

Plant roots link stronger with microorganisms in leaf litter rather than soil across tropical land-use systems

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Abstract

Tropical soil microorganisms are major recyclers of biosphere organic carbon. However, the link of tropical microorganisms to the two primary carbon inputs (roots and litter) remains unclear. Here, we studied the effects of excluding living roots and litter on microorganisms in leaf litter and topsoil in rainforests and plantations in Sumatra, Indonesia. Unexpectedly, excluding living roots strongly decreased microbial biomass and basal respiration in litter but not in soil, indicating that tropical trees prioritize mining for nutrients in litter layer rather than mineral soil. Contrary to litter, soil microbial communities were predominantly influenced by long-term factors related to land-use history. Litter removal neither significantly affected microbial biomass nor community structure in soil, suggesting compartmentalized carbon processing between litter and soil. Our study sheds new light on fundamental-ecosystem processes in the tropics and calls for greater consideration of the litter layer and for including root-derived resources in global carbon cycling models.

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Abstract

Tropical soil microorganisms are major recyclers of biosphere organic carbon. However, the link of tropical microorganisms to the two primary carbon inputs (roots and litter) remains unclear. Here, we studied the effects of excluding living roots and litter on microorganisms in leaf litter and topsoil in rainforests and plantations in Sumatra, Indonesia. Unexpectedly, excluding living roots strongly decreased microbial biomass and basal respiration in litter but not in soil, indicating that tropical trees prioritize mining for nutrients in litter layer rather than mineral soil. Contrary to litter, soil microbial communities were predominantly influenced by long-term factors related to land-use history. Litter removal neither significantly affected microbial biomass nor community structure in soil, suggesting compartmentalized carbon processing between litter and soil. Our study sheds new light on fundamental-ecosystem processes in the tropics and calls for greater consideration of the litter layer and for including root-derived resources in global carbon cycling models.

Keywords: roots; litter; land use; deforestation; microbial biomass; basal resources; trenching

Introduction

Approximately ninety percent of the biomass produced by terrestrial plants is entering the belowground system, where it fuels complex food webs and supports a quarter of the total biodiversity on Earth (Cebrian 1999; Guerra *et al.* 2021). Soils store approximately twice as much carbon as the atmosphere (Schimel 1995; Crowther *et al.* 2019), with the soil carbon storage and cycling being mainly driven by microorganisms (Bardgett & Wardle 2010; Liang *et al.* 2017). Soil microorganisms are key players in the transformation and processing of carbon and nutrients in belowground food webs, serving as the core of terrestrial ecosystem functioning (van der Heijden *et al.* 2008; Delgado-Baquerizo *et al.* 2016). Plant carbon enters the belowground

system via litter and roots – the two principle resources for soil microorganisms – both controlling the size, activity and composition of the soil microbial community (Bever *et al.* 2010; Schlatter *et al.* 2015; Hassani *et al.* 2018).

Plant litter represents the major source of carbon entering the soil from aboveground that fuels numerous litter decomposers and shapes the structure of microbial communities (McBride *et al.* 2020). After transformation and translocation into the mineral soil, litter compounds including cellulose, lignin, lipids and sugars, together with microbial-derived carbon are stabilized in soil organic matter which is protected by minerals and aggregates (Angst *et al.* 2021). However, plant carbon may reach mineral soil layers much more rapidly via the root pathway. A major fraction of photosynthetically fixed carbon is allocated to roots and is exuded into the soil actively or passively (Bais *et al.* 2006). The labile carbon compounds exudated by roots activate microorganisms in the rhizosphere and also affect microbial communities in bulk soil (Haichar *et al.* 2008; Zhou *et al.* 2020). It has been estimated that root-derived resources are responsible for up to half of soil respiration in temperate forests (Hanson *et al.* 2000; Díaz-Pinés *et al.* 2010). Changes in microbial activity also result in changes in the composition of microbial communities, particularly in the root vicinity (Paterson *et al.* 2007; Haichar *et al.* 2008). The interactions between roots and microorganisms cause distinct populations in the rhizosphere compared to those elsewhere in the soil (Ballauff *et al.* 2021). As known from temperate forests, root-associated fungi also affect decomposition of leaf litter by speeding up or slowing down decomposition processes via Priming or Gadgil effects (Talbot *et al.* 2008; Fernandez & Kennedy 2016; Lang *et al.* 2021). Plant roots and associated microorganisms exploit nutrients from soil and litter, and thereby interact with microorganisms in both soil and litter layers. Root associated microorganisms in soil may translocate nutrients into the litter thereby affecting litter decomposition and plant mining for nutrients (Bunn *et al.* 2019). However, little research has been conducted on the impact of living roots on microorganisms in litter. Further, there generally is a lack of studies investigating interactive effects of litter and root resources on the structure and functioning of microbial communities in soil as most studies either manipulated litter- or root-derived resources but not both. This is especially true for tropical ecosystems, where dominant mycorrhiza, climate and nutrient limitation differ significantly from temperate regions (Malloch *et al.* 1980; Camenzind *et al.* 2018).

