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Global distribution patterns of mycorrhizal associations: abundance, environmental drivers and ecological impacts

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CHAPTER 3

Mycorrhizal tree impacts on topsoil biogeochemical properties in tropical forests

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Abstract

1. In tropical regions, the patterns of carbon (C) and nutrient properties among ecosystems dominated by distinct mycorrhizal associations are unknown. We aim to reveal whether the dynamics differ and the ecological drivers and ecosystem functioning implications of such differences.
2. Based on a dataset of 97 tropical forest sites, we related EcM trees abundance (as a proxy for the transition from AM to EcM trees dominance) to different topsoil properties, climatic conditions, and microbial abundance proxies through Generalized Additive Models.
3. Higher abundances of EcM trees was correlated with lower topsoil concentrations of total nitrogen and C, extractable phosphorus and potassium, $\delta^{13}C$, mean annual temperature, precipitation, microbial (bacterial and fungal) biomass, and the relative abundance of saprotrophic fungi.
4. *Synthesis.* Our results reveal consistent differences in carbon and nutrient content between arbuscular mycorrhizal (AM-) and EcM-dominated vegetation across the tropical biome, pointing to lower soil fertility and lower rates of C and nutrient transformation processes in EcM-dominated forests. These patterns associate with lower topsoil C accumulation when compared to AM vegetation, which contrasts with patterns reported for temperate forests. We suggest that different mechanisms of soil organic matter accumulation explain the contrasting impacts of EcM dominance on topsoil properties of temperate and tropical biomes. Global vegetation and C models should account for the contrasting impacts of distinct mycorrhizal vegetation in different climatic zones.

3.1. Introduction

Mycorrhizal associations play a major role in the maintenance and functioning of terrestrial ecosystems. Arbuscular mycorrhiza (AM) and ectomycorrhiza (EcM) are the most taxonomically and geographically widespread mycorrhizal associations, being dominant in the majority of terrestrial biomes (Soudzilovskaia et al. 2019). It has been estimated that approximately 80% of the Earth's plant species form mycorrhizal associations with AM or EcM fungi (Brundrett and Tedersoo 2018). These two types of mycorrhizal fungi differ in physiology and morphology (Smith and Read 2008), which leads to strong differences in nutrient acquisition strategies. While EcM fungi produce exudates with extracellular lytic enzymes that can break down organic compounds (Read et al. 2004), AM fungi generally lack these saprotrophic abilities, obtaining nutrients mostly from inorganic compounds (Smith and Smith 2011).

The fundamental differences between AM and EcM fungal traits have led to the hypothesis that ecosystems dominated by EcM or AM plants have significant differences in processes of soil carbon (C) and nutrient cycling and storage (Read and Perez-Moreno 2003, Phillips et al. 2013, Brzostek et al. 2015). Consequently, this hypothesis predicts that ecosystems dominated by EcM are characterized by a slow turnover of soil organic matter, allowing the accumulation of larger pools of organic C and nitrogen (N) bound in complex recalcitrant organic molecules within the surface soils (Phillips et al. 2013, Averill and Hawkes 2016, Craig et al. 2018). These conditions select for a soil microbial community primarily dominated by ectomycorrhizal fungi with high enzymatic activity related to organic N and P liberation (Cheeke et al. 2017) and retarded activities of decomposers (Rousk and Bååth 2007). In contrast, AM-dominated systems have a rapid nutrient cycling, where labile plant litter is rapidly transformed into inorganic forms by both bacteria and saprotrophic fungi (Read 1991, Phillips et al. 2013).

Various studies examining soil biogeochemical cycles of AM- and EcM-dominated forest stands near each other suggest an active role of plant mycorrhizal types in soil carbon and nutrient cycling. However, this hypothesis has been primarily tested and corroborated in temperate forests of the northern hemisphere where AM and EcM

trees usually coexist and where data collection is generally facilitated by well-established long-term plots (Tedersoo and Bahram 2019). Extrapolations of this theory to other biomes remains controversial, especially so for tropical rainforests (Corrales et al. 2018).

Similarly to temperate forests, tropical forests feature gradients from AM to EcM-dominated forest stands (Brearley 2012, Nouhra et al. 2019, Steidinger et al. 2019). But, higher temperature and rainfall, limitation of P and other rock-derived nutrients (Camenzind et al. 2018) and hyper-diverse biological communities (Gentry 1992) make biogeochemical processes in tropical forests intrinsically different from temperate forests. Consequently, it is unclear whether this transition in plant mycorrhizal traits reflects (or promotes) changes in soil biogeochemical cycling.

A few local-scale studies have specifically, addressed this issue, with contrasting results. Torti *et al.* (2001) showed that soils in a monodominant EcM forest had a lower availability of N for plants than the contiguous AM mixed forest. Along the same lines, Corrales *et al.* (2016) hypothesized that the higher abundance of the EcM tree *Oreomunnea mexicana* in a Panamanian tropical forest was linked to the depletion of available N as a result of lower mineralization rates. However, other studies in tropical biomes have not found differences in soil properties when comparing EcM-dominated plots with adjacent AM plots (Hart et al. 1989, Moyersoen et al. 1998, Henkel et al. 2002, Read et al. 2006, Peh et al. 2011). Moreover, Keller & Phillips (2019) found that, in tropical areas, AM trees and EcM trees did not differ in litter decay rates, which contradicts the patterns in temperate regions.

Altogether these studies suggest that the mechanisms that lead to the remarkable differences in soil biogeochemical cycling between AM and EcM tree stands in temperate forests may be different or may be weakened in the tropics. However, to the best of our knowledge, no study has been conducted that includes the whole tropical biome and covers the full gradient of AM to EcM tree dominance to evaluate the existence of consistent functional differences between the two mycorrhizal types. Given the ecological and economic value of tropical forests, understanding whether dominant mycorrhizal traits can be used as proxies of soil biogeochemical cycling will

lead to a better prediction of changes in carbon and nutrient cycling in the future climate, land-use changes or species introductions.

Here, we aim to evaluate changes in soil chemical and biological properties along the gradient from AM to EcM-dominated tropical systems. We base our research on a dataset containing information on topsoil properties, climatic conditions, microbial abundance, and mycorrhizal tree abundance from all four continents that contain tropical forests. Based on our analysis, we suggest a new framework explaining the role of EcM in global patterns of soil C accumulation and nutrient turnover.

