
- Binkley D, Ernesto JLS, Takahashi N, Ryan MG. 2006. Tree-girdling to separate root and heterotrophic respiration in two eucalyptus stands in Brazil. *Oecologia* 148: 447–454.
- Brando PM, Nepstad DC, Davidson EA, Trumbore SE, Ray D, Camargo P. 2008. Drought effects on litterfall, wood production and belowground carbon cycling in an amazon forest: results of a throughfall reduction experiment. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences* 363: 1839–1848.
- Cavelier J. 1989. *Root biomass, production and the effect of fertilization in two tropical forests*. PhD thesis, University of Cambridge, UK.
- Cavelier J. 1992. Fine-root biomass and soil properties in a semideciduous and a lower montane rain-forest in Panama. *Plant and Soil* 142: 187–201.
- Chambers JQ, Tribuzy ES, Toledo LC, Crispim BF, Higuchi N, dos Santos J, Araujo AC, Kruijt B, Nobre AD, Trumbore SE. 2004. Respiration from a tropical forest ecosystem: partitioning of sources and low carbon use efficiency. *Ecological Applications* 14: S72–S88.
- Coleman DC, Crossley DA. 2003. *Fundamentals of soil ecology*. London, UK: Academic Press.
- Desrochers A, Landhausser SM, Lieffers VJ. 2002. Coarse and fine root respiration in aspen (*Populus tremuloides*). *Tree Physiology* 22: 725–732.
- Ekblad A, Högberg P. 2001. Natural abundance of C-13 in CO₂ respired from forest soils reveals speed of link between tree photosynthesis and root respiration. *Oecologia* 127: 305–308.
- Friese CF, Allen MF. 1991. The spread of VA mycorrhizal fungal hyphae in the soil: inoculum types and external hyphal architecture. *Mycologia* 83: 409–418.
- Grimoldi AA, Kavanova M, Lattanzi FA, Schaufele R, Schnyder H. 2006. Arbuscular mycorrhizal colonization on carbon economy in perennial ryegrass: quantification by ¹³CO₂/¹²CO₂ steady-state labelling and gas exchange. *New Phytologist* 172: 544–553.

- Hanson PJ, Edwards NT, Garten CT, Andrews JA. 2000. Separating root and soil microbial contributions to soil respiration: a review of methods and observations. *Biogeochemistry* 48: 115–146.
- Heinemeyer A, Hartley IP, Evans SP, De la Fuente JAC, Ineson P. 2007. Forest soil CO₂ flux: uncovering the contribution and environmental responses of ectomycorrhizas. *Global Change Biology* 13: 1786–1797.
- Heinemeyer A, Ineson P, Ostle N, Fitter AH. 2006. Respiration of the external mycelium in the arbuscular mycorrhizal symbiosis shows strong dependence on recent photosynthates and acclimation to temperature. *New Phytologist* 171: 159–170.
- Högberg P, Nordgren A, Buchmann N, Taylor AFS, Ekblad A, Höglberg MN, Nyberg G, Ottosson-Lofvenius M, Read DJ. 2001. Large-scale forest girdling shows that current photosynthesis drives soil respiration. *Nature* 411: 789–792.
- Husband R, Herre EA, Turner SL, Gallery R, Young JPW. 2002. Molecular diversity of arbuscular mycorrhizal fungi and patterns of host association over time and space in a tropical forest. *Molecular Ecology* 11: 2669–2678.
- Ineson P, Cotrufo MF, Bol R, Harkness DD, Blum H. 1996. Quantification of soil carbon inputs under elevated CO₂: C₃ plants in a C₄ soil. *Plant and Soil* 187: 345–350.
- Jarvis P, Rey A, Petsikos C, Wingate L, Rayment M, Pereira J, Banza J, David J, Miglietta F, Borghetti M *et al.* 2007. Drying and wetting of mediterranean soils stimulates decomposition and carbon dioxide emission: the “Birch effect”. *Tree Physiology* 27: 929–940.
- Johansson JF, Paul LR, Finlay RD. 2004. Microbial interactions in the mycorrhizosphere and their significance for sustainable agriculture. *Fems Microbiology Ecology* 48: 1–13.
- Johnson NC, Graham JH, Smith FA. 1997. Functioning of mycorrhizal associations along the mutualism-parasitism continuum. *New Phytologist* 135: 575–586.
- Johnson D, Leake JR, Ostle N, Ineson P, Read DJ. 2002. In situ (CO₂)-C-13 pulse-labelling of upland grassland demonstrates a rapid pathway of carbon flux from arbuscular mycorrhizal mycelia to the soil. *New Phytologist* 153: 327–334.
- Johnson D, Leake JR, Read DJ. 2001. Novel in-growth core system enables functional studies of grassland mycorrhizal mycelial networks. *New Phytologist* 152: 555–562.
- Kuzakov Y. 2002. Separating microbial respiration of exudates from root respiration in non-sterile soils: a comparison of four methods. *Soil Biology & Biochemistry* 34: 1621–1631.
