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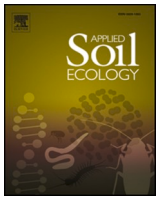

Short Communication

Large variability of soil microbial diversity and functions in an over 20-year old *Eucalyptus grandis* plantation

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ABSTRACT

The spatial heterogeneity in soil properties as well as in molecular and catabolic diversities of the soil microbial community were investigated by soil analyses, Illumina MiSeq sequencing and MicroResp™ profiling, respectively, in 51 plots of a 20 year-old eucalyptus plantation in south Brazil. The relationships between these parameters were assessed to test whether small scale variation in soil properties and understory vegetation generated heterogeneity in the soil microbial community. The spatial variability of soil microbial community and functioning was shown to be substantial. A greenness index, used as a proxy of the density of the understory biomass, explained very little of the soil microbial parameters. Variability in soil properties (mainly C availability, pH) partly explained shifts in molecular and catabolic parameters of the soil microbial community. Bacterial (and, to a lower extent, fungal) molecular parameters were the main factors explaining the catabolic capabilities of the soil microbial community. But overall, the sequencing data was of little use in explaining C processes in these soils, underlying the difficulty in predicting the contribution of large tree-plantations to the global C cycle.

1. Introduction

Soil is the largest terrestrial carbon (C) pool and a major compartment for durable C sequestration in the biosphere. In the context of climate change, it is critical to understand and evaluate the C fluxes and storage dynamics in terrestrial ecosystems (Jansson and Hofmockel, 2020). Land cover and land use influence soil microbial communities that carry out multiple biogeochemical processes. In turn, microbial community composition and processes influence the amount of C ultimately stored in soil and thus the ability of an ecosystem to act as net C sink (Basu and Behara, 1993; Subke et al., 2006; Nogueira et al., 2011). For instance, conversion of primary or secondary forests to plantations or to agricultural systems consistently shifts soil microbial community composition, resulting in significant implications for soil microbial processes including C cycling (Zhou et al., 2018). However, the mechanisms underlying these shifts in C storage in the soil remain poorly understood (Li et al., 2011; Basile-Doelsch et al., 2020). Understanding the relationships between soil characteristics, composition of the soil microbial community and associated functions, as well as their spatial heterogeneity, is then critical for accurately assessing ecosystem processes and predicting their response to changing environmental conditions or anthropogenic disturbances (Wieder et al., 2014; Cavicchioli et al., 2019).

The current knowledge gap on the drivers of soil C dynamics partly

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lies in the fact that soil biochemical processes are highly heterogeneous in space and time (Groffman et al., 2009; Fromin et al., 2013) and at various scales (Ettema and Wardle, 2002; Hugon et al., 2021), even in homogeneously managed environments (*e.g.* Baldrian et al., 2010). Elucidating the role of different drivers of the variability of soil microbial processes is essential to understand the mechanisms by which soil communities influence carbon and nutrient cycles in ecosystems, in particular in response to local environmental parameters within one land use type (Li et al., 2019).

The links between microbial biodiversity and soil microbial processes are very poorly understood, because soil microbial diversity is so great, but also because the microbial taxa effectively involved in the measured processes are unknown (Nannipieri et al., 2003). In this context, the question of whether a change in soil microbial community structure has functional consequences is critical (Schimel & Schaeffer, 2012), and we crucially lack knowledge on how taxonomic diversity of microbial communities relates to its activities and functional diversity (Wang et al., 2019). There probably exists some degree of functional redundancy among some microbial groups, but the taxa responsible for different processes under given environmental condition are mostly unique, and any shift in the diversity or composition of the soil microbial community is usually accompanied by changes in the processes they carry out and the generated fluxes (Delgado-Baquerizo et al., 2016; Wagg et al., 2019). For instance, studies showed that shift in the structure of the soil microbial community led to altered soil processes such as litter decomposition (Creamer et al., 2015) or degradation of different C substrates (Langenheder et al., 2006; Chaer et al., 2009). The taxonomic characterization of the soil microbial community would then be useful for predicting processes such as soil respiration.

Eucalyptus is the most planted tree genus in the tropics. It is used to supply the pulp and metallurgical industries with wood, cellulose and charcoal, and is of high economic importance in many regions. In Brazil, the area cultivated with Eucalyptus covers about 7.4 million hectares (IBGE, 2020). The effect of eucalyptus plantations on soil C dynamics and sequestration remains weakly understood (but see Epron et al., 2015) and needs further attention. Although Eucalyptus plantations are clonal, (*i.e.*, composed of individuals with the same genotype) and even-aged, their soil C balance and its microbial determinants may exhibit substantial variability, especially if the understory is allowed to develop. But empirical evidence is scarce. A better understanding of the determinants of soil respiration variability is important to estimate the contribution of these plantations to the global C balance.