3.2. Methods

3.2.1. Sample and data collection

We used 97 topsoil samples originating from a global set of soil samples presented in the study of Tedersoo *et al.* (2014). We selected all samples collected in tropical (n=83) and subtropical (latitude between 23.5° and 34°N/S; n=14) moist (mean annual precipitation (MAP)> 1500mm y^{-1}; n=8) and dry (MAP< 1500mm y^{-1}; n=89) lowland (altitude <800m; n=79) and lower montane (altitude >800m and <1500m; n=18) forests (henceforth referred to as tropical forests) (Figure 3.1). A detailed description of soil sampling can be found in Tedersoo *et al.* (2014). In short, soil samples were collected from 2500-m^2 plots. After removing loose debris from the forest floor, 40 topsoil cores were collected using PVC tubes (5 cm deep, 5 cm in diameter) from each of the selected plots. The soil cores included both organic and mineral horizons. These 40 soil cores were pooled, homogenised, and subsequently examined for microbial community composition and soil properties.

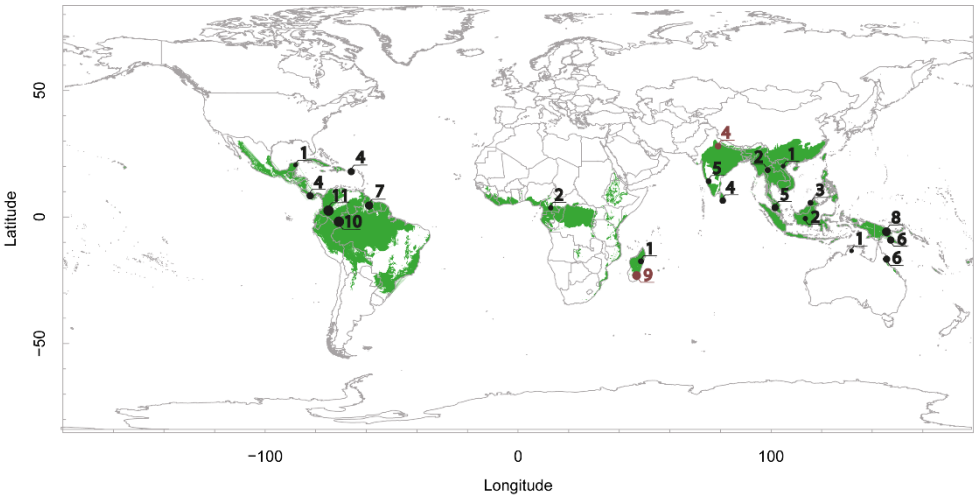


Figure 3.1: *Map with the 97 independent sample locations. The green area shows the tropical and subtropical moist and dry broadleaf forest biome derived from the World Wildlife Fund (WWF) ecoregions layer (Olson et al. 2001). For better visualization, each circle indicates areas with one or more sample locations and numbers indicate the number of independent sampling locations located at each black circle. Brown circles indicate subtropical samples.*

At each of the 97 plots, known EcM trees and shrubs were identified and their relative contribution to stand basal area was estimated as a proxy for EcM-associated trees abundance. Due to the low abundance of non-mycorrhizal trees and other mycorrhizal types in tropical areas (Soudzilovskaia et al. 2019, Soudzilovskaia et al. 2020), we consider all non-EcM plants as being AM hosts. Therefore, the relative abundance of EcM trees is seen as complementary to the relative abundance of AM trees (i.e. 20% coverage of EcM trees equals 80% of AM trees).

3.2.2. Soil properties and climate data

Soil chemical properties were determined as described in Tedersoo *et al.* (2012). The concentration of soil total carbon (total C), total nitrogen (total N), ammonium lactate soluble phosphorus (P), exchangeable potassium (K), calcium (Ca), and magnesium (Mg) cations were examined to assess the effect of distinct types of mycorrhizal trees on the amount and composition of soil organic matter. Additionally, $\delta^{13}C$ and $\delta^{15}N$

were examined, as these reflect soil organic matter (SOM) recalcitrance (Boström et al. 2007) and mineralization rates (Houlton et al. 2006), respectively.

To control for climate in addition to soil conditions (Conant et al. 2011), we included data on the mean annual temperature (MAT) and MAP at 30 arc-seconds resolution originally retrieved from the WorldClim database (www.worldclim.org).

3.2.3. Microbial abundance

3.2.3.1.Phospholipid fatty acid (PLFA) analysis

To explore the impacts of distinct types of mycorrhizal plants on soil microbial communities, we obtained data on the abundance of the main microbial groups (fungi and bacteria) by performing a phospholipid fatty acid (PLFA) analysis. From that original batch, 34 samples could not be recovered or there was not enough material for analysis. Therefore, 63 samples were finally used for PLFA analysis. The lipid extraction from soil was performed using a one-phase mixture following Bligh & Dyer (1959) as modified by White *et al.* (1977) and Frostegård *et al.* (1991). Lipids were extracted from 1, 2, or 3 g of dried soil (depending on their SOM content) with 10 ml of Bligh & Dyer solution ($CHCl_3$: MeOH: citrate buffer 1:2:0.8 v/v/v). The extracted lipids were fractionated into neutral lipids, intermediate lipids, and polar lipids on pre-packed silica columns by eluting with 1.5 ml chloroform, 6 ml acetone, and 1.5 ml methanol, respectively (van Aarle and Olsson 2003). The neutral and polar lipids (containing phospholipids) were evaporated again after adding 100 µl of fatty acid methyl ester (FAME) 19:0 as internal standard. The intermediate lipids were discarded. The samples were dissolved in 1 ml toluene:methanol (1:1) and KOH in methanol (0.2 M), and incubated at 37ºC for 15 min. After cooling, 2 ml of hexane:chloroform (4:1, v/v), 0.3 ml of acetic acid (1 M) and 2 ml of water were added. The upper (organic) phase obtained was transferred to a new test tube and evaporated. The samples were dissolved with 100 µl of hexane and analysed on a Hewlett Packard 6890a gas chromatograph.

The sum of the PLFA i15:0, a15:0, 15:0, i16:0, 16:1S9, i17:0, a17:0, cy17:0, 18:1ω7, and cy19:0 was used as a proxy of bacterial biomass (Frostegård and Bååth 1996). The amount of PLFA 18:2ω6 was used as a proxy of saprotrophic (and EcM) fungal biomass The ratio 18:2ω6 to bacterial PLFA was used as an index of the F:B abundance ratio in the soil.