The tropics account for over half of the global annual production of CO₂ of terrestrial ecosystems, and tropical rainforests play an important role as carbon sink in the global carbon cycle (Baccini *et al.* 2017; Mitchard 2018). Tropical ecosystems harbour some of the oldest and most nutrient-impooverished soils, which may also be severely depleted in soil organic matter, whereas large amounts of C is stored in living biomass, i.e., trees (West *et al.* 2010; Hartshorn 2013). Further, tropical soils are more limited by phosphorus compared to soils of temperate and boreal regions, where nitrogen typically is the primary limiting element (Camenzind *et al.* 2018). Nutrient limitation results in competition for litter-derived nutrients between saprotrophic and mycorrhizal microorganisms. To access nutrients released during litter decomposition, plant roots may colonise litter, which is likely to be more prevalent in tropical P-limited than in high latitude N-limited ecosystems (Xu *et al.* 2018; Langet *et al.* 2021). This may result in a stronger linkage between root-associated microbes and litter resources in the litter layer in tropical than in high latitude ecosystems, but this hypothesis remains untested.

Meanwhile, the tropics are at the forefront of agricultural expansion (Laurance *et al.* 2014; Grass *et al.* 2020). Conversion of tropical rainforest into agricultural production systems greatly reduces the amount of litter in organic layers and changes carbon cycling (Guillaume *et al.* 2018; Potapov *et al.* 2019; Veldkamp *et al.* 2020), and thereby the structure of above- and belowground communities (Rembold *et al.* 2017; Zhou *et al.* 2022). The composition of soil microbial communities changes strongly with the conversion of rainforest into agricultural land-use systems (Krashevska *et al.* 2015; Schneider *et al.* 2015; Ballauff *et al.* 2021). However, the roles of the two principle resources, i.e., living roots and litter, in modifying belowground microbial communities in response to land-use change remain unknown. Whereas short-term resource supply, such as root exudates, provides energy for microbial growth and activity (Hanson *et al.* 2000; Díaz-Pinés *et al.* 2010), long-term factors, such as land-use type and soil development, may pose general limits on microbial community composition and biomass (Bissett *et al.* 2011; Lauber *et al.* 2013). Understanding the dynamics

of both short-term resource supply and long-term factors is essential for predicting the response of soil microbial communities to environmental change and for maintaining soil ecosystem services. Therefore, the role of litter- and root-derived resources for the structure and functioning of microbial communities needs to be investigated across ecosystems of different land-use history.

In this study, we investigated the effects of the deprivation of resource input via living roots and aboveground leaf litter on microbial biomass, respiration and community composition in the litter layer, and in 0-5 and 5-10 cm of the underlying mineral soil. We conducted a full-factorial root-trenching and litter-removal experiment in rainforest and plantations of rubber and oil palm in Sumatra, Indonesia (Zhou *et al.* 2023). We focused on three critical knowledge gaps: (1) unknown relative importance of root- and litter-derived resources for microbial communities in soil and litter of different tropical land-use types, (2) unknown shifts in the role of root-derived resources for soil and litter microorganisms under land-use change, and (3) the contribution of short-term resource supply (root and litter resources) *vs* long-term factors (land use and soil layers) in shaping soil microbial communities. By assessing microbial biomass, basal respiration and phospholipid fatty acid (PLFA) patterns across litter and soil layers as well as land-use types, we analysed changes in microbial community structure and functioning after the deprivation of root and litter resources. Specifically, we tested the following hypotheses:

- 1) Root trenching causes a reduction in microbial biomass and soil respiration, and induces changes in the microbial community structure, with the changes being more pronounced in soil than in litter due to closer spatial association of roots with soil than with litter.
- 2) Effects of litter removal on soil microbial activity and community composition are weaker in plantations than in rainforest, as a result of reduced litter resources.
- 3) Long-term factors (land-use type and soil layer) are more important than the deprivation of short-term resource supply (root and/or litter resources) for microbial community structure, whereas short-term resource supply has a greater impact on total microbial biomass and soil respiration.

Materials and methods

1. Study sites, experimental set-up and soil sampling

The study was conducted in Jambi province, Sumatra, Indonesia. Over the past 25-35 years, the lowland rainforests in Sumatra have been largely replaced by intensively managed plantations, primarily consisting of oil palm and rubber (Margono *et al.* 2012; Drescher *et al.* 2016). Our experiment was established across three distinct land-use types, i.e., rainforest, rubber plantations and oil palm plantations located southeast of the city of Jambi ('Harapan landscape'; 1@ 55° 40' S, 103@ 15' 33' E), in October 2016. The experiment was set-up in a factorial design with four experimental treatments: control, root trenching, litter removal and both root trenching and litter removal. Each land-use type was replicated four times resulting in a total of 12 sites (48 experimental plots) spread across an area of ca. 35 km diameter with adjacent sites spaced apart by 0.5–5 km. For more details of the experimental design see Zhou *et al.* (2023).