- Kuzakov Y. 2006. Sources of CO₂ efflux from soil and review of partitioning methods. *Soil Biology & Biochemistry* 38: 425–448.
- Lovelock CE, Andersen K, Morton JB. 2003. Arbuscular mycorrhizal communities in tropical forests are affected by host tree species and environment. *Oecologia* 135: 268–279.
- Melillo JM, McGuire AD, Kicklighter DW, Moore B, Vorosmarty CJ, Schloss AL. 1993. Global climate-change and terrestrial net primary production. *Nature* 363: 234–240.
- Moyano FE, Kutsch WL, Schulze ED. 2007. Response of mycorrhizal, rhizosphere and soil basal respiration to temperature and photosynthesis in a barley field. *Soil Biology & Biochemistry* 39: 843–853.
- Pearson JN, Jakobsen I. 1993. Symbiotic exchange of carbon and phosphorus between cucumber and 3 arbuscular mycorrhizal fungi. *New Phytologist* 124: 481–488.
- Phillips OL, Lewis SL, Baker TR, Chao KJ, Higuchi N. 2008. The changing Amazon forest. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences* 363: 1819–1827.
- Pregitzer KS, Laskowski MJ, Burton AJ, Lessard VC, Zak DR. 1998. Variation in sugar maple root respiration with root diameter and soil depth. *Tree Physiology* 18: 665–670.
- Pyke CR, Condit R, Aguilar S, Lao S. 2001. Floristic composition across a climatic gradient in a neotropical lowland forest. *Journal of Vegetation Science* 12: 553–566.
- Raich JW, Potter CS, Bhagawati D. 2002. Interannual variability in global soil respiration, 1980–94. *Global Change Biology* 8: 800–812.
- Rillig MC. 2004. Arbuscular mycorrhizae and terrestrial ecosystem processes. *Ecology Letters* 7: 740–754.
- Rillig MC, Wright SF, Shaw MR, Field CB. 2002. Artificial climate warming positively affects arbuscular mycorrhizae but decreases soil aggregate water stability in an annual grassland. *Oikos* 97: 52–58.
- Santiago LS, Kitajima K, Wright SJ, Mulkey SS. 2004. Coordinated changes in photosynthesis, water relations and leaf nutritional traits of canopy trees along a precipitation gradient in lowland tropical forest. *Oecologia* 139: 495–502.
- Sayer EJ, Powers JS, Tanner EVJ. 2007. Increased litterfall in tropical forests boosts the transfer of soil CO₂ to the atmosphere. *PLoS ONE* 2: e1299.
- Silver WL, Thompson AW, McGroddy ME, Varner RK, Dias JD, Silva H, Crill PM, Keller M. 2005. Fine root dynamics and trace gas fluxes in two lowland tropical forest soils. *Global Change Biology* 11: 290–306.
- Smith SE, Read DJ. 1997. *Mycorrhizal symbiosis*. London, UK: Academic Press.
- Söderström B, Read DJ. 1987. Respiratory activity of intact and excised ectomycorrhizal mycelial systems growing in unsterilized soil. *Soil Biology & Biochemistry* 19: 231–236.
- Staddon PL, Ramsey CB, Ostle N, Ineson P, Fitter AH. 2003. Rapid turnover of hyphae of mycorrhizal fungi determined by AMS microanalysis of C-14. *Science* 300: 1138–1140.
- Subke JA, Ingleton I, Cotrufo MF. 2006. Trends and methodological impacts in soil CO₂ efflux partitioning: a meta-analytical review. *Global Change Biology* 12: 921–943.
- Valentini CMA, Sanches L, de Paula SR, Vourlitis GL, Nogueira JD, Pinto OB, Lobo FD. 2008. Soil respiration and aboveground litter dynamics of a tropical transitional forest in northwest Mato Grosso, Brazil. *Journal of Geophysical Research-Biogeosciences* 113: G00B10.
- Vasconcelos SS, Zarin DJ, Capanu M, Littell R, Davidson EA, Ishida FY, Santos EB, Araujo MM, Aragao DV, Rangel-Vasconcelos LGT *et al.* 2004. Moisture and substrate availability constrain soil trace gas fluxes in an eastern Amazonian regrowth forest. *Global Biogeochemical Cycles* 18: GB2009.1–GB2009.10.
- Veldkamp E, Becker A, Schwendenmann L, Clark DA, Schulte-Bispung H. 2003. Substantial labile carbon stocks and microbial activity in deeply weathered soils below a tropical wet forest. *Global Change Biology* 9: 1171–1184.
- Vitousek PM, Sanford RL. 1986. Nutrient cycling in moist tropical forest. *Annual Review of Ecology and Systematics* 17: 137–167.
- Wilsey BJ, Parent G, Roulet NT, Moore TR, Potvin C. 2002. Tropical pasture carbon cycling: relationships between C source/sink strength, above-ground biomass and grazing. *Ecology Letters* 5: 367–376.
- Windsor D. 1990. Climate and moisture availability in a tropical forest, long term record for Barro Colorado island, Panama. *Smithsonian Contributions to the Earth Sciences* 29: 1–145.