Here, we aimed to investigate the spatial variations in molecular and catabolic diversities of soil microbial communities within a eucalyptus plantation that has been established for over 20 years. We characterized the spatial heterogeneity of soil physico-chemical parameters, and of soil microbial taxonomic and functional parameters. For the latter, we explored the use of C-substrates for microbial respiration, parameters that are more specific than processes such as decomposition or soil respiration, and which should respond better to changes in microbial diversity. We hypothesized that, even in a supposedly homogeneous plantation, spatial heterogeneity in soil properties and understory vegetation biomass generates heterogeneity in soil microbial community composition, and in soil microbial processes. Because contrasting microbial communities are supposed to carry out different processes, we hypothesized that communities that were taxonomically more similar were also functionally more similar. Clarifying these questions in vast tree-planted areas, such as in eucalyptus plantations, is crucial to understand their importance in the global C balance, considering the surface they occupy.

2. Material and methods

The study was conducted in the south of Brazil, at the experimental station of forest sciences of Itatinga (ESALQ, University of São Paulo, Appendix A1), which has a tropical humid climate (Alvares et al., 2013). The soils are deep Ferralsols (F.A.O., 1998), and their mineralogy and physico-chemical characteristics are detailed in Maquere et al. (2008). The site was planted in 1998 with *E. grandis* at a tree density of 1666 individual trees ha^{-1}. Last fertilization at the site occurred in 2008, with no management for the last 10 years, meaning that the understory vegetation was allowed to develop. A 198 × 276 m stand of rather homogeneous topography was defined and divided in 108 experimental plots of 20 m × 23 m each (Appendix A2), which correspond to the different plots of a biodiversity experiment set up in this area in October 2019 (MataDIV, https://treedivnet.ugent.be/ExpMataDIV.html, data not shown). Plots were distributed in three blocks separated by a 9 m wide interrow (the blocks were not used in the present study). The sampling was done in March 2018, during the dry season. The 10 cm-depth surface soil was collected using an auger in a total of 54 plots spread throughout the stand (data could be processed for 51 plots only) (Appendix A2). Each sample was a mixture of 9 soil cores taken in the plot, with three cores yielded in three inter-rows in the center of the plot. All samples were sieved at 2 mm and immediately frozen or air-dried for storage before molecular and other analyses, respectively. To approximate the heterogeneity of the vegetation understory biomass, a greenness index (GI) based on the proportion of green pixels in photographs taken in the plots (Appendix B) (Li et al., 2024).

Total C and nitrogen (N) were analyzed using an elemental analyzer (CHNS Flash2000 Thermo Scientific, USA). Dissolved organic carbon (DOC) and dissolved nitrogen (DN) were analyzed with a TOC analyzer equipped with a supplementary module for N (CSH E200V, Shimadzu, Kyoto, Japan). Soil labile P was estimated according to Olsen et al. (1954) (see Waithaisong et al., 2020 for complete procedure). Soil samples were analyzed for additional physical-chemical properties (texture, pH in H_2O, aluminum by extraction in potassium chloride 1 mol/L, organic matter by the dichromate- titrimetric method, and cation exchange capacity, CEC) at the Laboratory of Chemical Analysis in the Soil Science Department at the Luiz de Queiroz College of Agriculture (ESALQ / USP) (van Raij et al., 2001).

The MicroResp™ system (Campbell et al., 2003) was used for the functional characterization of the soil microbial community (Barratella and Pinzari, 2019) as previously described (Gillespie et al., 2023). MicroResp™ substrate-induced respiration rates across a range of organic substrates provides a measure of the catabolic diversity of the microbial community that has been shown to be linked to pools of organic C in soils (Degens et al., 2000). Briefly, soil respiration rates were measured with fifteen different C-substrates (substrate-induces respiration, SIR) or water (as a proxy of basal respiration). The substrates were: glucose, cellulose, xylan, L-serine, L-glycine, L-asparagine, L-lysine, L-glutamine, *N*-acetylglucosamine, uric acid, oxalic acid, malic acid, caffeic acid, vanillic acid, syringic acid. Various functional microbial parameters were computed for each soil sample: i) the global catabolic activity across the 15 substrates (sum15) as the sum of the 15 SIR rates; ii) the contribution of each C-substrate to the global metabolic activity as $\%SIR_i$ (for substrate i) = SIR_i / sum15 (expressed as % of sum15), iii) the enhancement (or not) of respiration by each substrates (by comparing the SIR rate to the respiration rate with water only, *t*-test $p < 0.05$), and iv) the number of substrates used by the soil microbial community.

In order to characterize bacterial and fungal communities, total soil genomic DNA was extracted from 500 mg of soil with the FastDNA SPIN™ kit for soil (MP Biomedicals Santa Ana, CA, USA) as described by Tournier et al. (2015). Extracted DNA was quantified using PicoGreen fluorescence (Molecular Probes, Paris, France) and kept at −20 °C until amplification. The abundance of bacteria and fungi was determined by quantitative PCR (qPCR). Details on the primers used as well as qPCR conditions are given in Appendix C. Initial DNA quantity (2.5 ng) was amplified in a total reaction volume of 10 µL containing 1× of Sso advanced SYBR Green supermix (BioRad, Hercules, CA, USA) and 0.5 µM of each primer. qPCR assays were run in duplicate using a Biorad CFX96 Real-time PCR System and the results were analyzed with the