In September-October 2017, i.e., one year after the experimental setup, litter and soil (0–5 and 5–10 cm depth) samples were collected using a corer of a diameter of 5 cm. To account for potential spatial variability, three cores were taken per plot and the material from respective layers were pooled as one sample. In total, 144 pooled samples were collected (i.e., 4 treatments × 3 land-use types × 3 layers × 4 sites). The samples were transported to the laboratory within 1-2 days after collection, where the soil samples were sieved through a 2 mm mesh and litter samples were cut into pieces < 25 mm². Then, all samples were stored at -20°C for later analysis.

Water content was measured by drying samples at 65 °C for 48 h. Water content in soil was significantly increased after root trenching in rainforest and rubber plantations ($F_{1, 63}=31.78$, $p<0.001$), but this was not the case in litter. Litter removal did not significantly affect soil moisture in the two soil depths.

We also assessed the biomass of fine roots (diameter < 2 mm) in the litter layer and in 0-5 cm soil depth next

to the experimental plots by taking 16 cm × 16 cm soil cores from three sub-sites in each study site. Soil was weighed and washed through a 1-mm sieve to extract roots; roots from litter were hand-picked. After drying at 65 °C for 48 h, roots of < 2 mm diameter were weighed and the results are given as the amount of dry roots per kilogram of dry litter or soil, i.e., root density.

2. Basal respiration and microbial biomass

Prior to respiration measurements, an aliquot of the pooled samples, i.e., 3 g soil and 0.5 g litter (dry weight equivalent) were thawed at 4°C for three days and then placed at room temperature for seven days to allow equilibration. Basal respiration was measured as O₂ consumption and microbial biomass was measured by substrate-induced respiration (Anderson & Domsch 1978; Scheu 1992). The microbial respiratory response was measured at hourly intervals at 22 degC for 24 h. Basal respiration (µg O₂ g⁻¹ h⁻¹) was determined without addition of substrate and measured as mean O₂ consumption rates 11–21 h after attachment of the vials to the measurement system. Microbial biomass was determined based on the maximum initial respiratory response (MIRR; µg O₂ g⁻¹ h⁻¹) after adding glucose (10 mg per gram of soil and 80 mg per gram of litter dry weight) to saturate microbial glycolytic enzymes; MIRR readings were taken 4–7 h after glucose addition. MIRR was converted to microbial biomass (C_{mic}; µg C_{mic}g⁻¹) as 38 × MIRR (Beck *et al.* 1997). Microbial specific respiration was calculated as the ratio between microbial basal respiration and microbial biomass (qO₂; µg O₂ µg⁻¹ C_{mic}h⁻¹).

3. Phospholipid fatty acid analysis

Our study emphasizes the functional perspective of microbial communities, and accordingly, we opted to use phospholipid fatty acid (PLFA) analysis as quantitative and highly sensitive method for analysing microbial community composition (Orwin *et al.* 2018; Joergensen 2022). PLFAs were extracted from the frozen samples of litter (2 g) and soil (4 g) using a modified Bligh and Dyer method (Frostegård *et al.* 1993). Briefly, lipids were fractionated into neutral lipids, glycolipids and phospholipids by elution through silica acid columns using Bligh/Dyer solvent [chloroform, methanol, citrate buffer (pH 4); 1:2:0.8]. Phospholipids were then subjected to mild alkaline methanolysis and fatty acid methyl esters were identified by chromatographic retention time compared to standards (FAME CRM47885, C11 to C24; BAME 47080-U, C11 to C20; Sigma-Aldrich, Darmstadt, Germany) using a GC-FID Clarus 500 (PerkinElmer Corporation, Norwalk, USA) equipped with an Elite 5 column (30 m × 0.32 mm inner diameter, film thickness 0.25 µm). The temperature program started with 60 °C (held for 1 min) and increased by 30 °C per min to 160 °C followed by 3 °C per min to 280 °C. The injection temperature was 250 °C and helium was used as carrier gas. PLFA data of seven of the 144 samples are missing due to an analytical error (Supplementary data table).

PLFA abundances were calculated as nmol per gram dry weight of soil and litter. Total PLFAs included all identified PLFAs (nmol g⁻¹), which was used to complement C_{mic} analysis as an additional measure of microbial biomass. The PLFAs were separated and used as markers for Gram-positive bacteria (Gram⁺), Gram-negative bacteria (Gram⁻), fungi and arbuscular mycorrhiza fungi (AMF). And the ratio between some particular PLFAs were used as indicators of fungi/bacteria ratio, carbon limitation, physiological or nutritional stress, for more details see Appendix 1.

