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Abstract

Trees residues decomposition is a key step in forested ecosystems. In the context of sclerophyllous Mediterranean forests, the effects of residue quality on soil organic matter and microbial communities is still poorly documented. Using a mesocosm experiment, we explored microbial responses (enzyme activities, basal respiration, molecular fingerprints and catabolic profiles) and variations in soil chemical properties (pH, $CaCO_3$, organic C and N contents, quality of soil organic matter *via* ^{13}C CP/MAS NMR) induced by coarse woody debris (W) or fine residues (i.e. twigs -leaves/needles, hereafter TL) from mono-specific or mixed stands of *Pinus halepensis* and *Quercus ilex*, *P. sylvestris* and *Q. pubescens*. After a nineteen-month incubation period, we found that the origin of residues (*Pinus spp*. stand, *Quercus spp.* stand or mixed stand) shaped the genetic structure of both fungal and bacterial communities. TL inputs led to higher soil N content and increased enzyme activities and bacterial biomass. Aromatic compounds and C to N ratio increased with W inputs, strongly shaped fungal communities and increased tyrosinase activity. Thus, the type of residue and the tree species identity (*Quercus spp vs Pinus spp*) appeared to modulate the balance between fungal and bacterial biomass and soil microbial diversity. Our results revealed that logging residues management, by modulating the type of residues remaining on forest floor, could have significant impacts on soil functioning by modifying both chemical properties (e.g. soil C/N ratio) and microbial structure and functions. These findings need to be supported by *in natura* studies since deciphering such mechanisms controlling C fluxes in forest soils has important consequences under the context of climate change.

Keywords: Logging residues; Mediterranean forests; microbial communities; mesocosm; soil organic matter.

1. Introduction

Forest soils are important carbon (C) sinks: more than 70% of forest organic C is stored in soils and forested ecosystems store more than 80% of the terrestrial C (Lal, 2005). Forest adaptation and mitigation have been addressed in the scientific agenda as two important measures to cope with climate change effects. Forest managers are now facing the need to assess the role of silvicultural practices in this context. Forest type and associated management regimes are known to have an effect on the quantity of C that can be sequestrated (Noormets et al., 2015). Mediterranean forests, which contribute strongly to global C sink (Allard et al., 2008), own distinct characteristics resulting from climate, fire frequency and forest management practices that largely affect the quality and quantity of plant C inputs (Muñoz-Rojas et al., 2015) with potential consequences on soil C storage.

Soil C stocks in forests largely result from a dynamic balance between plant inputs, transformation and stabilization of organic matter by soil microorganisms and loss of carbon by respiration (e.g. Batjes, 1996; Jandl et al., 2007; Jobbágy and Jackson, 2000; Six et al., 2002). Soil microorganisms thus play a fundamental role in forest soil C dynamics. Studies regarding organic matter (OM) decomposition have highlighted that fungi drive chemical changes occurring at the early stage of decomposition due to their ability to colonise plant residues *via* hyphae and to extensively produce extracellular ligninolytic enzymes. Bacterial communities appear to be important later in the oxidation of smaller molecules (Bani et al., 2018; Tláskal et al., 2016). Light is partly shed on mechanisms governing transformation, stabilization and mineralization of OM by microorganisms and the major role of OM quality on resulting C dynamics has been pointed out.

In Mediterranean ecosystems, the chemical composition of plant material is driven by strategies of resistance to climate stress (heat waves and drought events) including thick foliar cuticle, increased concentrations in tannins and emission of VOCs (Genard-Zielinski et al.,

2018; Vicente et al., 2018). Differences in organic matter content have been observed between Mediterranean tree species (resinous, evergreen and deciduous species), particularly in terms of nitrogen content, recalcitrant and allelopathic compounds. Salamanca et al. (1998) found that nutrient mobility differed between litters from either *Quercus spp*. and *Pinus spp*. This is supported by the study of Schwendener et al. (2005), who indicated that N release and transfer to soil strongly depend on the quality of leaves and that lignin proportion is a good predictor of decomposition in litters. Understanding the mechanisms of OM decomposition in Mediterranean forests is also complicated by the potential non-additivity effects of mixing litter from various tree species (Brunel et al., 2017). For instance, Versini et al. (2016) demonstrated that litter mixtures including several species are decomposed at different rates from what is expected from the dynamics of each species separately.

Forest management strategies further impact the type and quality of plant residues remaining on the floor after forest operations. In particular, leaf or wood residues can impact soil C dynamics since coarse wood (hereafter W) or fine residues (i.e. twigs and leaves/needles, hereafter TL) differ both physically and chemically. Higher proportions of minerals, which are known to increase OM turn-over (Freschet et al., 2013), are found in leaves, needles and twigs than in coarse wood (Ma et al., 2014). Conversely, tree organs with high contents in recalcitrant C (e.g. lignin) such as trunk and branches, are known to decay slowly (Berg and McClaugherty, 2014; Cornwell et al., 2008). However, dead coarse wood play vital roles in forest ecosystems by regulating soil temperature and water content, leading to more favorable habitats for microorganisms (Kwak et al., 2015).

While the interactions among microbial communities and chemical composition of fine and coarse residues have largely been studied in boreal and temperate forests (Bani et al., 2018), they remain elusive in the Mediterranean context, limiting our understanding of consequences of logging residues management on soil C dynamics in such ecosystem. Here,

89 we set up a laboratory experiment to determine how the quality of different inputs (i.e. W or
90 TL) from four Mediterranean tree species affects OM decomposition in forest soils. We
91 assumed i) that inputs of either coarse or fine residues would distinctively shape microbial
92 community structures and functioning and result in different C dynamics, ii) that the relative
93 effect of both type of residues will vary depending on fungal and bacterial communities and
94 iii) that these modifications would also rely on the chemical prints of tree-species
95 (broadleaves, evergreens or conifers) in admixture or not, which would indicate that the
96 impact of litter quality on soil functioning vary depending on the forest stand of typical
97 Mediterranean species. Mesocosms were set up with soils from mono-specific and mixed
98 stands of *Pinus halepensis* and *Quercus ilex* on one hand and of *P. sylvestris* and *Q.*
99 *pubescens* on the other hand and incubated for 19 months with different types of plant
100 residues to test their influence on soil chemical and microbial properties.