3.2.3.2.Sequencing Data

The soil fungal community was characterised using 454 pyrosequencing (Tedersoo et al. 2014). This data contains sequences of the ITS2 regions classified taxonomically and functionally, differentiating 12 relevant lifestyles, including arbuscular mycorrhizal, ectomycorrhizal, and saprotrophic fungi (ericoid and orchid mycorrhiza were not included). We used the total number of fungal OTUs as a proxy of the fungal richness and the relative abundance of saprotrophic fungi (calculated as the total reads of saprotrophic fungi relative to the total fungal reads) as a proxy for the abundance of saprotrophic fungi. For a detailed protocol on DNA extraction, sequencing, and bioinformatics procedures see Tedersoo *et al.* (2014).

3.2.4. Statistical analysis

A set of univariate Generalized Additive Models for Location, Scale and Shape (GAMLSS) were fitted to relate the relative abundance of EcM trees to individual soil, climatic, and microbial proxies of biogeochemical cycling using the "gamlss" package (Stasinopoulos and Rigby 2007) in R Studio. A GAMLSS approach was selected as it allows fitting flexible regression models using a beta distribution, which is appropriate for modelling continuous proportions (Douma and Weedon 2019). As the beta distribution assumes values of the response variable to be >0 and <1 while our dataset contains 0 (i.e. absences of EcM trees in the plot) and 1 (i.e. only EcM trees in the plot) values, we used the transformation $x' = \frac{x(N-1) + 0.5}{N}$ where N is the sample size (Smithson and Verkuilen 2006). The existence of non-linear relationships was explored by plotting residual vs fitted values of the univariate models. As no clear trends were detected, GAMLSS were fitted using linear predictors. Logarithmic

transformations were applied to total N, total C, P, K, Mg, and Ca to meet model assumptions. We further tested differences among tropical systems (tropical vs subtropical; moist vs dry; lowland vs lower montane) on the univariate models by including these factors as interaction terms. We did not find any influence of these distinctions on the main trends (see Supporting information Table S3.1).

To visualize the relationship between the soil properties and EcM trees abundance we also performed a Principal Component Analysis (PCA) using the "prcomp" function of R package "stats".

To determine the importance of the selected biogeochemical drivers on explaining EcM trees abundance, we performed a multivariable model selection. The full GAMLSS model contained only a set of non-correlated climatic and soil variables (i.e. Pearson correlation <0.6 and a variance inflation factor (VIF) <3) that were significant in the univariate models. As total N and total C were highly correlated (Pearson correlation = 0.967), only total N was included in the multivariable model due to its higher univariate R^2. Bacterial and fungal PLFA data were excluded from the multivariable model and interpreted independently, due to the high collinearity with soil C and N (see Supporting Information Table S3.2). Therefore, only MAT, MAP, Total N, C/N, $\delta^{15}N$, P, Mg, Total fungal OTUs and the relative abundance of saprotrophic fungi were included in the final model. A forward selection was performed removing non-significant variables from the model.

The presence of spatial autocorrelation (SAC) in the multivariable model residuals was tested using Moran's I test with the "moran.I" function in the "ape" package. The test confirmed the presence of SAC in the model residuals (Observed =0.259; Expected=-0.01; *P*-value = 0.002) which was alleviated by explicitly including spatial coordinates in the model as a two-dimensional smooth surface (Observed =0.059; Expected=-0.01; *P*-value = 0.42).

3.3. Results

3.3.1. Single variable effects on EcM trees abundance

As a first exploratory analysis, the PCA showed that distinct EcM trees abundances were separated in a multivariate space (Figure 3.2) spanned by the chemical and biological soil properties.

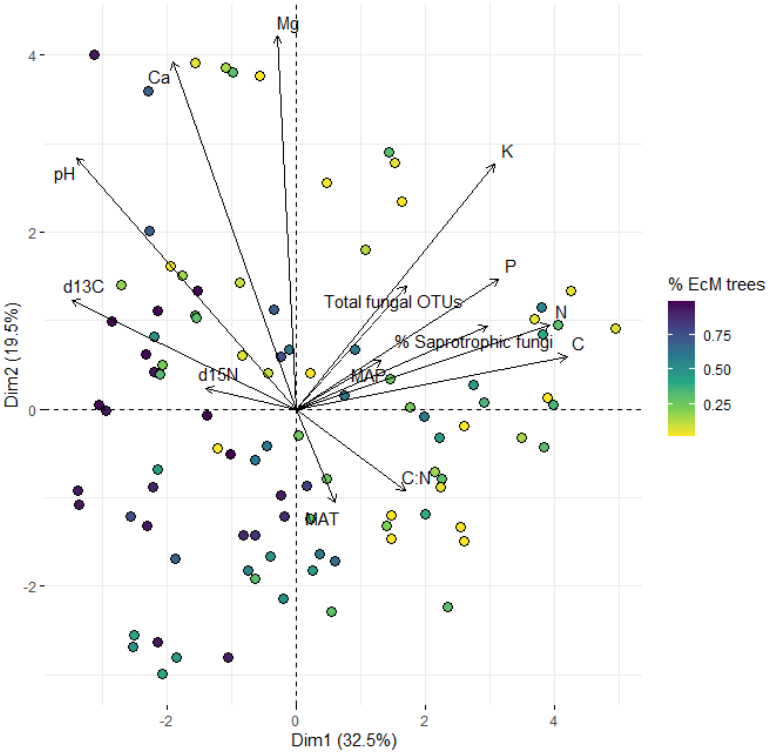


Figure 3.2: *Principal components analysis (PCA) ordination plot of soil properties showing the differences in soil biogeochemistry imposed on an EcM trees abundance gradient. The vector length indicates the relative importance of the variables.*

Subsequent individual GAMLSS models showed that total topsoil C and N, exchangeable P and K, MAT, and MAP decreased linearly with increasing abundance of EcM-associated trees in tropical forests (Table 3.1; Figure 3.3a-f). $\delta^{13}C$ was significantly positively related to EcM tree abundance (Figure 3.3g). Among the

predictors, topsoil total N and $\delta^{13}C$ provided the strongest models, explaining 24.1% and 22.6% of the total variance, respectively (Table 3.1).

Using the 454 sequencing data, we found that both the total number of fungal OTUs and the relative proportion of saprotrophic fungi were significantly and negatively related to the abundance of EcM trees (Table 3.1; Figure 3.3h-i).