Software Bio-Rad CFX Manager 2.0. Bacterial and fungal community diversity were assessed by sequencing the V3-V4 region of the 16S rDNA and the internal transcribed spacer ITS2 of the nuclear ribosomal RNA on a MiSeq Illumina sequencer. Amplicon libraries were constructed following a two PCR step protocol according to the Illumina 16S Metagenomic Sequencing Library Preparation Guide (Ref.15044223 Rev. B). Briefly first rounds of PCR were performed using the locus specific primers with a 5′ nucleotide overhangs. These extensions aimed at anchoring a second round of PCR that introduced indexes and completed Illumina adapters. Details on the primers used as well as PCR conditions are given in Appendix C. To improve recovery and limit PCR bias, three PCR replicates per sample were pooled. After a magnetic bead purification (Clean PCR, Proteigene, France) with a bead-to-sample ratio of 0.8 X, the second round of PCR was performed in a total volume of 18 µL (5 µL of first round PCR products, 9 µL Phusion® High-Fidelity PCR Master Mix, NEB, France, 2 µL I5 index-adapter, 2 µL I7 index-adapters. Primers and PCR conditions areg given in Appendix C. The indexed products were multiplexed and purified with magnetic beads (ratio: 0.7). The final libraries were then sequenced on a MiSeq sequencer using MiSeq Reagent Kit v2 (500-cycles; Illumina).

Illumina sequencing data were analyzed as follows. Briefly, paired Illumina MiSeq reads were assembled with vsearch v2.14.2 (Rognes et al., 2016), and cutadapt v4.4 (Martin, 2011). Only reads containing both primers were retained. Operational taxonomic unit (OTU) representative sequences were searched for chimeras with vsearch's command uchime_denovo (Edgar et al., 2011). In parallel, representative sequences were assigned using the stampa pipeline (https://github.com/frederic-mahe/stampa/) against the reference database Silva SSU NR99 v138 (Quast et al., 2013) or against the fungal reference database UNITE v9.0 (https://unite.ut.ee/) (Kõljalg et al., 2013). Clustering results, expected error values, taxonomic assignments and chimera detection results were used to build a raw OTU table. The raw sequence data are available under the BioProject PRJEB72151. Richness (number of OTU) was estimated using the R vegan package (Oksanen et al., 2020). See Supplementary HTML file bioinfo for more details.

All statistical analyses were performed using XL-STAT version 19.6 (Addinsoft) and R Studio version 1.1.463 (R Development Core Team, 2020). Normality of the distribution of soil and microbial variables was estimated by a Shapiro-Wilkinson' test, and a Box-Cox transformation was applied to the variables that did not meet this assumption.

Ten soil physico-chemical parameters (C, C:N ratio, P_{Olsen}, DOC, DN, pH, Al, OM, CEC, sand) were incorporated into a principal component analysis (PCA). The first two axis scores (soil-PC1 and soil-PC2) were extracted and used as integrated proxies of soil quality in our statistical analyses to take account of the spatial heterogeneity of the soil, as previously proposed in Gillespie et al. (2020). Dissimilarity matrices were computed on the soil functional parameters (%SIR for the 15C substrates used in MicroResp™ catabolic profiling) and on the soil bacterial and fungal OTU composition (based on ten independent rarefactions with the function rrarefy() of the vegan R package). Euclidian distance was used to compute the dissimilarity matrix for %SIR, while Bray Curtis dissimilarities were computed for bacterial and fungal composition. Dissimilarity matrices were visualized using non-metric multi-dimensional scaling using the R vegan package metaMDS() function. NMDS scores for the first two dimensions were further used in addition to individual parameters to explore the relationships between parameters.

Linear regression models were run to test for the relationship between soil, molecular parameters and catabolic parameters. The physical distances between two plots were computed as Euclidian distance using the coordinates taken in the middle of the plots (x_i, y_i) and (x_j, y_j): $d_{(i,j)} = \sqrt{(xi - xj)^2 + (yi - yj)^2}$. To determine if closer topsoil locations displayed more similar microbial communities, the dissimilarity between all pairs of soil locations in bacterial and fungal community composition on the one hand, and in microbial catabolic properties on the second hand (using Bray-Curtis dissimilarity) was plotted against the physical distance among sampling locations (Euclidian distance). We used Mantel test from the distance and dissimilarity matrices to assess the correlation between microbial parameters and physical distance between samples (10,000 permutations, Pearson correlation). Finally, we also used partial Mantel tests to disentangle the influence of soil physico-chemical parameters and of bacterial and fungal composition on the catabolic profiles (SIR%-NMDS scores) of the soil microbial community.