101 **2. Material and methods**

102 *2.1 Soil sampling*

103 Forest soil sampling was conducted in spring 2013 in South-Eastern France (Provence-
104 Alpes Côte d'Azur), an area characterized by frequent and intense droughts and heat waves
105 typical of the Mediterranean climate. Mean annual temperature and mean annual precipitation
106 characterizing the region are respectively 11.3°C and 799mm (worldclim data). We focused on
107 the most represented forest stands of the two bioclimatic areas (meso-Mediterranean and supra-
108 Mediterranean areas) found in this region: monospecific and mixed stands (60/40) of *Pinus*
109 *halepensis* / *Quercus ilex*, and *Pinus sylvestris* / *Quercus pubescens,* respectively (*Qi*, *Ph*, *Qi-Ph*,
110 *Qp*, *Ps*, *Qp-Ps*). We selected 3 plots (20m x 20m) for each of the six forest stands (18 plots)
111 with similar management (60 ± 10 years-old, no harvest/clear cutting since 35 years), and soil
112 carbonate pedofeatures. To avoid the "home-field" advantage (Ayres et al., 2009; Chomel et
113 al., 2015), soils used for mesocosms were collected from the 0-to-10-cm layer of the eighteen

114 natural forests from which the plant materials were collected. In each of the eighteen forest
115 plots, we sampled ten subsamples of A horizon soils (0-10 cm) which were pooled and sieved
116 (4 mm mesh) to set up mesocosms. The chemical characteristics of soils (calcaric Cambisol,
117 Blum et al., 2018) are given in Table 1. Two physico-chemical characteristics of soils varied
118 with forest stand: alkyl C contents of soils from *Pinus halepensis* stands were lower than
119 those from mixed *P. halepensis* and *Q. ilex* stands (21.0 ± 0.1 *vs.*26.5±0.7 respectively, F=4.6
120 p=0.01) and pH was more acid (pH =5.20 ± 0.79, F=8.47, p<0.005) in soil from *Q. pubescens*
121 stands than pH from other soils (pH around 7).

122 *2.2 Experimental set up*

123 Mesocosms were prepared by weighing 700 g (dry mass equivalent) of 4 mm-sieved
124 soil in pots of 15 cm mean diameter and 20 cm in depth. Plant materials were collected from
125 *Pinus halepensis* (*Ph*), *Quercus ilex* (*Qi*), *Pinus sylvestris* (*Ps*) and *Quercus pubescens* (*Qp*).
126 Coarse branches (4 to 10 cm diameter) were separated from twigs (<0.5 cm diameter)
127 supporting leaves/needles. After 3 weeks of drying at ambient temperature, each type of
128 residues was crushed and sieved at 2 cm to increase their decomposability and mimic residues
129 from forest harvesting operations. To test the influence of different residues (W and TL), each
130 soil was amended with the species-specific residues that corresponded to its stand of origin.
131 Mesocosms without residues were used as controls. Dried residue samples were analysed for
132 total carbon content to homogenise carbon input in each soil i.e. 35 g $C.kg^{-1}$ soil DW
133 (Hakkila, 1991; Thiffault et al., 2011; Wall, 2008). Since a higher decomposition was
134 expected when residues are incorporated to soil (Nicolardot et al., 2007), we mixed plant
135 material with the top part of soils (0-10cm). The 54 mesocosms were then incubated for 19
136 months from March 2013 to October 2014 at room temperature (oscillation from 27°C in
137 summer to 17°C in winter). Mesocosms were watered twice a week to maintain constant soil
138 humidity to 60% of the water holding capacities. The water holding capacity of soils was

139 determined by the amount of water held in the saturated soil sample vs. the dry weight of the
140 sample. At the end of the incubation, soils were sieved at 2 mm before being analyzed. For
141 each mesocosm, a sub-sample of each mesocosm was stored at 4°C before microbial activity
142 measurements, a second one at -20°C for molecular analyses and a third one was dried at
143 60°C before physico-chemical analyses.

144 *2.3 Soil chemical characterization*

145 The pH of soil samples was determined in distilled water and in a KCl solution (2 M)
146 after a 45 min equilibration. For each soil, total C and N contents were measured using a C/N
147 elementary analyzer (Flash EA 1112 Thermo Scientific series). Soil carbonate ($CaCO_3$) was
148 quantified by acid dissolution (HCl 4N) followed by the volumetric analysis of the released
149 carbon dioxide (Chaney et al., 1982). The percentage of carbon in $CaCO_3$ was calculated as
150 follows: % C-$CaCO_3$ = 11.991 / 100 x % $CaCO_3$. Then organic carbon was calculated as the
151 difference between total C content and carbonate C content. The OM was characterized with
152 solid-state ^{13}C NMR spectroscopy using cross-polarization and magic angle spinning (CP-
153 MAS ^{13}CNMR). This method enables the linkages between emitted spectra and the structure
154 of C compounds and their relative quantity. The solid state ^{13}C NMR spectra were obtained
155 on a Bruker Avance 400MHz NMR spectrometer operating at a ^{13}C resonance frequency of
156 106 MHz and a Bruker double-bearing probe. 80 mg of dry ground soil was placed in a
157 zirconium dioxide rotor of 4 mm outer diameter and spun at a magic angle spinning (MAS)
158 rate of 10 kHz. The cross-polarization (CP) technique (Schaefer and Stejskal, 1976) was
159 applied with a ramped 1H-pulse starting at 100% power and decreasing to 50% during the
160 contact time (i.e. 2 ms) to avoid Hartmanne Hahn mismatches (Cook et al., 1996; Peersen et
161 al., 1993). To improve the resolution, a dipolar decoupling GT8 pulse sequence (Gerbaud et
162 al., 2003) was applied during the acquisition time. To obtain a good signal-to-noise ratio in
163 the ^{13}C CPMAS experiment, 12 000 scans were accumulated using a delay of 2.5 s. The ^{13}C

chemical shifts were referenced to tetramethylsilane (at 0 ppm) and calibrated with a glycine carboxyl signal set at 176.5 ppm. Each spectrum was integrated using Dmfit software (Massiot et al., 2002) to determine the relative intensity of each chemical region. The relative distribution of carbon groups in different structures was determined by integrating the signal intensities over defined chemical shift windows. The ^{13}C CP/MAS NMR spectra were divided according to Mathers and Xu (2003) into 4 regions: alkyl-C (0-45 ppm),O-alkyl-C (45-112 ppm), aromatic-C (112-160 ppm) and carboxyl-C (160-185 ppm). To describe organic matter quality, the following ratios of humification (HR1 and 2) and aromaticity ratio (AR) were calculated according to Baldock and Preston (1995): AR= aromatic C/ (alkyl C+ O-alkyl C+ aromatic C), HR_1=alkyl C/ carboxyl C and HR_2= alkyl C/ O-alkyl C.