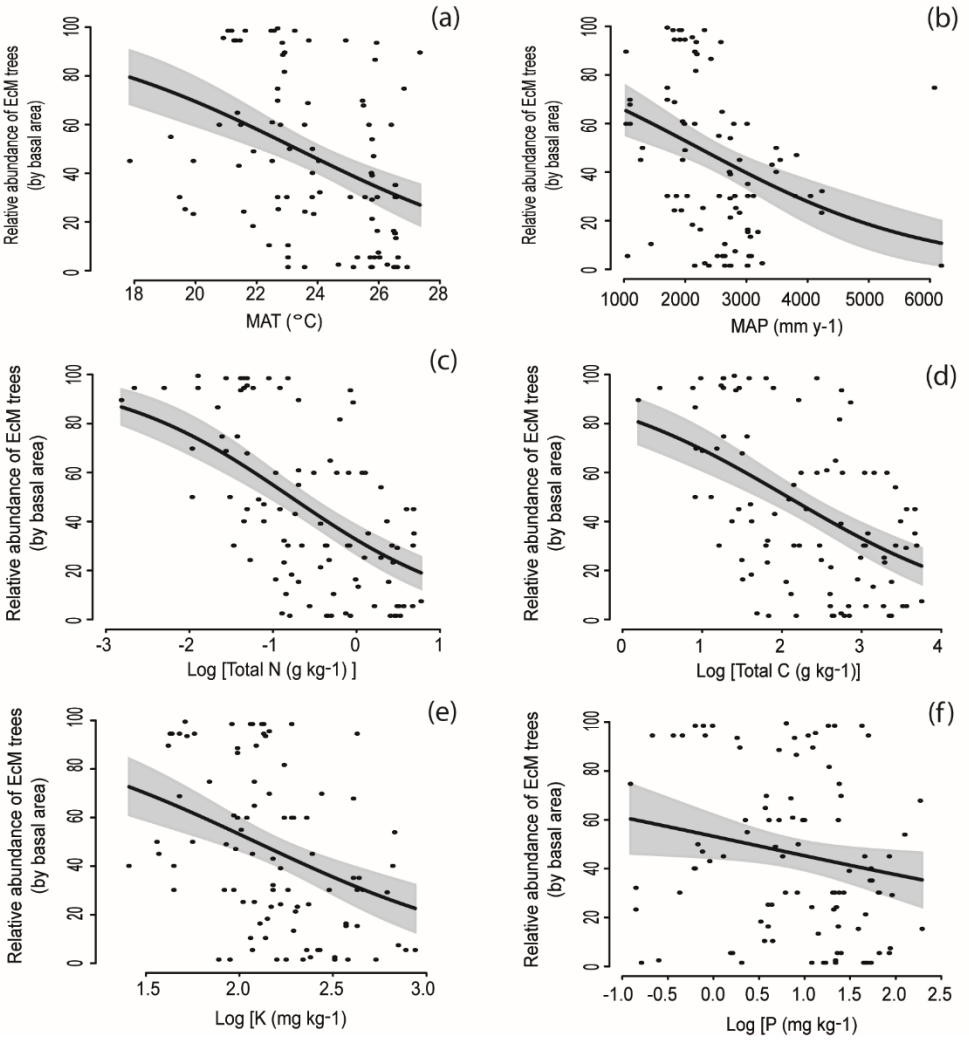
GAMLSS models of microbial abundances showed that relative EcM tree abundance was significantly (*P*-value < 0.05) negatively related to bacterial biomass (bacterial PLFA) (Table 3.1; Figure 3.3j). Fungal PLFA showed a marginally significant (*P*-value > 0.05 but < 0.1) negative relationship with EcM tree abundance, whereas F/B ratio did not show any significant relationship (Table 3.1).

Table 3.1: *Regression coefficients, standard errors, t-values, P-values, and pseudo-R^2 for single variable GAMLSS models predicting EcM tree abundances based on individual soil biogeochemical proxies.*

	Coefficient	Std. Error	t-value	*P*-value	Pseudo-R^2
MAT (°C)	-0.241	0.058	-4.138	**<0.001**	0.159
MAP (mm y-1)	-0.0005	0.0001	-4.933	**<0.001**	0.121
Total N* (g kg^{-1})	-1.198	0.229	-5.23	**<0.001**	0.241
Total C* (g kg^{-1})	-0.052	0.011	-4.497	**<0.001**	0.186
C/N	0.035	0.029	1.194	0.235	0.014
C/P	-0.009	0.005	-1.784	0.08	0.042
N/P	0.024	0.013	1.862	0.07	0.04
$\delta^{15}N$ (‰)	-0.081	0.071	-1.141	0.257	0.013
$\delta^{13}C$ (‰)	0.391	0.081	4.819	**<0.001**	0.226
pH	0.203	0.104	1.947	0.0545	0.038
P* (mg kg^{-1})	-0.332	0.158	-2.105	**0.0379**	0.044
K* (mg kg^{-1})	-1.472	0.369	-3.985	**<0.001**	0.147
Mg* (mg kg^{-1})	-0.290	0.271	-1.07	0.287	0.011
Ca* (mg kg^{-1})	0.063	0.223	0.283	0.778	0.001
Total fungal OTUs	-0.01	0.002	-4.287	**<0.001**	0.17

Relative abundance of saprotrophic fungi (%)	-0.043	0.008	-5.633	**<0.001**	0.272
Bacterial PLFA (nmol g^{-1})	-0.011	0.003	-4.134	**<0.001**	0.235
Fungal PLFA (nmol g^{-1})	-0.058	0.031	-1.835	0.071	0.053
log(F/B+1)	3.683	3.482	1.058	0.294	0.017