4. Statistical analysis

All analyses were done in R 4.0.3 (R Core Team 2020). To assess the effect size of litter removal and root trenching on microbial responses, we fitted linear mixed-effects models (LMMs) and then applied contrasts between resource exclusion (either no litter or no root) and resource inclusion (either litter or root). Response variables included basal and specific respiration, microbial biomass, PLFA markers (Gram⁺ bacteria, Gram⁻ bacteria, total bacteria, fungi and AMF) and PLFA indicators (carbon limitation, fungi/bacteria ratio, physiological and nutritional stress). All PLFA markers were analysed as proportions of the total PLFA amount in the sample (mole percentage) and these proportional data were logit-transformed prior to analyses (Warton & Hui 2011). Microbial biomass, PLFA indicators, basal and specific respiration data were log-transformed due to a left-skewed distribution. The litter removal effect was calculated as the difference between ‘control’ + ‘trenching’ vs ‘litter removal’ + ‘litter removal and trenching’. The root trenching effect

represents the difference between ‘control’ + ‘litter removal’ vs ‘trenching’ + ‘litter removal and trenching’. The residuals of all mixed models were visually checked and met the assumption of homogeneity of variance.

The models were run separately for litter and soil (0-5 and 5-10 cm soil depth), as litter was excluded in the litter removal treatment. For litter, the LMMs included root trenching (trenching/no trenching), land-use type (rainforest, rubber and oil palm plantations) and their interaction as fixed factors, with site as random factor to account for interdependence of treatments from the same site. For soil, the LMMs included root trenching (trenching/no trenching), litter (removal/no removal), land-use type (rainforest, rubber and oil palm plantations), soil depth (0-5 and 5-10 cm), their pairwise interactions and moisture content as fixed factors, with treatment nested within site as random effect to account for interdependence of layers from the same soil core and treatments from the same site. We included moisture as fixed factor to account for potential side effects of trenching on microbial communities via changes in soil moisture.

To analyse the influence of land use and treatments on microbial community structure, PLFA composition were analysed by Bray–Curtis dissimilarity-based PERMANOVA and visualized by non-metric multidimensional scaling (NMDS). Only PLFAs present in more than half of the plots were included in the analysis. PERMANOVA was used to first analyze changes in PLFA composition with land use and treatments in each layer (litter, 0-5 and 5-10 cm soil depth). Thereafter, we analyzed the changes in PLFA composition with depth and land use in the two soil depth in a single model without litter.

To assess differences in root density between layers and land-use types, we used two-way analysis of variance with land-use type and layers as independent variables followed by post hoc comparison of means using Tukey’s honestly significant difference (HSD) test to inspect differences between means. Data were log-transformed to fit normal distribution prior to the analysis.

The *vegan* package was used for NMDS and PERMANOVA analysis (Oksanen *et al.* 2020); the *nlme* package was used to fit LMMs (Bates *et al.* 2015; Pinheiro *et al.* 2022) and the *emmeans* package was used to conduct planned contrasts (Lenth 2021). Pairwise comparisons of root biomass among land-use types were done using post hoc HSD.test function from the package *agricolae* following analyses of variance (Mendiburu 2020). Results were visualized using *ggplot2* (Wickham 2016).

Results

1. Changes in basal respiration and microbial biomass

In the litter layer, root trenching reduced microbial basal respiration, microbial biomass and total PLFAs by 37.7 ± 9.4 % (estimated mean \pm standard error), 31.9 ± 10.6 % and 37.1 ± 12.3 % across land-use types (Figs 2, 3a; Tables S1, S2); there were generally no significant interactions between root trenching and land use, but the effects of root trenching on microbial biomass were in trend stronger in oil palm plantations ($F_{2,9}=3.73$, $p=0.066$; Tables S1). In the two soil depths studied, by contrast, both root trenching and litter removal did not significantly affect microbial basal respiration, microbial biomass and total PLFAs (Figs 2, 3a; Table S1). Microbial specific respiration in litter and soil generally was not significantly affected by the experimental treatments, but varied significantly with land use and soil depth (Fig. 2; Table S1).

Land-use type and soil depth (as ‘long-term’ factors) strongly affected microbial biomass across treatments (Figs 2, 3b, S2; $F_{2,10}=5.2$, $p=0.027$ and $F_{1,48}=18.79$, $p<0.001$, respectively). Basal respiration and microbial biomass in both litter and soil were about two times higher in rainforest and rubber plantations than in oil palm plantations (Figs. S2; Table S5). Consistently across land-use types and treatments, basal respiration, microbial biomass and total PLFAs decreased with soil depth, each being about ten times higher in litter than in soil (Fig. 3b), and two times higher in 0-5 than 5-10 cm soil depth (Fig. S2; Table S4).