2.4 Soil microbial activities

Catabolic-level physiological profiles (CLPP) were analysed using BIOLOG® Ecoplates (BIOLOG Inc., Hayward, CA) using a procedure adapted from Garland and Mills (1991). Briefly, the bacterial suspension was prepared by homogenizing 5 g (dry weight equivalent) of soil with 100 ml of sterile 0.1% sodium pyrophosphate (pH 7.0). This suspension was agitated 2 hours on a rotary shaker (80rpm) and centrifuged 6 min at 500*g*. A 1/50 dilution (in 0.85% NaCl) was used to inoculate the plates (150 µl per well). Plates were incubated for up to 5 days at 25°C and the absorbance was measured twice a day (Infinite Tecan) at 590 nm. The average well colour development (AWCD) of each internal replicate block of 31 wells in each plate was calculated at each reading to determine the incubation time corresponding to AWCD = 0.3. The absorbance value at t0.3 in each well was then normalized. To assess microbial catabolic diversity and evenness, a Shannon (H') and Simpson (D) diversity indexes were calculated as follow: $H' = -\sum pi\ (ln*pi)$ and $D = 1 - \sum pi^2$ where pi is the ratio of the OD on each substrate (ODi) to the sum of OD on all substrates $\sum OD$.

Microbial biomass (MB) was estimated using Substrate-Induced Respiration (SIR). 10 g (dry weight equivalent) of standardized samples at 60% of WHC were placed in 117 ml flushed-air glass jars and amended with a powder of talc and glucose (1000 µg C g^{-1} soil). After ninety minutes, 1 ml of air was sampled with a syringe and injected into a gas chromatograph (Chrompack CHROM 3 – CP 9001) to determine CO_2 production. The gas chromatograph was equipped with a thermal conductivity detector and a packed column (Porapack). The carrier gas helium flow was regulated at 60 ml h^{-1}. CO_2 concentration of flushed air was subtracted from the CO_2 concentration of each sample and resulting values were adjusted to 22°C according to Ideal Gas Laws using $Q_{10} = 2$. SIR rates were converted into MB using equations given by Beare et al., (1990). Basal respiration was estimated using the same method to calculate the metabolic quotient qCO_2 (the ratio of basal respiration to microbial biomass), which is a sensitive ecophysiological indicator of soil stress induced by environmental conditions (Anderson, 2003).

To determine the catabolic potential of microbial communities, four extracellular enzyme activities (EEA) involved in soil organic carbon and nitrogen cycles were assessed: tyrosinase, cellulase, urease and protease activities. Enzyme activities were expressed as µmoles of reaction products released per minute (U) per gram of dry soil (U g^{-1} DS). All the experiments were performed in triplicate for each soil sample. Tyrosinase activity was assessed according to the modified method of Saiya-Cork et al. (2002). 2 ml of 25 mM L-DOPA solution (L-3,4-dihydroxyphenylalanine) in potassium phosphate buffer (50 mM, pH 6.5) were added to 0.4 g of soil (fresh weight), mixed and incubated for 30 min, in darkness at 25°C. The mixture was centrifuged for 3 minutes at 12 000 g before absorption was measured at 590 nm. Cellulase activity was assayed using CarboxyMethylCellulose (CMC) 1% in 2 mL of sodium acetate buffer (50 mM, pH 6) added to 0.5 g of soil (fresh weight) incubated for 4 h at 50°C. Glucose was quantified according to the Somogyi-Nelson method and absorption

was read at 870 nm (Farnet et al., 2010). Protease activity was measured using 5 g of soil (fresh weight) in 5 mL of casein at 2% in TrisHCl buffer (50 mM, pH 8.1). The mixture was incubated for 3 hours at 50°C and then the reaction was stopped with 5 mL of Trichloroacetic acid solution (at 15%) and the mixture centrifuged (2min, 12 000g). Aromatic amino acids were detected using Folin reagent (33%) at 700 nm (Ladd and Butler, 1972). Tyrosine was used as standard. Urease activity was assessed using 0.5 g of soil (fresh weight) in 2mL of urea solution (80 mM) in a sodium phosphate buffer (50 mM, pH 6). The mixture was incubated for 2h at 37°C and then centrifuged (2 min, 12 000 g). Ammonium was revealed in microplates using an adapted Mulvaney method (1996): 15µL of EDTA solution, 60 µL of Na-salicylate solution and 30 µL of hypochlorite solution were added to 30 µL of the supernatant. After 45 min, mixture absorption was measured at 667 nm.

2.5 Fungal and bacterial abundances

To further describe fungal and bacterial communities, DNA was extracted from 0.25 g of frozen sample, using the PowerSoil DNA Isolation Kit (MoBio, USA) according to the manufacturer's instructions. The quality of DNA extractions was checked by electrophoresis on 1% agarose gels stained with GelRed (Molecular Probes, USA) using a Gel Doc image analyser (BioRad,USA). The DNA concentration was quantified using Quant-iT^{TM} dsDNA High-Sensitivity Assay Kit (Invitrogen, Canada). All DNA extracts were then diluted to 0.5 ng mL for subsequent analysis. Copy numbers of bacterial and fungal small subunit ribosomal genes were assessed by quantitative PCR (qPCR) with a StepOne^{TM} Real-time PCR (Applied BioSystem, USA) targeting the 16S rRNA gene for bacteria and the internal transcribed spacer (ITS) gene for fungi. The primers used in the amplifications were 341F 5'-CCTACGGGAGGCAGCAG-3' and 534R 5'-ATTACCGCGGCTGCTGGCA-3' in the bacterial assay, and ITS3 5'-GCATCGATGAAGAACGCAGC-3' and ITS4 5'-TCCTCCGCTTATTGATATGC-3' in the fungal assay. The qPCR have been performed as

239 described in (Changey et al., 2018). The results were analyzed using StepOne V 2.2.2
240 software.
241 *2.6 Bacterial and fungal community structures*
242 The effect of the different residues on the structure of bacterial and fungal
243 communities were analysed using a terminal restriction fragment length polymorphism (T-
244 RFLP) and automated ribosomal intergenic spacer analysis (ARISA), respectively, as
245 described in Moni et al. (2015). Bacterial 16S rRNA gene was amplified using primers 63F
246 (5'-CAGGCCTAACACATGCAAGTC-3') fluorescently labelled at the 5' end with FAM dye
247 and 1389R (5'-ACGGGCGGTGTGTACAAG-3') (Marchesi et al., 1998). Fungal internal
248 transcribed spacers (ITS) were amplified using primers ITS1F (5'-
249 CTTGGTCATTTAGAGGAAGTAA-3') (Gardes and Bruns, 1993) fluorescently labelled at
250 the 5' end with Yakima Yellow® dye and ITS4 (5'-TCCTCCGCTTATTGATATGC-3').
251 PCR were performed using Biorad T100 thermal cycler, with 2 µL of diluted DNA template
252 (0,5 ng.µL^{-1}) in a total volume of 15 µL (Master Mix Kit, Qiagen) and 0.05 mM of each
253 primer. Amplification, quality check and sequencing processes were performed according to
254 Moni et al., 2015. Fragments and amplicons were binned with 0.5bp interval and only peaks
255 height □50 fluorescence units were considered in the analysis. Fragments between 50 and 500
256 bp and amplicons between 500 and 800 bp were included in T-RFLP and for ARISA analysis,
257 respectively. As measures of alpha diversity, we calculated the OTU richness and the
258 Shannon index, H'(Shannon, 1948).