3. Results - discussion

Our study has evidenced that spatial variability of soil microbial community and functioning can be substantial, even in a supposedly homogeneous eucalyptus plantation and despite a 20-year history of eucalyptus plantation. This is in line with other studies showing a great deal of spatial variation in soil and microbial parameters in ecosystems originally considered as relatively homogeneous (*e.g.* Li et al., 2019 in afforested land). However, the magnitude of the variability strongly depends on the parameter considered (Appendix D). GI showing the importance of understory vegetation ranged from 17.1 to 59.4 %. All soil parameters varied across the plots, with substantial variations in P_{Olsen} ranging from 1.49 to 12.27 mg P kg^{-1} soil (CV = 44 %), DN from 9.3 to 40.7 mg N kg^{-1} (CV = 38 %), DOC from 22.5 to 124.8 mg C kg^{-1} (CV = 35 %), and OM content from 4.0 to 16.0 g kg^{-1} (CV = 33 %) (Appendix D). Other soil parameters such as C:N ratio or proportion of sand exhibited less spatial heterogeneity (CV = 9 % and 5 %, respectively). PCA biplot on soil parameters is shown on Appendix E1, with soil-PC1 axis explaining 45.59 % of variance, and soil-PC2 explaining 16.90 % of variance. High soil-PC1 scores were obtained for soils with high C %, and Al^{3+} contents, and high CEC (highest loadings for these 3 variables), and with low pH and fine texture (low sand content) compared to soils with low soil-PC1 scores. High Soil-PC2 scores were recorded for soils with high DN and P_{Olsen} concentrations.

Soil microbial parameters also varied substantially (Appendix D). Bacterial and fungal abundances both varied by a factor of 4 across the 51 sampling locations. Bacterial and fungal richness ranged from 1148 to 1638 OTUs and from 96 to 222 OTUs, respectively. The bacterial community mainly included Proteobacteria (29.6 to 42.4 % of reads), Acidobacteriota (21.0 to 32.6 %), Actinobacteriota (10.3 to 19.0 %), while other phyla representing <10 % of reads (Fig. 1a). The dominance of these three phyla is in line with that observed by Li et al. (2018) in Eucalyptus plantations along a chronosequence. Pereira et al. (2017), characterizing the bacterial community composition in monospecific stands of *Eucalyptus grandis* growing on a sandy ferrasol (Sao Paulo state, Brazil) showed the dominance of Firmicutes and Proteobacteria, while Acidobacteria and Actinobacteria were less frequent than in the present study. The fungal composition also varied across the experimental plot and was dominated by Basidiomycota (17.3 to 66.2 % of reads), Ascomycota (23.5 to 63.0 %). These two phyla were also identified as the most abundant groups in Eucalyptus plantations by Pereira et al. (2020) and Rachid et al. (2015). Additionally, the proportion of Mucoromycota varied widely across the plots (0.2 to 52.9 %) (Fig. 1b). The NMDS plots on soil bacterial and fungal composition are shown in Appendix E2.

Alike, catabolic parameters varied, with the sum15 varying by a factor 1.5 (CV = 11 %), and the number of substrates that enhanced the soil respiration ranging from 0 to 14 (Appendix F). Glucose, xylanase and asparagine were the most frequently used substrates (in 32, 28 and 28 samples, respectively) and syringic and vanillic acids the most rarely used ones (in 3 samples among 51 for both substrates) (Appendix F). NMDS plot displaying the dissimilarities between catabolic profiles is shown in Appendix E3. Previous studies have suggested that the variability of soil microbial parameters in forest soil occurs at various scales, depending on the parameters considered (Fromin et al., 2013; Kivlin and Hawkes, 2016). This question of spatial variability was rarely addressed, to our knowledge, in tropical tree plantations (but see Goodrick et al.,