2. Microbial community structure and functioning

As indicated by the proportional PLFAs composition, microbial community structure in the litter layer was not affected by root trenching but by land-use types (PERMANOVA: $F_{1,14}=0.97$, $p=0.421$ and $F_{2,14}=4.16$, $p=0.002$, respectively; Fig. 4a). By contrast, microbial community structure in the two soil depths

was significantly affected by root trenching (PERMANOVA: $F_{1,62} = 4.56$, $p = 0.002$) and strongly changed with land use and depth (PERMANOVA; $F_{2,59} = 11.45$, $p < 0.001$ and $F_{1,59} = 14.53$, $p < 0.001$, respectively; Fig. 4b,c,d) but not after litter removal (PERMANOVA: $F_{1,59} = 0.98$, $p = 0.463$).

In soil, root trenching decreased the relative abundance of fungi by $41.8 \pm 9.1\%$ and $35.9 \pm 8.7\%$ in 0-5 and 5-10 cm depth in comparison to the control, respectively (Fig. 5a, Table S3). Trenching also decreased the relative abundance of AMF by $17.3 \pm 4.2\%$ and $15.5 \pm 3.9\%$ in 0-5 and 5-10 cm depth, respectively, with the effects being significant in rainforest and oil palm plantations ($-22.1 \pm 5.3\%$ and $-22.1 \pm 5.3\%$) but not in rubber plantations (Figs 2, 5a, Table S3). In addition, the effects of root trenching on AMF were influenced by the removal of litter; in the presence of litter, trenching decreased AMF by $23.4 \pm 3.3\%$, whereas the effect of trenching was not significant when litter was absent (Fig. 2).

Litter removal had no general effects on soil microorganisms across land-use types and depths (Fig. 2). Litter removal increased the relative abundance of fungi but only in soil at 0-5 cm depth ($+42.9 \pm 18.8\%$) and increased the relative abundance of AMF but only in soil at 5-10 cm depth ($+9.0 \pm 4.6\%$). Litter removal decreased the relative abundance of bacteria by $12.2 \pm 4.9\%$ and Gram⁻bacteria by $20.2 \pm 7.4\%$ in rainforest (Fig. 2). In addition, the relative abundance of fungi was higher in litter than in soil and the relative abundance of bacteria was higher in soil than in litter (Fig. 5b).

3. Fine root density

Fine root density per mass of substrate was significantly different between layers ($F_{1,18} = 113.55$, $p < 0.001$) and land-use types ($F_{2,18} = 13.94$, $p < 0.001$), being more than ten times higher in litter (76.5 ± 29.6 g kg^{-1}) than in soil (4.9 ± 0.9 g kg^{-1}) across land-use types. In the litter layer, fine root density was higher in oil palm plantations (166.7 ± 73.4 g kg^{-1}) than in rainforest (41.5 ± 9.2 g kg^{-1}) and rubber plantations (21.4 ± 4.6 g kg^{-1}), while in soil it did not vary significantly among land-use types (Fig. S3).

Discussion

We assessed the effects of litter- and root-derived resources on the biomass, respiration and functional group structure of microbial communities by root trenching and litter removal in tropical rainforest and plantations. For the first time we tested the different roles of living roots on tropical microbial communities in both litter and mineral soil. In contrast to our first hypothesis, effects of the exclusion of living roots on microbial biomass and respiration in litter were more pronounced than in soil, and this was consistent across the studied tropical land-use systems. By contrast, root trenching did not significantly affect microbial biomass and basal respiration in soil, but reduced the relative abundance of saprotrophic and mycorrhizal fungi. Contrasting our second hypothesis, litter removal generally did not significantly affect soil microorganisms, however, the effects of root trenching on soil fungi were weaker when litter was removed. Overall, across the studied tropical ecosystems plant roots were more strongly linked to litter than to soil microorganisms, while biomass and community structure of soil microorganisms were affected primarily by ‘long-term’ factors, i.e., land use and soil depth, partly supporting our third hypothesis.