259 *2.7 Statistical analyses*
260 Statistical analyses were performed with R version 3.0.0 (R Development Core Team,
261 2007). The statistical significance of the forest stand influences on the initial soil properties
262 was determined by one-way analysis of variance (ANOVA) and the Tukey HSD test was used
263 to distinguish significant differences between treatments. When the dataset remains non-

parametric after transformations, the GLM test with the appropriate model and link function was used to compare data between treatments, i.e. stands of origin and residues' types (Crawley, 2012). Residue quality was described by principal component analysis (PCA). PCA was performed on a covariance matrix to observe the variations induced by the organ (wood or twigs/leaves) and stand origin of each residue, and the proportion of variance explained by each principal component was calculated (Factominer package, Lê et al., 2008). For microbial genetic fingerprinting, between class analysis (ade4 package, Dray and Dufour, 2007) were performed on PCA using each tree residues as data matrix and stand origin as class. The differences among stand origin were tested using the Monte Carlo Permutation Test. The similarity of Euclidian distances matrices obtained with or without organic input was determined using a Mantel test performed on 10^5 permutations.

Differences in organic matter quality and in microbial parameters induced by the types of residues were observed by comparing differences between each mesocosm and their control (without residues) after 19 months. Values of chemical markers obtained from amended soils were subtracted to the corresponding control to find out variations in OM quality and quantity potentially induced by the residue inputs. Variations were tested using a t-test. Analyses of variance were also performed to determine effects of the types (W or TL), the stand origin (*Qi*, *Ph*, *Qi-Ph*, *Qp*, *Ps*, *Qp-Ps*) of residues and of their interaction. Post-hoc tests were realised to identify such differences.

We used a non-parametric method of multivariate analysis of variance based on pairwise distances (PERMANOVA) to detect influence of the type and the stand origin of residues on the CLPPs, EEAs and genetic composition of fungal and bacterial communities. Then differences induced by the stand origin or type of residues were determined as described above using two-way analyses of variance. The importance of the OM quality of residues (organic C, carboxyl C, alkyl C, O-alkyl C, aromatic C, pH, and total N and the ratio AR,

289 HR1, HR2 and the C to N ratio) in determining CLPP, EEAs patterns, bacterial and fungal
290 composition of soils was evaluated by variation partitioning using db-RDA on Bray-Curtis
291 distance matrices (Vegan package, Dixon, 2003). The significance of explanations was
292 determined by ANOVA of the partial RDA test using 999 permutations. Then, relationships
293 between soil microbial and chemical variables were analyzed by Pearson linear regression
294 models and reported when significant ($P<0.05$ and $r>|0.4|$).
295

296 **3. Results**

297 *3.1 Effect of plant residues addition on soil organic matter*

298 Differences in the chemical patterns of plant residues were described depending on the
299 forest stand (*P. halepensis, Q. ilex, P.halepensis/Q. ilex, P. sylvestris, Q. pubescens,*
300 *P.sylvestris/Q. pubescens*) and the type of residues (i.e. wood *vs* twigs and leaves/needles).
301 Figure 1 A and B shows a factorial map from PCA based on the first two axes that accounted
302 for 87.96% of variance. Residues composed of wood (W) and of twigs and leaves/needles
303 (TL) were clearly separated on the first axis (51.61%). TL residues were positively correlated
304 to the C fraction relevant to cutin compounds (i.e. the alkyl C fraction and COOH), HR2
305 (alkyl-C/O-alkyl-C) and N content, while wood residues were associated to polysaccharide
306 signal (O-alkyl-C) and C to N ratio. The second axis (36.35%) described differences
307 associated with the tree species depending on the plant material: few differences were found
308 for W while discrimination between TL was clearly observed. Total C content and recalcitrant
309 compounds (aromaticity ratio and alkyl-C/COOH i.e. lipids) were strongly associated to *Pinus*
310 *spp*. TL and pH was positively correlated to *Quercus spp* TL.

311 Both W and TL inputs indeed induced a significant increase in soil organic C (Fig.
312 2.A) but both did not result in significant variations in the recalcitrant fraction of OM i.e.
313 aromatic C (Fig. 2.D). On the other hand, certain soil chemical properties varied depending on

W or TL inputs. TL inputs increased soil N content (Fig. 2.B) but did not result in a decreased C/N ratio (Fig. 2.C). W inputs increased polysaccharide content (O-alkyl C fraction, Fig. 2.E) and reduced lipid content (alkyl C fraction, Fig. 2.F). Consequently, a decrease in alkyl C to O-alkyl C ratio was observed in W amended soils, which was not found in TL amended soils (Fig. 2.G). Thus, TL and W addition resulted in different soil chemical variations compared to controls: an increase in N availability was found in soils amended with TL, while an increase in more labile C sources was observed in W amended soils. It has to be noted that W addition also led to an increase in pH compared to control (Fig. 2.H).

Depending on the tree species origin of residues, variations in carboxyl C fraction of OM (and thus in alkyl C to carboxyl C ratio) was observed: it decreased after addition of residues from *Qi*, *Ph* and *Qp-Ps* mixture compared to what was observed with *Qp* residues (Fig. 3.A). Consequently, in soils amended with *Qi*, *Ph* and the *Qp-Ps* mixture, a higher alkyl C to carboxyl C ratio linked to longer alkyl chains (lipids) were found, while in *Qp* amended soils, a low alkyl C to carboxyl C ratio indicated the production of short-chain acids potentially linked to a more intense mineralization (Fig. 3.B). No effect of the interaction between species origin and type of tree residues was found on soil organic matter characteristics. This is a result of importance since it shows that the effects of the type of tree residues on soil chemical properties were not driven by tree species.