*Bold P-values indicate significant relations. *Variables log-transformed*



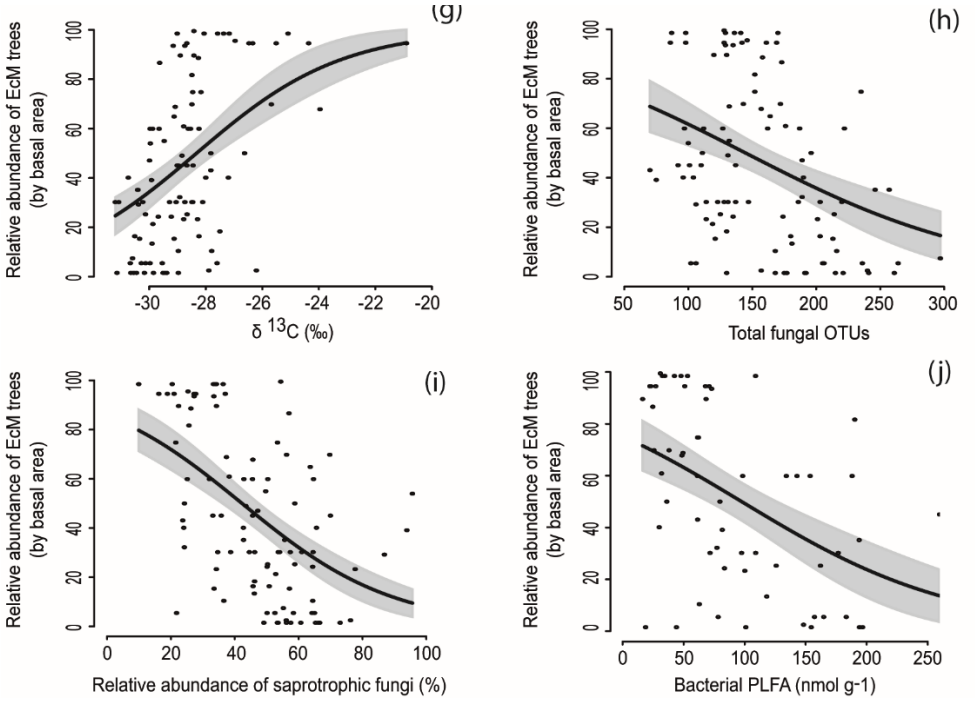


Figure 3.3: *Predicted relationships between the proportion of EcM-associated trees and biogeochemical proxies in the single variable models. Each relation was calculated by setting the other variables to the mean value. Light coloured shades show the region within the 95% confidence interval.*

3.3.2. Multivariable model

When all non-correlated biogeochemical proxies were included in one model, only total N, MAT, and the relative abundance of saprotrophic fungi remained in the best model (Table 3.2). Together, these three predictors explained 50% of the total variance in EcM trees abundance. When substituting total N by total C, very similar patterns with only slightly lower R^2 (0.45) were obtained. In both cases, total N and total C were the most important predictors. MAT and the relative abundance of saprotrophic fungi accounted for 12.3% and 11.6%, respectively, of the model variance.

Table 3.2: Predictors, coefficients, standard errors, t-values, p-values, and total pseudo- R^2 of the final multivariable GAMLSS model and the pseudo-R^2 that is attributed to each variable.

	Coefficient	Std. Error	t-value	*P*-value	Pseudo-R^2	Contribution to pseudo-R^2[a]
MAT (°C)	-0.16	0.046	-3.482	<0.001	0.50	0.123
Total N (g kg^{-1})	-0.668	0.134	-4.976	<0.001		0.186
Relative abundance of saprotrophic fungi (%)	-0.023	0.007	-3.517	<0.001		0.116

[a]Due to the presence of joint effects (which refers to the shared contribution in the final model), the sum of the independent contribution of each variable to the model Pseudo-R^2 does not necessarily approximate to the pseudo-R^2 of the final model.

3.4. Discussion

During the last decades, the amount of information about the mycorrhizal status of tropical plants has been growing rapidly (Brearley 2012, Corrales et al. 2018, Nouhra et al. 2019). However, the influence of distinct types of mycorrhizal associations on the ecological processes in tropical areas is still poorly understood compared to that in temperate and boreal biomes. Using a large dataset that covers the full gradient of EcM trees relative abundance, we provide the first evidence that topsoil chemistry and topsoil microbial characteristics, along with climate, are linearly related to EcM abundance across the tropical biome.

3.4.1. Biogeochemical patterns in tropical EcM forests

We found that topsoil total C and total N (two closely linked variables, see Supporting Information Table S3.2), were the main factors explaining EcM tree abundances, exhibiting a clear negative relation therewith. This suggests that in tropical forests, topsoil OM concentrations in EcM tree stands are generally lower than in AM systems. Moreover, the negative correlation found between EcM trees abundance and the concentration of extractable P and K, emphasizes the role of EcM fungi in the weathering of rock-derived nutrients (Landeweert et al. 2001, van Schöll et al. 2008) in tropical systems where these nutrients are known to be limited (Camenzind et al.

2018). Finally, the significant changes in microbial biomass and community composition of main decomposers suggest that SOM transformation rates may vary from AM to EcM dominated forests. In summary, our results reflect consistent differences associated with carbon and nutrient cycles between AM- and EcM-dominated vegetation in the tropical biome, pointing to lower soil fertility and lower C accumulation in forests dominated by EcM. Lower nutrient concentrations in EcM systems are consistent with the idea that the EcM symbiosis is an adaptation to low nutrient environments (Read 1991, Read and Perez-Moreno 2003).

The ecosystem properties that trigger these distinct biogeochemical syndromes found in the tropics remain unresolved. Here we explore several potential (non-mutually exclusive) mechanisms that are likely to explain associations between EcM trees and low nutrient and C concentrations in tropical systems:

1. **Litter quality pools**: Differential C and nutrient cycling in EcM tropical stands may be triggered by a distinct quality of the litter pool compared to AM stands (Cornelissen et al. 2001, Phillips et al. 2013). The lack of consistent relations between soil C/N ratio, F/B ratio and, pH with EcM tree abundance in our dataset does not support this hypothesis. In addition, previous studies found no differences in litter chemistry (Averill 2016, Keller and Phillips 2019) nor litter decay rates (Keller and Phillips 2019, Seyfried et al. 2021) between tropical AM and EcM plant species.

2. **SOM decomposition:** Generally, the lower C and nutrient concentrations found in EcM stands could be related to faster SOM transformation rates. However, the decrease in the total biomass of bacteria may indicate that the rate and magnitude of C and nutrient transformation processes are lower in EcM tropical forests than in AM forests. Moreover, the lower relative abundance of saprotrophic fungi in EcM-dominated plots is consistent with the so-called "Gadgil effect", which refers to the inhibition of saprotrophic fungal activity by EcM fungi due to the competition for nutrients, resulting ultimately in reduced rates of SOM breakdown (Gadgil & Gadgil, 1971; Fernandez & Kennedy, 2015). Alternatively, topsoil SOC turnover may be enhanced by the saprotrophic abilities of EcM fungal communities (Tedersoo et al. 2014) that complement or even replace

saprotrophic communities as main decomposers (Lindahl et al. 2021). In a context of low fertility, rapid decomposition will minimize nutrient losses via leaching (Chuyong et al. 2000). Unfortunately, our dataset does not allow us to explicitly test the effect of EcM abundance on organic carbon turnover rates. The potential connections between microbial abundance and process rates should be further explored and tested.