1. Effect of root and litter resources on microorganisms in litter and soil

In contrast to our first hypothesis, root trenching more strongly affected microbial biomass and respiration in litter than in soil suggesting that living roots of tropical trees are supplying carbon predominantly to litter and not to soil microorganisms. Conform to this pattern, root density, i.e., root biomass per unit litter or soil, also was higher in litter than in soil. Microorganisms in litter either may have directly benefitted from root exudates or from mycorrhizal fungi using plant carbon to facilitate associated microorganisms for capturing nutrients from decomposing litter (Lang *et al.* 2021). As known from temperate forests, plant roots may grow into the litter where the associated mycorrhizal fungi participate in litter decomposition by exuding enzymes themselves, or by stimulating the free-living associated community of saprotrophs (priming effect; (Talbot *et al.* 2008; Zhou *et al.* 2020; Langet *et al.* 2021). In contrast to our findings, root trenching in temperate forests was associated with a decrease in microbial biomass both in litter and in soil (Bluhm *et al.* 2019), and root-derived resources have been shown to be responsible for about one half of soil respiration

(Hanson *et al.* 2000; Díaz-Pinés *et al.* 2010). The lack of response of soil microbial biomass to root trenching in our experiment indicates that the linkage between living roots and free-living soil microorganisms in the tropics is weaker than in temperate forest ecosystems. Probably, this is due to strongly weathered soils in the tropics which are poor in nutrients (West *et al.* 2010; Hartshorn 2013) as also the case at our study sites (Allen *et al.* 2016). High temperature accelerates weathering processes and the often long history of tropical ecosystems results in soils depleted in nutrients, especially in phosphorus (Stoops 2003; Camenzind *et al.* 2018). This likely makes root exudation inefficient for acquiring nutrients from mineral soils via priming (Kuzyakov 2002). In contrast to the nutrient-poor tropical soils, litter represents a nutrient-rich substrate worth being exploited by investing carbon resources via priming or by fostering nutrient exploitation by mycorrhizal fungi.

Despite root trenching did not significantly alter microbial biomass and respiration in mineral soil, it changed microbial community structure as indicated by PLFAs patterns, which contrasts previous studies in temperate forests where the deprivation of root-derived resources did not affect microbial community structure (Hart & Sollins 1998; Bluhm *et al.* 2019). In our study, root trenching specifically decreased the relative abundance of fungal and AMF biomarkers in mineral soil suggesting that both saprotrophic and mycorrhizal fungi in soil critically depend on plant roots in the tropics. This is conform to recent findings that fungi benefit greatly from root-derived resources (Lemanski & Scheu 2014; Birgander & Olsson 2021), however, this has only been shown in temperate ecosystems. Notably, the effects of trenching on AMF abundance were weak when litter was absent, which indicates that soil AMF may not rely solely on nutrients originating from soil, but also on those originating from litter. Overall, the results point to the critical role of plant roots in supporting saprotrophic and mycorrhizal fungi in soil, but also highlight their reliance on litter resources.

In contrast to root trenching not affecting overall microbial biomass in soil, root trenching resulted in a decline in animal abundances (meso- and macrofauna) in soil by 30-40% as shown in our previous study based on the same experiment (Zhou *et al.* 2023). This may indicate that soil meso- and macrofauna predominantly rely on fungi (which declined after trenching) and are unable to efficiently switch to bacteria as an alternative food resource. Supporting this conclusion, soil detritivorous microarthropods have been shown to predominantly consume fungi in temperate forest ecosystems (Ferlian *et al.* 2015; Pollierer & Scheu 2021). Generally, the rhizosphere is the hotspot of microbial and animal activity in soil (Kuzyakov & Blagodatskaya 2015). Animals are mobile, and therefore may generally benefit from root-derived resources even though predominantly living in bulk soil distant to roots. This contrasts unicellular microorganisms which may only benefit from root-derived resources in close vicinity of roots. In contrast to unicellular bacteria, saprotrophic, but in particular mycorrhizal fungi may proliferate away from roots and thereby contribute to spreading root-derived resources in space (Cardon & Whitbeck 2007; Bonfante & Anca 2009). The strong response of animals in soil to root trenching as found by Zhou *et al.* (2023) therefore likely reflects the mobility of animals and potentially also the spread of root-derived resources by fungi into bulk soil and litter.

In contrast to root trenching, litter removal neither significantly affected soil microbial biomass nor microbial community structure, which contrasts our second hypothesis. This resembles our previous results on animals in soil which also remained unaffected by litter removal (Zhou *et al.* 2023). These results suggest that litter resources are predominantly used locally and that the 'litter' energy channel is isolated from energy channelling in soil, highlighting the compartmentalization of carbon processing between litter and soil in tropical ecosystems. However, previous studies from other tropical region showed that in the long-term (9 years) litter removal may also detrimentally affect soil microorganisms (Sheldrake *et al.* 2017).