3.2 Soil microbial abundance, structure and activities

No variation in fungal nor bacterial biomass (assessed via qPCR of ITS or 16S rDNA, respectively) or in active biomass (*via* substrate-induced respiration) was found after addition of plant residues whatever their type (W *vs* TL) or origin (tree species), data not shown. On the other hand, the main factor shaping bacterial and fungal community structures was the origin of the tree organ as shown by the between class analysis performed using T-RFLP and ARISA fingerprints (Fig. 4) and the Monte Carlo test (simulated $P<0.001$). When using T-

RFLP subset data separately for control, C, TL and W treatments, the degrees of similarity (Mantel r coefficients) obtained between treatments C and TL and between treatments C and W were 0.76 ($P<0.001$) and 0.71 ($P<0.001$), respectively. This result indicates that treatments TL and W induced similar effects on bacterial community structure. When using ARISA subset data separately for C, TL and W treatments, the degrees of similarity (Mantel r coefficients) obtained between treatments C and TL and between treatments C and W were 0.49 ($P<0.001$) and 0.31 ($P<0.001$), respectively. This result indicates that treatment W induced a stronger effect on the fungal community structure than treatment TL.

We further determined to what extent the type of tree residues, W and TL, influenced microbial markers using variance partitioning analyses on CLPP, EEAs and the genetic structure of fungal and bacterial communities (ARISA and T-RFLP respectively). Results from db-RDA, and their significances (using ANOVA-like permutation test) are shown in Figure 5 and Table 2 respectively. The amount of variation explained by the type of tree organ on bacterial properties (both CLPP and T-RFLP) ranged from 24% to 41% for W and from 20% to 34% for TL, but the only significant effect was observed for W residues on CLPP (W explained 41 % of the variance in CLPP). Variations in fungal community structure were explained by both W (36%) and TL (34%).

3.3 Relationships between soil organic matter properties and microbial communities

To better understand how the type of tree residues in litter modified soil microbial communities, we determined the Pearson correlation coefficients between microbial markers and soil physico-chemical properties (Table 3). Most enzyme activities were positively correlated to organic C and/or total N amount. More particularly N content, which was higher in TL amended soils (Fig 2.B), was positively correlated with cellulase, protease, and urease activities, thus enhancing enzymes involved in mineralization of the labile fraction of organic matter and N cycle. Lignocellulolytic activities (cellulase and tyrosinase) and urease activities

were also positively correlated to organic C amount that increased in both W and TL amended soils (Fig 2.A). Similarly, tyrosinase was also positively correlated to C/N ratio, which was higher in W and TL amended soils (Fig 2.C).

The active microbial biomass (assessed *via* SIR) and the bacterial biomass (determined *via* the 16S copy numbers) were positively correlated to both organic C and total N contents. It has to be noted that a significant relationship was found between active microbial biomass and bacterial biomass ($r=0.55$, $P<0.001$, data not shown) and not between active microbial biomass and fungal biomass, which suggests that fungal communities were weakly involved in SIR measurement. C/N ratio increased with tyrosinase activities and the Fungi to Bacteria ratio. This result is corroborated with the correlation linking tyrosinase activities to the fungal biomass ($r=0.64$, $p>0.001$, data not shown). The active microbial biomass was negatively correlated to pH and a pH increase was found in W amended soils (Fig 2.H): together, such results suggest that W inputs are likely to down regulate the active microbial biomass (and thus particularly bacteria).

Our results also pointed out that an increase in carboxyl C was associated to a decrease in catabolic diversity (H') and in the relative fungal biomass (assessed *via* the fungi to bacteria ratio) but positively associated to fungal richness. We also observed an increase in fungal diversity associated with the alkyl C fraction of organic matter (OM). Since our previous results highlighted that modification in the carboxyl C fraction of OM was linked to tree species origin of residues (more -COOH in *Pinus spp*. residues) and that the alkyl fraction also differed between soil origin, this result indicates that tree species chemical 'print' could affect soil processes through both microbial catabolic potential and fungal population shaping.

386 **4. Discussion**

387 *4.1 Changes in soil chemical characteristics induced by tree residues*

388 After a 19 month incubation period, we observed higher C content in soils amended by
389 both fine (TL) and coarse (W) residues compared to non-amended soils, attesting the
390 decomposition of residues and transfer of degradation products from plant materials to soil.
391 Plant residues are obviously supposed to be degraded more slowly *in natura via* litter decay.
392 In our experiment, we crushed and sieved plant residues before mixing them into the top soil
393 of mesocosms under controlled humidity over time (60% humidity). We expected this process
394 to enhance plant decay rate by increasing the surface available for microbial colonization (Ma
395 et al., 2014) and thus microbial activities. The standardized quantity of C inputs across
396 treatment can explained similar level of C increase in all amended soils.

397 On the other hand, it is noteworthy that fine and coarse residues led to a different soil
398 OM quality. After wood addition, higher soil C quality was found as shown by a lower alkyl
399 C to O-alkyl C ratio, while addition of fine residues did not lead to modification in C quality,
400 as described by NMR data, but in higher N content. Tree mineral nutrients are known to be
401 mainly stored in leaves and in smallest twigs (Ma et al, 2014). Higher N content in fine
402 residues and higher C/N ratio and O-alkyl C in wood residues were found back as a 'chemical
403 print' in soil. OM turnover is known to be strongly regulated by C and N stoichiometry, and
404 many studies showed that C/N was a strong predictor of decomposition of wood and leaves
405 (Jackson et al., 2013; Sundqvist et al., 2011). Thus, our study demonstrated that, under our
406 experimental conditions, coarse or fine residues modified C/N, a major factor regulating
407 microbial activities and thus OM turnover in soil. The contrasting effects of fine and coarse
408 residues suggest a major role of such residue as drivers of soil properties (Freschet et al.,
409 2013). Concerning the aromatic fraction of soils, no variation after residue addition has been
410 observed and the weak 'print' of lignin from plant residues on soil chemical properties is

411 probably linked to a slower degradation of such recalcitrant compounds (Kalbitz et al., 2006).
412 At the early stage of decomposition, polysaccharides are preferentially used over lignin (Berg
413 and McClaugherty, 2014).