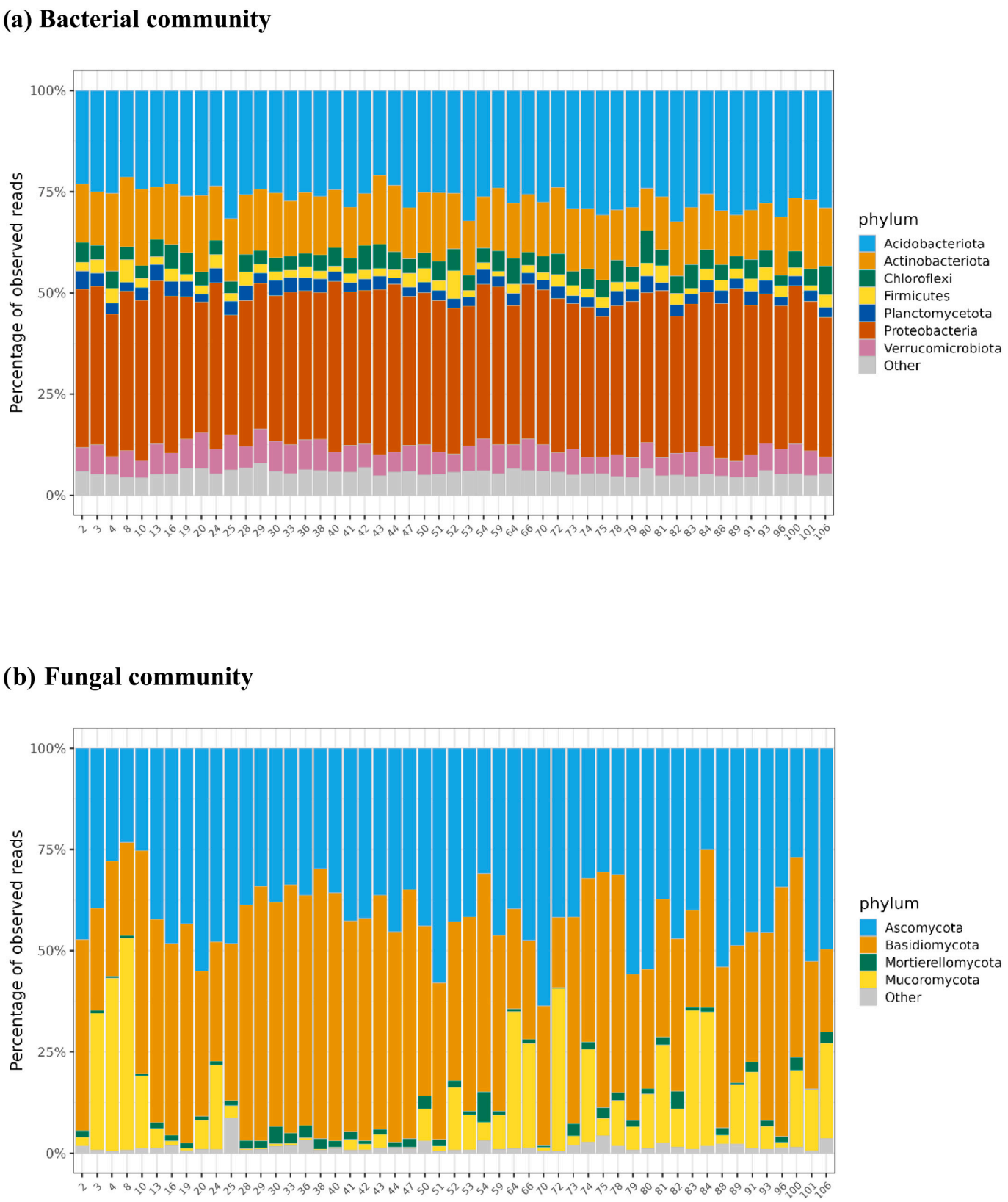

Fig. 1. Composition (by phyla) of bacterial (a) and fungal (b) community for the 51 plots.
(1a) Bacterial community
(1b) Fungal community.

2016 on oil palm plantation). It is also worth noting that our results are a snapshot, at a given moment, of the state of the microbial community, which varies over time. Qu et al. (2020) showed that soil bacterial diversity and functionality are influenced by eucalyptus plantation age, and with a more diverse community with increasing plantation age. However, other studies (*e.g.* Kivlin and Hawkes, 2020) suggest that the temporal variability of bacterial communities would be lower in tropical than in temperate soils.

This work also enabled the exploration of relationships between plot (soil physico-chemical and vegetation), and microbial molecular and functional parameters. It should be noted here that, because of the experimental design (existence of confounding factors), also given the limitations of correlation-based analyses, our results should be treated with caution. Although the density of the understory vegetation (estimated by the greenness index, GI) varied by more than a factor of three, it explained very little of the soil microbial parameters, except for a positive relationship with bacterial richness ($R^2 = 0.132$, $p = 0.009$), and very little of the catabolic parameters (Appendix G). This result suggests either that the greenness index is not a good proxy for the influence of the understory vegetation (this index does not take the composition of the understory vegetation into account), or that the density of understory vegetation is not so important for the soil microbial community as compared to the influence of Eucalyptus trees. Working in a 3-year-old eucalyptus plantation in Congo, Epron et al. (2015) found that the spatial heterogeneity of soil respiration was correlated with above-ground litter rather than with root biomass and soil C content, and clearly affected by management practices, especially by detritus management. The composition of the understory vegetation, with different species producing quantitatively and qualitatively different organic inputs to the soil (Wardle et al., 2004; Thoms et al., 2010; Urbanová et al., 2015) could help explaining the variability of the microbial parameters, as shown by Bréchet et al., 2009 for tropical tree species. However, Kivlin and Hawkes (2016) concluded, in their study examining tree species effects in experimental tree monocultures and secondary forest in Costa Rica, that the dependence of soil bacteria on plant identity is only partial. In the present study, the understory vegetation was rather variable in composition, but its contribution to litter input was rather low as compared to that of Eucalyptus trees (data not shown).

Linear regression models showed several significant relationships between soil and molecular or catabolic microbial parameters. Soil-PC1 scores were significantly related to all catabolic parameters (Appendix G), with negative relationship with sum15 ($R^2 = 0.086$, $p = 0.037$) and positive one with number of substrates used ($R^2 = 0.170$, $p = 0.003$). Soil-PC1 scores were also positively related to NMDS1-%SIR and negatively NMDS2-%SIR ($R^2 = 0.202$ and 0.136, $p < 0.001$ and $= 0.008$, respectively). Soils with higher soil-PC1 scores were also associated with higher abundance, but lower richness of bacteria ($R^2 = 0.120$, $p < 0.05$, and 0.495, $p < 0.001$), higher richness of fungi ($R^2 = 0.092$, $p < 0.05$), as well as contrasted molecular bacterial ($R^2 = 0.461$, $p < 0.001$ for B-NMDS1) and fungal community structures ($R^2 = 0.190$ and 0.115, $p <$ 0,01 for F-NMDS1 and F-NMDS2, respectively) as compared to soils with low soil-PC1 scores (Appendix G). Into more details, soils with low soil-PC1 scores had a lower proportion of Acidobacteriota ($R^2 = 0.150$, $p <$ 0.01) and higher proportions of Actinobacteria $R^2 = 0{,}113$, $p < 0.05$) and of Verrucomicrobiota ($R^2 = 0{,}111$, $p < 0.05$). The importance of soil C content, and especially of easily available C forms (DOC), both integrated into soil-PC1 scores, to explain soil microbial parameters (Appendix G) is in line with other studies showing the relevance of C availability for soil bacterial and fungal community (Prescott and Grayston, 2013) or its functional properties, especially in tropical soils (*e.g.* Fanin et al., 2014). For instance, Murugan et al. (2014) showed that removal of understory plants or tree girdling, by reducing labile C input to the soil, was a key determinant of the soil microbial community in eucalyptus plantations, promoting bacteria and saprophytic fungi.