3. **Litter inputs:** Lower topsoil C and N concentrations in EcM-dominated stands may be related to lower aboveground or belowground litter inputs. Lin et. al., 2016 found no differences in litter inputs between EcM and AM-dominated tropical stands, although this result is based on relatively few data points (n=19). Unfortunately, no relevant litter input proxy was available at sufficiently high resolution to allow testing this mechanism at the plots examined in our study.

4. **Role of N-fixing trees:** A higher proportion of N-fixing trees is expected in AM tropical stands (Veresoglou et al. 2012). This higher abundance of N-fixers has been related to an increased accumulation and stabilization of soil organic matter (Resh et al. 2002, Binkley 2005, Levy-Varon et al. 2019). Although the mechanism behind this relationship remains unclear, increased N availability may lead to greater fresh C inputs to the soil and a preferential decomposition of the newer and more labile forms of C over the older and recalcitrant forms (Resh et al. 2002). The role of facultative N-fixing trees and free-living N-fixers should be taken into consideration when testing this hypothesis.

5. **Climatic control:** The significant relationships between EcM abundance and temperature and precipitation suggest at least a partial climatic control on soil biogeochemical processes. Lower temperature and precipitation may favour the establishment of ectomycorrhizal associations through ecological mechanisms that improve the performance and survival of ectomycorrhizal plants and fungi (Barceló et al. 2019, Steidinger et al. 2019).

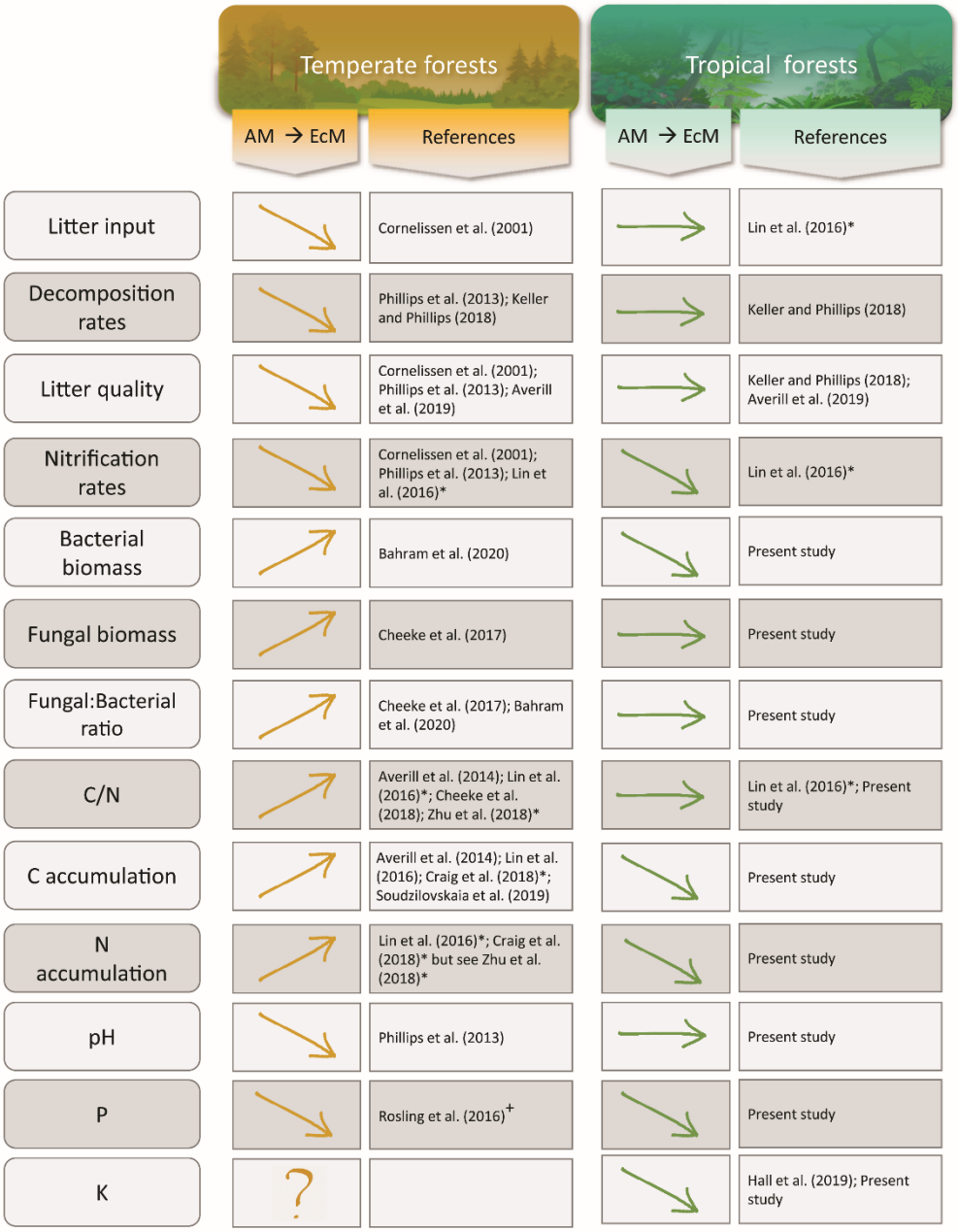
Further large scale research in tropical areas targeting EcM communities and their functional roles and specific ecosystem processes (e.g. litter inputs, decomposition rates and productivity) is key to disentangle the mechanisms underpinning the patterns

found in the tropics and may provide insights into the generic impacts of mycorrhizal associations on biogeochemical cycles.