414 Tree species origin of residues modified alkyl C to carboxyl C ratio which is a
415 chemical marker of decomposition : a low ratio indicates intense mineralization leading to
416 short-chain acids (Mourik et al., 1998; Preston et al., 2002). A low ratio was found after
417 addition of *Q. pubescens* residues only. Thus, *Q. pubescens,* the only deciduous species
418 considered, produced residues which favored OM turnover compared to the other tree
419 residues tested. Polyakova and Billor (2007) studied the impact of deciduous tree species on
420 litter quality and decomposition processes in pine stands and found that deciduous species,
421 more than evergreen, favored decomposition because of higher mineral content and easily-
422 degradable OM amount.

423 The type of residues (i.e. wood or twigs with leaves or needles) modified more
424 strongly soil OM quality than tree species origin. Interestingly, no modification in soil
425 chemical properties associated to fine or coarse residues depended on the tree species (no
426 effect of interaction). This result is sustained by the study of Pietsch et al. (2014), who
427 demonstrated the importance of wood and leaf residue decomposability as 'after-life' effects
428 of plant traits, which could potentially lead to variations in OM turn-over rates. These
429 observations are substantial since they suggest that the type of residues is likely to modify
430 differently C and N cycling, and moreover that their impacts on soil chemical properties are
431 similar whatever the forest stands considered (pure or mixed stands, coniferous, deciduous or
432 evergreen species).

433 *4.2 Fine and coarse residues shape catabolic and genetic structures of microbial communities*

434 Microbial community fingerprinting (ARISA and T-RLFP) showed that the coarse or
435 fine residues clearly shaped fungal communities, while it did not significantly explain

variations in bacterial community structure. Moreover, the origin of the residues (tree species) also modified the genetic structure of fungal communities and not that of bacteria. The particular sensitivity of fungal communities to residues addition is probably associated to the potential of mycelia to actively colonize plant residues in bulk soil. Mycelium growth rates in laboratory mesocosm can reach up to 8 mm per day (Donnelly et al., 2004). Thus, fungi are able to produce propagule networks which ramify at the interface between soil and residues, making nutrient resources available over wide surfaces (Boddy et al., 2009). Conversely, bacteria are connected to their immediate surroundings, i.e. micro-niches with soil-pore size (Vos et al., 2013), with very specific conditions (i.e. pH, conductivity), which differ from the average properties of their environmental matrix (Urbanová et al., 2011). This lack of connectivity is probably enhanced in our mesocosms where no mesofauna can favor OM circulation.

While the limitations of BIOLOG methodology for the characterization of whole communities are well known, CLPP remain useful to analyse the dynamic of copiotrophic bacteria from the soil (Lladó and Baldrian, 2017). Here, variance in CLPP was significantly explained by the quality of coarse residues (wood). Fanin et al. (2014) examined the effects of litter with different chemical prints on CLPP and highlighted that the catabolic potential of communities was influenced by residue quality and particularly by the C, N, and P stoichiometry. Higher labile C fraction content (O-alkyl) was found in wood residues, and thus the availability of labile carbon substrate may have favored fast-growing decomposers such as copiotrophic bacteria and consequently modified the functional structure of microbial communities.

4.3 Coarse or fine residues modulate microbial enzymes involved in C and N cycling

The strong correlation observed between N content and urease, protease and cellulase suggest that nitrogen was the major factor stimulating extracellular enzyme activities involved

in mineralization of labile fraction, but did not affect lignin-modifying enzyme (tyrosinase). Cellulase is secreted by a large number of microorganisms, but only a small number are able to produce lignin-modifying enzyme (e.g. white-rot basidiomycetes and xylacarious ascomycetes) (Carreiro et al., 2000). Thus, this difference in enzyme response could be related to the changes in microbial community structure described above. More precisely, N content was related to the active microbial biomass, here mainly referring to bacterial communities. Conversely, fungal communities and their activities seemed favored by high C/N and more precisely the potential of aromatic compound transformation (tyrosinase) and the balance between fungal and bacterial biomass. Since C/N was higher in soils amended with wood than with fine residues, coarse residues may facilitate the settlement of fungal populations and their lignocellulolytic activities. Moreover, as described by the positive relation between alkyl C content and fungal diversity, the reduction in alkyl C observed in wood amended soils may affect fungal equitability by selecting specific taxa. Our results are supported by the microcosm experiment of Kooijman et al. (2016), who demonstrated, using selective inhibition of either bacterial or fungal communities, a higher N demand by bacteria than by fungi. Thus, it is noteworthy that coarse or fine residues modified differently the structures and functions of bacterial and fungal communities.

In their meta-analysis, Chen et al. (2018) showed that soil N addition reduced phenoloxidase activities and that this N-induced enzyme repression was associated with increases in soil C. This is consistent with our result as we found that the proportion of N (via C/N ratio) was negatively associated to lignin-modifying enzyme. Moreover, we found deep modification in enzyme activities and soil microbial properties (including soil respiration SR, which represents one of the largest C fluxes from terrestrial ecosystems to the atmosphere) related to OM quality variation due to the type of residues. Since lignin and cellulose are the two most abundant organic resources derived from plants, differences induced by the type of

residues on C-releasing activities of soil microbes and their C-acquiring enzymes have potential to deeply modify soil C and N cycling.

5. Conclusion

Our study revealed that, under laboratory conditions, coarse or fine residues differently shaped soil microbial communities. It is noteworthy that in a more natural setting, the material would fall on the surface of the soil and work its way into the soil over time. Thus, the rate of OM decomposition and resulting effects on microbial communities and soil C and N cycling would occur on a longer time step which would differ between residues sizes. However, our findings remain of importance since we show that the type of residues modulates soil microbial responses to plant residues, prevailing over the species origin of residues. Moreover, C/N ratio (characterizing the chemical print of fine inputs) appeared to strongly control the balance between fungal and bacteria biomass. This is particularly noteworthy since fungal communities are supposed to be k-strategists which favor humification and soil C storage. Tree residues can thus be considered as important drivers of soil microbial functioning and *in natura*, the type and amount of such residues can be modified by sylvicultural practices. This should be considered to enhance carbon sequestration in forest soils under the context of climate change.