Although it is challenging to classify all bacterial groups in copiotrophic (that use labile C sources and that are found in high quality C environments) or oligotrophic types (decomposing more recalcitrant C) (Ho et al., 2017; Westoby et al., 2021), some phyla can be typically considered as copio- or oligotrophs. For instance, Proteobacteria (Fierer et al., 2007), Actinobacteria (Bastida et al., 2016) and Bacteroidetes (Männistö et al., 2016) are more competitive under copiotrophic condition. Conversely, Acidobacteria (Fierer et al., 2007), Chloroflexi (Pepe-Ranney et al., 2016) or Verrucomicrobia (Ho et al., 2017) are rather classified as oligotrophs. Although we studied a presumed homogeneous environment, we found different proportions of these groups across the plots, sometimes explained by C availability parameters. Following previous studies mentioned above, we found higher proportions of Actinobacteria and lower proportions of Acidobacteria in soils with high Soil-PC1 (*i.e.* with high C, OM or DOC content). Soil PC1 is also largely explained by soil pH (Appendix E1), with soil pH positively related to B-NMDS1 as well as to bacterial richness ($R^2 = 0.261$ and 0.203, respectively, Appendix H). The importance of soil pH, nutrients and organic C for the soil microbial community has been widely reported (*e.g.* Delgado-Baquerizo et al., 2017; Gillespie et al., 2020), while soil pH was suggested to be less important to the fungal than to the bacterial community (*e.g.* Rousk et al., 2010). Fierer and Jackson (2006) reported that soil pH explained the variances of diversity and richness of soil bacterial communities by 70 and 58 %, respectively. Here we found that soil pH explained both bacterial and fungal community composition, and with significant relationships with some fungal groups (Appendix H). Soil-PC2 scores (explaining 16,90 % of variance of soil parameters only and related to P_{Olsen} and DN) failed to explain microbial parameters (except for a higher proportion of Firmicutes in soils with high soil-PC2 scores, Appendix G). Even though P availability is considered as critical in old, nutrient-impoverished tropical soils (Vitousek et al., 2010; Fanin et al., 2011), P_{Olsen} by itself only displayed a negative relationship with B-NMDS1 and was positively related with the relative abundance of Verrucomicrobiota (Appendix H).

Finally, both bacterial and fungal molecular parameters slightly but significantly explained the catabolic (MicroResp™) parameters of the soil microbial community (Table 1). Sum15 was mainly related to the bacterial parameters, with higher sum15 values associated to more abundant ($R^2 = 0.092$, $p < 0.05$) and less diverse ($R^2 = 0.110$, $p < 0.05$) bacterial communities that displayed lower B-NMDS1 scores ($R^2 =$ 0.087, $p < 0.05$), and, to a lower extent, to higher F-NMDS2 scores ($R^2 =$ 0.059, $p < 0.10$) (Table 1). Soil catabolic profiles (NMDS1-%SIR) were significantly related to bacterial abundance ($R^2 = 0.102$, $p < 0.05$), richness ($R^2 = 0.170$, $p < 0.01$), composition (B-NMDS2, $R^2 = 0.111$, $p <$ 0,05), and proportion of Actinobacteriota ($R^2 = 0.222$, $p < 0.001$). The relevance of fungal parameters in explaining catabolic parameters was less than that of bacterial parameters, in line with Liu et al. (2015), and even though fungi have been suggested as key drivers in decomposition process in forests (Baldrian et al., 2012). In our study, NMDS1-SIR% was also related to fungal abundance ($R^2 = 0.125$, $p < 0.05$) and composition (F-NMDS2, $R^2 = 0.116$, $p < 0.05$). Additionally, the relative proportions of Ascomycota, and, to a lower extent, of Basidiomycota were negatively and positively (respectively) related to NMDS1-SIR% scores (($R^2 = 0.102$, $p < 0.05$ and $R^2 = 0.071$, $p < 0.10$, respectively) (Table 1). Into more details, these two groups were also related to different preferential substrate use (Table 2), confirming that Ascomycota and Basidiomycota occupy differential 'nutritional' niches, as previously shown by Bastida et al. (2016) using a metaproteomics approach in Mediterranean soils. The relative abundance of some phyla also directly explained the contribution of some substrates to the total catabolic activity (Table 2), with SIR% for ten of the 15 substrates being partly but significantly related to the relative abundance of at least one bacterial or fungal phyla. For instance, the relative abundance of Acidobacteriota explained about 10.0 % of variation in %LYS (with negative relationship), that of Actinobacteria 15.2, 17.0, 10.7 and 8.2 % of variation in %ASP, %LYS, %CAF and %VAN, respectively, with positive relations for %ASP and %LYS and negative ones for %CAF and %VAN