2. Long-term vs. short-term effects on soil microorganisms

Contrasting our third hypothesis, exclusion of root- or litter-derived resources little affected microbial basal respiration, microbial biomass and only moderately affected microbial community structure in soil. By contrast, most of the microbial parameters studied varied strongly with land use and soil depth indicating that they are mainly driven by factors acting in the long-term. This is conform to earlier studies showing that microorganisms in soil are resistant to short-term disturbances, such as changes in root resource input, but only respond with a delay in time (Hart & Sollins 1998; Bluhm *et al.* 2019). This 'soil legacy effect' on

soil microbial properties has been observed in a number of ecosystems including arable systems, grasslands and forests (Dupouey *et al.* 2002; Eisenhauer *et al.* 2010; Fichtner *et al.* 2014). Land-use history may even more strongly impact soil microbial community composition than current aboveground vegetation and soil properties (Jangid *et al.* 2011). Rubber and oil palm plantations at our study sites were established about 20 years before our experiment started in 2017. The establishment of the plantations was associated by strong changes in physical and chemical soil properties, such as pH and soil organic carbon content, and also microclimatic changes (Drescher *et al.* 2016; Guillaume *et al.* 2018). Such land-use changes strongly affect microbial communities as documented previously (Brinkmann *et al.* 2019; Krashevskaa *et al.* 2022). Similarly, the formation of soil depth gradients across larger time scales has been shown to strongly change soil carbon and nutrient concentrations, especially in the tropics (Guillaume *et al.* 2015). Long-term exposure to changes in land use renders microbial communities in tropical soil relatively stable, enhancing their resistance to short-term changes in resource inputs. This underscores the critical role of sustainable long-term land management practices in promoting soil health and preserving microbial communities, particularly in tropical regions.

Long-term and short-term effects on microorganisms can interact. Root trenching had a greater impact on microbial biomass in oil palm plantations compared to rainforest and rubber plantations, which parallels the higher root density in litter in oil palm plantations than in the other two land-use types. Both higher root density and higher microbial biomass may be related to the comparatively high quality of litter in oil palm plantations largely comprising litter of understory vegetation, which is regularly eradicated by herbicide application, whereas fronds of oil palm are piled up in rows, with the litter of understory vegetation having lower lignin concentration than the litter in rainforest and rubber plantations (Krashevskaa *et al.* 2018) indicating higher decomposability (Thevenot *et al.* 2010; Talbot & Treseder 2012). Similarly, trenching effects on soil fauna abundance were also stronger in oil palm plantations (Zhou *et al.* 2023) indicating that living roots are of particular importance for the entire food web of oil palm plantations. Root trenching also reduced soil AMF relative abundance, but only in rainforest and oil palm plantations and not in rubber plantations. In rubber plantations the rubber trees are tapped to collect latex, which likely decreases the supply of assimilates to the roots and thus reduces root-derived resources (Nguyen *et al.* 2020). Although litter removal had no general effects on microbial biomass and community structure, it did result in a reduction in the relative abundance of bacteria in rainforest. Specifically, this decrease was primarily attributed to Gram⁻ bacteria, which are known to heavily rely on simple carbon compounds derived from plants (Fanin *et al.* 2019). Rainforests typically featured a thicker litter layer compared to plantations and this may explain the stronger impact on soil bacteria in the rainforest compared to plantations. Overall, the findings suggest that the quality of litter, root density and living roots are crucial factors that interact to determine the response of microorganisms and soil fauna to environmental perturbations in oil palm, rubber and rainforest ecosystems, whereas the importance of these factors are overridden by long-term changes in environmental factors such as changes in land use.

Conclusions

For the first time we documented the important role of root-derived resources for litter and soil microorganisms across tropical land-use systems. Our results showed stronger effects of root trenching on microbial biomass and respiration in litter than in soil, indicating that trees in the tropics predominantly allocate resources to roots for mining for nutrients in litter rather than from organic matter in soil. Little impact of litter removal on soil microorganisms suggests independent carbon processing in litter and soil, pointing to the vertical compartmentalisation of carbon cycling in tropical ecosystems. The close linkage of plant roots and microorganisms in litter highlights the importance of litter nutrients for plant nutrition, and positions leaf litter as a crucial component in plant-soil feedbacks in the tropics that is often overlooked. At the same time, long-term effects, such as land use or soil depth, continuously alter the role of litter and root resources and set limits on their impact on microbial community structure and function.

Overall, our study sheds light on the driving forces for the structure and functioning of microbial communities in tropical ecosystems, and highlights the importance of root-derived resources for energy channelling, carbon

cycling and nutrient exploitation by trees. This knowledge is crucial for understanding microbial processes in soil, modelling carbon and nutrient dynamics in tropical ecosystems, and for developing sustainable land-use systems in the tropics under global change.

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Figure legends

Fig 1. Experimental design. The experiment was established at 12 sites, four sites in each land-use type (rainforest, rubber and oil palm plantations). Four treatments/experimental plots were established per site: control (C), root trenching, litter removal, and combined treatment. Samples were separated into leaf litter layer and soil layer with two depth, i.e., 0–5 cm and 5–10 cm. We measured microbial biomass, respiration, and phospholipid fatty acid (PLFA) profiles.