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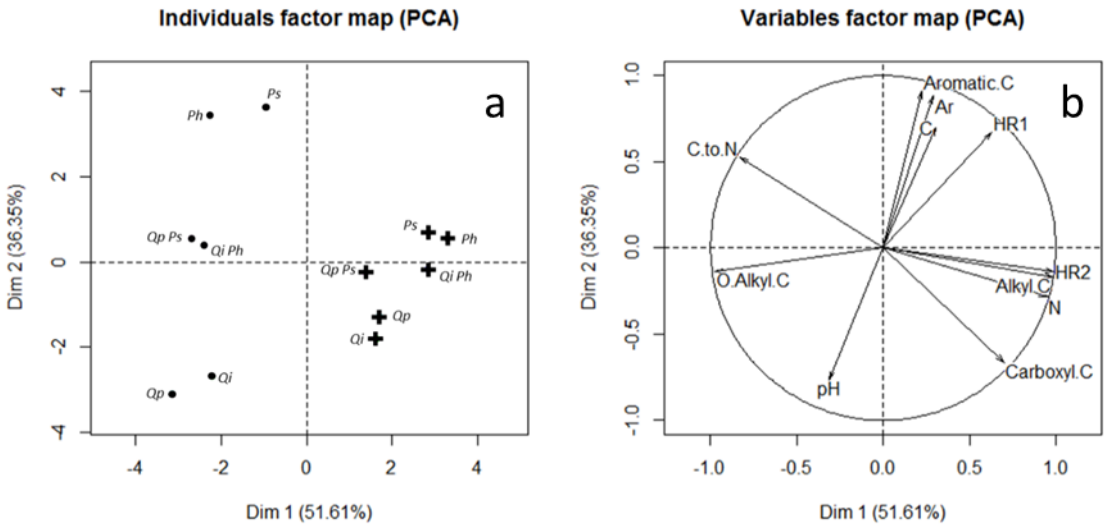

Figure 1: Scores (a) and loadings (b) plots of the Principal Component Analysis calculated on the chemical properties of plant residues. Tree-species and the type of input: (+) stands for Twigs and Leaves/needles (TL), and (•) for Wood (W), (*Qi) Quercus ilex. (Ph) Pinus halepensis. (Qp) Quercus pubescens* and *(Ps) Pinus sylvestris*. HR1 stands for Alkyl C to Carboxyl C ratio and HR2 for Alkyl C to O-Alkyl C ratio.

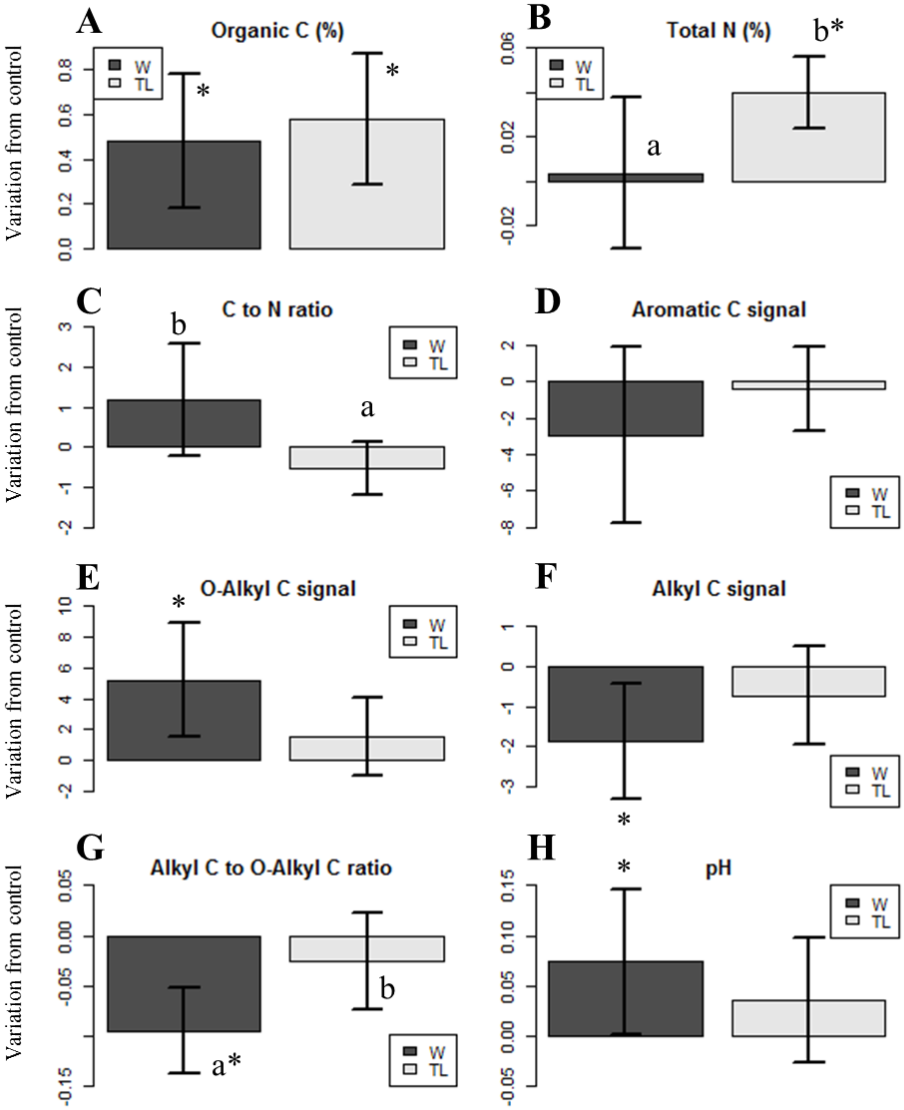

Figure 2: Variations in soil OM quality: (A) Organic C (%), (B) Total N %, (C) C to N ratio, (D) Aromatic-C signal, (E) O-alkyl-Csignal, (F) Alkyl-Csignal, (G) Alkyl-C to O-Alkyl-C ratio,(H) pH after19-month incubation depending on the different residues (W, wood, TL, Twigs and leaves/needles). Significant differences are indicated by different letters. Differences compared to the control soils are indicated by (*).

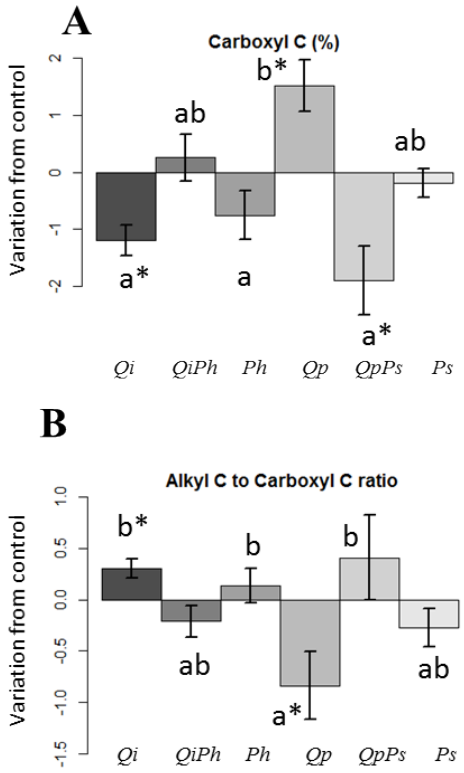

Figure 3: Variations in soil OM quality compared to the corresponding control soil, after amendments with debris from: (*Qi*) for *Quercus ilex*, (Ph) for *Pinus halepensis*, (*Qp*) for *Quercus pubescens*, (*Ps*) for *Pinus sylvestris*, and (*Qi-Ph*) and (*Qp-Ps*) for their respective mixture. Panel (A) show results for the Carboxyl C fraction, and panel (B) for the Alkyl C to Carboxyl C ratio. Significant differences between amended soils are indicated by different letters. Differences compared to control soils are indicated by (*).