Table 1
Linear regression (R^2 and associated *p*-values) to explain microbial catabolic parameters with molecular (bacterial and fungal) parameters. "sum15" represents the global catabolic activity (sum of the 15 substrate-induced respiration rates); "NMDS1-%SIR" and "NMDS2-%SIR" are the scores for the first two axes of NMDS ordination for %SIR, *i.e.* the contribution of each MicroResp to the global catabolic activity sum15; and "nb substrates" the number of substrates used by the soil microbial community, *i.e.* the substrates that significantly enhanced microbial soil respiration as compared to respiration with water addition only (see Material and methods". Negative relationships are indicated using minus sign. Significant regression values are shown in bold characters ("†" for *p-value* < 0.10 and > 0.05; "*" *p-value* < 0.05 and > 0.01; "**" *p-value* < 0.01 and > 0.001; "***" *p-value* < 0.001); models with non-significant *p-value* ($p \geq$ 0,05) are noted "ns".

Molecular parameters	sum15	NMDS1-% SIR	NMDS2-% SIR	nb substrates
B-qPCR16S	**0.092***	**0.102***	ns	ns
B-NMDS1	**- 0,087***	ns	ns	**- 0.109***
B-NMDS2	ns	**- 0.111***	ns	ns
B-richness	**- 0.110***	**- 0.170*****	ns	**- 0.097***
Acidobacteria	**- 0.082***	**- 0.083***	ns	ns
Actinobacteria	ns	**0.222*****	ns	ns
Chloroflexi	ns	ns	0.061†	ns
Firmicutes	ns	ns	ns	ns
Planctomycetota	ns	ns	ns	ns
Proteobacteria	ns	ns	ns	ns
Verrucomicrobiota	ns	ns	ns	**0.111***
F-qPCR18S	ns	**0.125****	ns	ns
F-NMDS1	ns	ns	ns	ns
F-NMDS2	**0.059†**	**0.116***	ns	**0.081***
F-richness	ns	ns	ns	ns
Ascomycota	ns	**- 0.102***	ns	ns
Basidiomycota	ns	**0,071†**	ns	ns
Mortiellomycota	ns	ns	ns	ns
Mucoromycota	ns	ns	ns	ns

(Table 2). Overall, our data confirm that the bacterial community is more important to explain the soil microbial functioning than the fungal community, as previously found in a rubber plantation (Wang et al., 2020). Our results also support the idea that soil microbial communities across the Eucalyptus plantation are functionally dissimilar with respect to the use of specific C compounds, in line with the literature showing functional redundancy of dissimilar communities for common processes performed by a wide range of microorganisms, but functional dissimilarity for more specific processes driven by a narrower range of organisms (Strickland et al., 2009; Fanin et al., 2015).

Finally, we examined if spatially closer samples were more similar, *i.e.* if there were relationships between the physical distance between soil sample locations and their dissimilarity in microbial composition or microbial catabolic parameters. We found that the Bray Curtis dissimilarity in bacterial and, to a lesser extent, in fungal communities increased with the physical distance between soil samples ($R^2 = 0.057$, $p < 0.0001$, and $R^2 = 0.025$, $p < 0.0001$, for fungal and bacterial composition, respectively). The results of the Mantel tests (Table 3) showed that dissimilarity in the bacterial composition was the variable that best explained the catabolic dissimilarity (%SIR) ($\rho = 0.130$, $p < 0.001$), followed by the dissimilarity in soil parameters ($\rho = 0.084$, $p < 0.01$), and that of the fungal community ($\rho = 0.082$, $p < 0.01$), while the physical distance between soil sample was not significantly correlated with catabolic dissimilarity (Table 3). And even, considering the physical distance between samples or the dissimilarity in soil parameters did not improve the relationships between microbial composition and catabolic dissimilarities (Table 3). Here again, in line with our hypothesis, molecular bacterial parameters better explained the catabolic profile of the soil microbial community. But overall, the composition of both bacterial and fungal communities poorly explain the functional (catabolic) capabilities of the soil microbial community, in line with other studies. For instance, Kivlin and Hawkes (2020) showed that shifts in the composition of the soil microbial community was not linked to

Table 2
Linear regression to explain the use of MicroResp substrates by the relative abundance of bacterial and fungal phyla. %GLU, %XYL, *etc.* are. the %SIR (*i.e.* the contribution of the C-substrates to the global catabolic activity sum15) that displayed significant relationship with at least one bacterial or fungal phyla: GLU for glucose, XYL for xylan, ASP for asparagine, SER for L-serine, LYS for lysine, and GLUT for L-glutamine, -NAC for acetylglucosamine, UR for uric acid, CAF for caffeic acid, SYR for syringic acid, and VAN for vanillic acid. Data for other (non-significant) C-substrates are not shown. Negative relationships are indicated using minus sign. Significant regression values ($p < 0,05$) are in bold characters; models with non-significant *p-value* ($p \geq 0,05$) are shown in white, and † is for *p-value* ≤ 0,010.