3.4.2. Tropical vs temperate biogeochemical patterns across the AM–EcM transition

It is well established that, within temperate ecosystems, a higher abundance of EcM plants is generally associated with lower organic C and nutrient turnover rates (Phillips et al. 2013, Averill and Hawkes 2016). This effect has been traditionally related to ecosystem processes directly mediated by EcM fungi such as low turnover rate of extraradical mycelium (Leake et al. 2004, Olsson and Johnson 2005, Ekblad et al. 2013) or competition with other microorganisms that lower decomposition rates, reducing nitrogen availability to saprotrophic organisms (Gadgil and Gadgil 1971, Fernandez and Kennedy 2015). In temperate forests, EcM plant communities are known to have lower NPP (Read and Perez-Moreno 2003, Vargas et al. 2010, Averill et al. 2014) and more recalcitrant and acidic litter (Cornelissen et al. 2001, Phillips et al. 2013, Averill et al. 2019), contributing to slower carbon and nutrient cycling. This has led to the supposition that, in temperate ecosystems, EcM-dominated forests store more C than AM-dominated ones (Phillips et al. 2013, Averill and Hawkes 2016). Recent investigations questioned the validity of this view, suggesting that the mechanisms through which AM and EM vegetation impact biogeochemical cycling vary with soil depth (Craig et al. 2018, Jo et al. 2019). While EcM temperate stands usually accumulate more C in the upper surface soils, the pattern is reversed in deeper soils (Craig et al. 2018, Jo et al. 2019).

Until now, it was unclear if and how differences in biogeochemical cycling also prevail between AM and EcM vegetation types in tropical systems, making it impossible to generalize the impact of EcM on ecosystem functioning at global scales. Existing literature and our analyses on the upper surface soils suggest that mycorrhizal impacts of SOM might be expressed through a different set of mechanisms in the tropics than in the temperate zone. Figure 3.4 reviews the differences in topsoil biogeochemical properties in the transition from AM- to EcM-dominated vegetation in tropical and temperate forests according to existing literature and the results of our analysis.



*Only topsoil patterns included

+Local scale study

Figure 3.4: *Visualization of the differences in soil biogeochemical properties in the transition from AM to EcM-trees dominance in tropical and temperate forests according to literature data. For consistency, only analyses containing multiple sites at regional or continental scale comparisons were included. Local patterns were only included when no other relevant study was found. When different soil layers were investigated, only patterns resulting from topsoil layers were taken into account. Horizontal arrows indicate evidence of the absence of patterns. Question marks indicate that no relevant studies were found.*

We showed that in tropical forests, a higher proportion of EcM trees do not result in a build-up of C in the topsoil when compared to AM systems, contrary to temperate patterns. We hypothesize that the contrasting impacts on upper surface soils C concentrations between EcM temperate and tropical forests may be explained by distinct mechanisms through which the two biomes accumulate SOM in the upper soil layers.

Traditionally, the formation of stable SOM has been related mainly to the recalcitrant compounds in the litter that are not assimilated by decomposing microorganisms (Berg and McClaugherty 2008). Under this paradigm, slow decomposition promoted by recalcitrant litter would result in higher SOM stocks. We suggest that this is the primary mechanism acting in temperate forests where temperature constrains decomposition (Koven et al. 2017) and litter inputs are likely to be the primary source of SOM stocks in the upper soil (Liang et al. 2019). In this way, a transition from AM- to EcM-dominated forest leads to a build-up of SOM in the topsoil (Lin et al. 2017, Craig et al. 2018, Zhu et al. 2018) due to the higher recalcitrance of EcM plants litter in temperate forests (Cornelissen et al. 2001, Phillips et al. 2013) and the competition with saprotrophs (Gadgil and Gadgil 1971, Fernandez and Kennedy 2015).

In contrast, in tropical forests, the litter of EcM plants does not decay slower than AM litter (Keller and Phillips 2019) and temperature is not likely to limit saprotrophic activity. Thus, overall decay rates themselves may be less strongly limiting the final concentrations of topsoil SOM. Instead, the microbial turnover processes may be more influential. In line with this, an emerging body of research argues that microbial products fixed to the mineral soil matrix may be the primary component of stable

SOM (Liang and Balser 2011, Cotrufo et al. 2013, Kallenbach et al. 2016, Sokol and Bradford 2019). Consequently, conditions enhancing rates of microbial biomass growth would promote the accumulation of microbial-stabilized SOM. In this scenario, the observed higher microbial biomass of tropical AM vegetation would lead to higher topsoil C accumulation. The lack of evidence of different recalcitrance between AM and EcM litter together with the higher bacterial biomass and higher C and N concentrations found in AM-dominated plots support this hypothesis. Additionally, the higher abundance of N-fixing trees in tropical than in temperate AM-dominated ecosystems (Steidinger et al. 2019) could induce microbial growth, promoting the stabilization and accumulation of surface SOM (Binkley 2005).

Additionally, the prevalence of AM associations in sites where topsoil SOM is accumulated may be related to the importance of leaching processes in tropical forests. Contrary to temperate forests, tropical systems feature significant losses of dissolved nutrients by leaching (Montagnini and Jordan 2005). As AM fungi lack saprotrophic capabilities (Smith and Read 2008), AM associations would be favoured in locations where the soil is stabilized and losses of dissolved nutrients by leaching are minimized. In contrast, EcM fungi can access nutrients directly from organic sources (Smith and Read 2008) and can effectively immobilize nutrients through a more extensive hyphal network (Agerer 2001), and are less susceptible to leaching losses.

We suggest that using the dominant type of mycorrhizal association to predict soil biogeochemical properties based solely on patterns from the temperate zone can be misleading. Further mycorrhizal research in tropical forests explicitly testing differences in SOM cycling and accumulation between temperate and tropical forests, and accounting for differences in soils depth is needed for a better understanding of mycorrhizal effects on ecosystem functioning in different climatic zones.

3.5. Concluding remarks

Our data show that in tropical forests, EcM associations are mainly found in non-fertile soils. This reinforces the view of EcM associations being dominant in ecosystems where plant communities are nutrient-limited. However, in contrast to the patterns

observed in the temperate zone, the dominance of EcM trees does not result in an accumulation of the topsoil C. We suggest that the contrasting effect of mycorrhizal type in topsoil C concentrations between temperate and tropical biomes is related to differences in their mechanisms of SOM accumulation in the upper surface soils, which are triggered by distinct climatic and biotic conditions. Global models of vegetation dynamics and SOM pools should account for the impacts of mycorrhizal associations on soil biogeochemical processes and their associated microbial mechanisms in different climatic zones.

3.6. Acknowledgements

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3.7. Conflict of Interest

The authors declare that there is no conflict of interest.

3.8. Author contributions

M.B., N.A.S. and P.M.v.B. conceived the manuscript. P.A.O. assisted with PLFA analysis. L.T. contributed data. M.B. performed the statistical analysis and wrote the manuscript with substantial input from all the authors.