Fig. 2. Wald test results of linear mixed-effects models on the effect of litter removal (NoLitter), root trenching (NoRoot) and land-use types (LU) on the microbial indicators in the litter and soil layers (24 models in total). The present of bubble indicate significant effects and bubble sizes represent the F-value of the corresponding factor effects. For the detail of modelling results see Table S1.

Fig 3. The effect sizes of litter removal (NoLitter) and root trenching (NoRoot) on basal respiration ($\mu\text{g O}_2\text{g}^{-1}\text{ h}^{-1}$), microbial biomass (C_{mic} ; $\mu\text{g C}_{\text{mic}}\text{ g}^{-1}$) and total PLFAs (nmol g^{-1}) amount in three layers: litter, 0-5 and 5-10 cm soil depth (a, upper panel), and cross-treatment average values \pm standard errors of these parameters per land-use type (LU) in three layers (b, lower panel). Effect sizes are given as back transformed log response ratios (percent changes). Asterisks in the panel a indicate significant effects, with (*) $p < 0.1$, * $p < 0.05$. X-axes in the panel b are square-rooted.

Fig 4. Non-metric multidimensional scaling (NMDS) of microbial community structure (PLFAs) in three land-use types in litter layer (a), soil layer with 0-5 cm soil depth (b), and soil layer with 5-10 cm soil depth (c), with and without living roots (Root: control + litter removal; NoRoot: trenching + litter removal and trenching). Positions of the treatments represent centroids ($n = 8$). The variation of microbial community structure with land-use types (rainforest - green, rubber - orange and oil palm - yellow) and soil depth (0-5 cm: solid-line ellipse; 5-10 cm: dash-line ellipse) across experimental treatments (d).

Fig 5. The effect sizes of litter removal (Litter-) and root trenching (Root-) on the relative abundance of arbuscular mycorrhiza fungi (AMF), bacterial PLFA in three layers across land-use types (a, upper panel), and cross-treatment average values \pm standard errors of these parameters per land use system in three layers: litter, 0-5 and 5-0 cm soil depth (b, lower panel). Effect sizes are given as back transformed logit response ratios (percent changes). Asterisks in the panel a indicate significant effects, with (*) $p < 0.1$, * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$.

Appendix legends

Appendix 1. The methodology for calculating microbial markers and indicators by PLFAs

Figure S1. Correlations between PLFA and NLFA 16:1 ω 5 in the same studied sites.

Figure S2. Effect sizes of land use (a, upper panel) and soil depth (b, lower panel) on basal respiration ($\mu\text{g O}_2\text{g}^{-1}\text{ h}^{-1}$), microbial biomass (C_{mic} ; $\mu\text{g C}_{\text{mic}}\text{ g}^{-1}$) and total PLFAs (nmol g^{-1}) amount across treatments. Effect sizes are given as back transformed log response ratios. Asterisks in the panel a indicate significant effects, with (*) $p < 0.1$, * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$.

Figure S3. Root density (amount of dry fine roots per kilogram of dry substrates; g/kg) in the litter and soil layer of each land-use type. Bars sharing the same letter within each pane are not significantly different (Tukey's HSD test following ANOVA, $p > 0.05$).

Table S1. Type III ANOVA table of F- and p-values of linear mixed-effects models on the effect of root trenching and land-use type (rainforest, rubber and oil palm plantations) on each microbial parameter in litter layer, with site as random effects; and root trenching, litter removal, land-use type, soil depth (0-5 and 5-10 cm) and soil moisture (both 0-5 cm and 5-10 cm) on each microbial parameter in soil layer, with treatment nested within site as random factor; num DF, numerator degrees of freedom; den DF, denominator degrees of freedom. Asterisks denote significance levels: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Table S2. Effect sizes of root trenching and litter removal on basal respiration ($\mu\text{g O}_2\text{ g}^{-1}\text{h}^{-1}$), microbial biomass (C_{mic} ; $\mu\text{g C}_{\text{mic}}\text{ g}^{-1}$) and total PLFAs (nmol g^{-1}) in each layer across land-use types. Asterisks denote significance levels: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Table S3. Effect sizes of root trenching and litter removal on PLFA indicators in each layer across land-use types. Asterisks denote significance levels: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Table S4. Effect sizes of soil depth (the ratio between 5-10 cm and 0-5 cm) on basal respiration, microbial biomass and total PLFAs in each and across land-use type. Asterisks denote significance levels: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Table S5. Effect sizes of land use (the ratio between oil palm plantation *vs* rainforest, rubber plantation *vs* rainforest and oil palm plantation *vs* rubber plantation) on basal respiration, microbial biomass and total PLFAs in litter and soil layer. Asterisks denote significance levels: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Table S6. Effect sizes of soil depth (the ratio between soil layer *vs* litter layer) on root density in each land-use type.