	Bacterial phyla							Fungal phyla			
Microresp substrates	Acidobacteria	Actinobacteria	Chloroflexi	Firmicutes	Planctomycetota	Proteobacteria	Verrucomicrobiota	Ascomycota	Basidiomycota	Mortiellomycota	Mucoromycota
%GLU	ns	ns	ns	ns	ns	**−0.118**	**0.083**	ns	ns	ns	ns
%XYL	−0.065†	ns	**0.140**	ns	ns	ns	ns	ns	ns	ns	ns
%ASP	ns	**0.152**	ns	ns	ns	ns	−0.062†	**−0.089**	ns	ns	ns
%SER	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
%LYS	**−0.100**	**0.170**	ns	ns	ns	ns	ns	**−0.089**	ns	ns	ns
%GLUT	ns	ns	ns	**0.138**	ns	ns	ns	ns	ns	ns	ns
%NAC	**0.083**	−0.058†	ns	ns	ns	ns	ns	ns	ns	ns	ns
%UR	ns	−0.073†	ns	ns	**0.131**	ns	ns	ns	ns	ns	ns
%CAF	ns	**−0.107**	ns	ns	ns	ns	ns	ns	ns	ns	ns
%SYR	**0,071**	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
%VAN	ns	**−0.082**	ns	ns	ns	0.056†	ns	ns	ns	ns	ns

Table 3
Summary of Mantel tests and partial Mantel tests comparing physical distances (D), dissimilarity in soil physico-chemical parameters (soil) and/or dissimilarity in bacterial or fungal community composition (Bcomp and Fcomp, respectively) to explain the catabolic profiles of the soil microbial community (SIR%-NMDS scores). We also tested Bcomp or Fcomp accounting for D or soil-PC.
(ρ and associated *p* values and p- Correlations were calculated with Mantel tests ($n = 10{,}000$ permutations). For geographical distance and %SIR data, dissimilarities were calculated using the Euclidian distance, while Bray-Curtis was used for bacterial and fungal composition. Significant r (rho, in greek character) values ($p < 0.05$) are given in bold.

	ρ	p-value
D	0.052	0.069
soil	**0.084**	**0.002**
Bcomp	**0.130**	**<0.0001**
Bcomp/soil	**0.106**	**<0.001**
Bcomp/D	**0.122**	**<0.001**
Fcomp	**0.082**	**0.003**
Fcomp/soil	**0.063**	**0.029**
Fcomp/D	**0.076**	**0.005**

Significant values ($p < 0.05$.

changes in functional, *e.g.* enzyme, activities. On their side, Lupatini et al. (2013) showed a large discrepancy between taxonomic and functional diversity of the soil microbial community. This might be because a large part of the microbial sequences recovered following DNA extraction correspond to inactive bacterial cells in soil (Lennon and Jones, 2011).

As a conclusion, we evidenced a large spatial variability of the soil physico-chemical and microbial parameters in the subsoil of the studied 20-years old eucalyptus plot. Our results show that important soil (*e.g.* total C or DOC) and microbial taxonomic and functional (catabolic) parameters, that are important for understanding and predicting C fluxes in soils, can vary substantially across the Eucalyptus plantation. This implies that samples need to be multiplied to correctly characterize these parameters. We also found that the microbial respiration parameters (characterized by the use of various C substrates by the soil microbial community) could be explained by both the soil parameters and bacterial communities (and, to a lower extent fungal communities). However, these parameters did little to explain the variability in the catabolic functions of the community, suggesting that determining the composition of the soil microbial community is of limited use to predict C processes in the soil. Finally, the results of our study do not allow us to formulate recommendations for the management of eucalyptus plantations, for example to optimize C sequestration. To do this, studies integrating other components of the carbon cycle, and their spatial and temporal variability, will have to be consired, and the contribution of the understory vegetation will have to be specified, with possible implications for the management of Eucalyptus plantation and their contribution to the global C cycle.

Credit authorship contribution statement

Margot Brondani: Writing – review & editing, Writing – original draft, Investigation, Formal analysis. **Agnès Robin:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Julie Marchal:** Formal analysis. **Anne-Laure Pablo:** Formal analysis. **Aline Personne:** Formal analysis. **Erick Desmarais:** Investigation, Formal analysis, Data curation. **Frédérique Cerqueira:** Formal analysis. **Frédéric Mahé:** Writing – review & editing, Visualization, Data curation. **Florine Degrune:** Data curation. **Joannès Guillemot:** Writing – review & editing, Supervision, Project administration, Investigation, Funding acquisition, Conceptualization. **Pedro H.S. Brancalion:** Writing – review & editing, Supervision, Project administration, Investigation, Conceptualization. **Nathalie Fromin:** Writing – original draft, Visualization, Supervision, Methodology, Funding acquisition, Data curation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.apsoil.2025.106008.

Data availability

Data available via the CIRAD Dataverse Repository https://doi.org/10.18167/DVN1/QN22CM (Brondani et al., 2025)

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