3.9. Data availability

The data that support the findings of this study are openly available in Figshare at http://doi.org/10.6084/m9.figshare.13260227

3.10. Supporting Information

Table S3.1: *Estimates and P-values of univariate GAMLSS models including data points from only tropical or subtropical areas, dry or moist climates and lowland or lower m ontane locations.*

	Sub-biome 1				Sub-biome 2				Sub-biome 3			
	Tropical		Subtropical		Dry		Moist		Lowland		Lower montane	
	Estimate	*P-value*	*Estimate*	*P-value*	*Estimate*	*P-value*	*Estimate*	*P-value*	*Estimate*	*P-value*	*Estimate*	*P-value*
MAT (°C)	-0.21	<0.001	-0.18	0.01	-0.26	<0.001	-0.25	<0.001	-0.3	<0.001	-0.33	<0.001
MAP (mm y-1)	-0.0004	<0.001	0.0001	0.73	-0.002	<0.001	-0.001	<0.001	-0.0005	<0.001	-0.0005	<0.001
Total N* (g kg−1)	-0.83	<0.001	-1.27	<0.001	-0.49	0.152	-0.989	<0.001	-0.94	<0.001	-0.855	<0.001
Total C* (g kg−1)	-0.76	<0.001	-0.43	0.032	-0.71	0.002	-0.76	<0.001	-0.79	<0.001	-0.68	<0.001
C/N	0.02	0.53	0.06	0.05	0.04	0.32	0.03	0.23	0.03	0.23	0.3	0.28
δ15N (‰)	-0.1	0.183	-0.01	0.93	-0.05	0.672	-0.097	0.207	-0.09	0.231	-0.09	0.613
δ13C (‰)	0.37	<0.001	0.33	<0.001	0.46	<0.001	0.43	<0.001	0.39	<0.001	0.39	<0.001
pH	0.16	0.11	0.41	<0.001	0.15	0.2	0.23	0.04	0.2	0.06	0.22	0.07
P*(mg kg−1)	-0.47	0.004	0.56	0.06	-0.07	0.86	-0.33	0.04	-0.35	0.03	-0.07	0.8
K* (mg kg−1)	-1.46	<0.001	-0.98	0.01	-1.41	<0.001	-1.45	<0.001	-1.45	<0.001	-1.39	<0.001
Mg* (mg kg−1)	-0.57	0.05	-0.07	0.79	-0.25	0.41	-0.26	0.36	-0.25	0.36	-0.22	0.5
Ca* (mg kg−1)	-0.07	0.75	0.32	0.16	0.07	0.78	0.11	0.63	0.1	0.66	0.15	0.55
Total fungal OTUs	-0.01	<0.001	-0.005	0.06	-0.01	0.006	-0.01	<0.001	-0.01	<0.001	-0.01	<0.001
Relative abundance of saprotrophic fungi (%)	-0.05	<0.001	-0.02	0.008	-0.04	<0.001	-0.04	<0.001	-0.04	<0.001	-0.04	<0.001
Bacterial PLFA (nmol g−1)	-0.01	<0.001	-0.005	0.2	-0.009	0.042	-0.012	<0.001	-0.012	<0.001	-0.01	0.012
Fungal PLFA (nmol g−1)	-0.072	0.039	0.01	0.84	-0.04	0.5	-0.06	0.08	-0.05	0.12	-0.06	0.27
log(F/B+1)	-0.81	0.88	5.63	0.14	3.3	0.78	4.04	0.26	4.52	0.21	-1.58	0.83

***Table S3.2**: Person correlation matrix of soil, climatic and microbial abundance variables.*

	NPP	MAP	MAT	N	C	C/N	$\delta^{15}N$	$\delta^{13}C$	pH	LogP	LogK	LogMg	LogCa	Saprotrophs	Total fungal OTUs	Bacterial PLFA	Fungal PLFA	F/B
NPP	1.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
MAP	0.28	1.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
MAT	0.23	0.40	1.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
N	0.11	0.11	0.13	1.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C	0.13	0.10	0.12	**0.97**	1.00	-	-	-	-	-	-	-	-	-	-	-	-	-
C/N	-0.06	-0.18	-0.11	0.10	0.30	1.00	-	-	-	-	-	-	-	-	-	-	-	-
$\delta^{15}N$	0.01	0.01	0.21	-0.17	-0.29	-0.53	1.00	-	-	-	-	-	-	-	-	-	-	-
$\delta^{13}C$	-0.19	-0.32	-0.35	-0.52	-0.56	-0.15	0.24	1.00	-	-	-	-	-	-	-	-	-	-
pH	-0.17	-0.12	-0.03	-0.45	-0.55	-0.46	0.23	**0.64**	1.00	-	-	-	-	-	-	-	-	-
LogP	-0.02	-0.29	0.18	0.54	0.57	0.15	-0.11	-0.35	-0.35	1.00	-	-	-	-	-	-	-	-
LogK	0.03	-0.02	0.26	**0.66**	**0.66**	0.07	-0.13	-0.29	-0.13	**0.64**	1.00	-	-	-	-	-	-	-
LogMg	-0.41	-0.18	-0.02	0.12	0.08	-0.02	-0.14	0.23	0.57	0.17	0.42	1.00	-	-	-	-	-	-
LogCa	-0.36	-0.15	-0.04	-0.16	-0.23	-0.21	0.02	0.45	**0.83**	-0.07	0.19	**0.82**	1.00	-	-	-	-	-
Saprotrophs	-0.06	0.03	0.23	0.44	0.46	0.19	-0.12	-0.40	-0.35	0.47	0.46	0.13	-0.08	1.00	-	-	-	-
Total fungal OTUs	-0.27	0.12	0.26	0.30	0.28	0.00	0.13	-0.25	-0.12	0.18	0.37	0.22	0.04	0.28	1.00	-	-	-
Bacterial PLFA	0.20	0.04	0.03	**0.86**	**0.85**	0.07	-0.17	-0.42	-0.40	0.36	0.52	0.03	-0.16	0.30	0.18	1.00	-	-

Fungal PLFA	0.13	-0.02	0.02	**0.62**	**0.71**	0.39	-0.25	-0.37	-0.53	0.40	0.46	0.03	-0.23	0.29	0.16	**0.70**	1.00	-
F/B	-0.19	-0.18	-0.10	0.01	0.13	**0.60**	-0.19	-0.12	-0.36	0.21	0.05	0.11	-0.08	0.21	0.09	-0.02	0.54	1.00

Numbers in bold indicate pairs of variables with correlation r >0.6