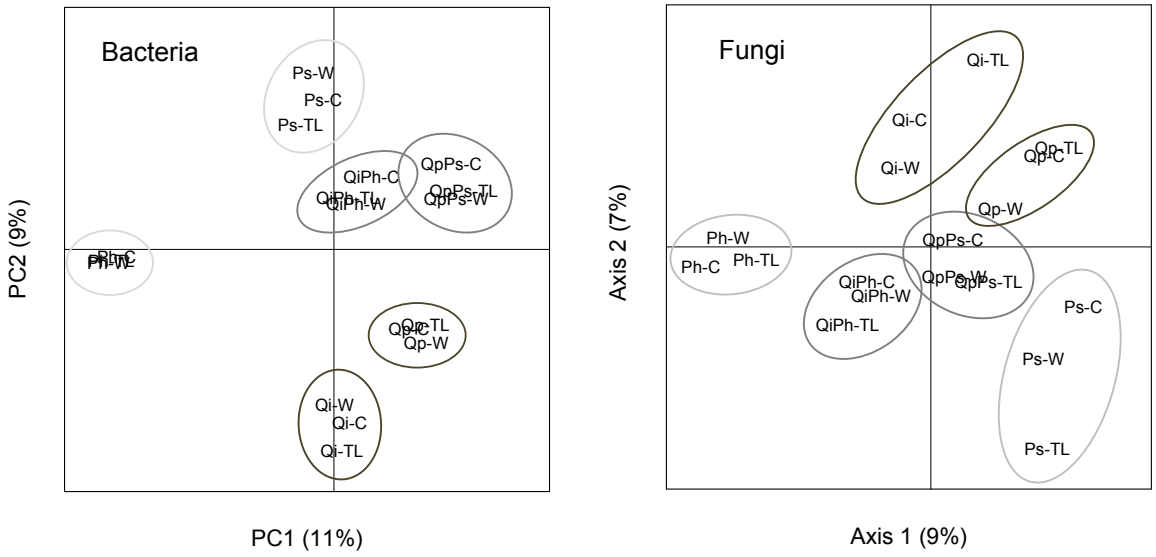

Figure 4: Differences in bacterial (left) and fungal (right) community structure represented by between class analysis (BCA) based on molecular profiling. Soft-grey-lined ellipses represent soils from pine stands (Ps and Ph), intermediate-grey-lined ellipses represent soils from mixed stands (QpPs and QiPh) and dark-grey-lined ellipses represent soil from oak stands (Qp and Qi). Suffix - C, -TL and - W correspond to non-amended samples (Control) or amended with twigs-leaves/needles or coarse wood respectively. Letters represent the barycenter of replicates (n=3) for each treatments. Monte Carlo test revealed significant differences among trees species (simulated $P < 0.001$) for both taxa.

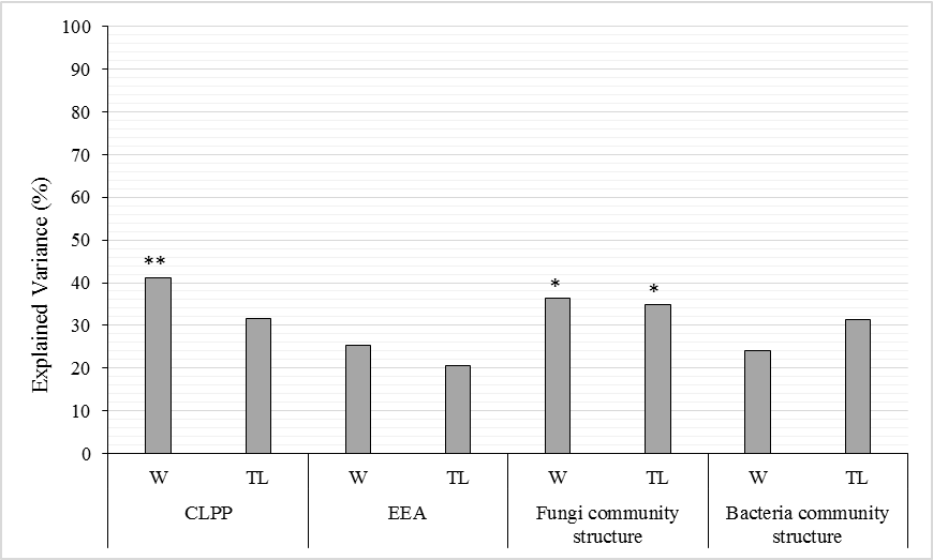

Figure 5: Variance partitioning of Catabolic Level Physiological Profile (CLPP), extracellular enzyme activities (EEAs), and fungal and bacterial community structure after addition of twigs-leaves/needles (TL) or coarse wood (W). Significance levels: P,0.05: *; P,0.01: **.

Table 1: Chemical properties (including solid state ^{13}C NMR data) of soils at T0. Soils used to set up the experiment were collected from different forest stands: *Qi* for *Quercus ilex*, *Ph* for *Pinus halepensis*, *Qp* for *Quercus pubescens* and *Ps* for *Pinus sylvestris*. Values are the mean ± standard deviation (n=18). Mean differences between control and amended soils were determined by Tuckey tests. Different letters indicate significant differences (P<0.05).

	Ph	***Qi Ph***	***Qi***	***Ps***	***Qp Ps***	***Qp***
Organic C %	4.62 (±1.14)	6.84 (±0.59)	9.67 (±1.03)	8.04 (±5.47)	7.83 (±3.88)	5.18 (±0.70)
Total N %	0.262 (±0.12)	0.417(±0.10)	0.537 (±0.12)	0.394 (±0.26)	0.406 (±0.24)	0.327 (±0.05)
C to N ratio	17.4 (±2.9)	17.1 (±3.4)	18.9 (±4.9)	22.7 (±8.1)	21.1 (±4.2)	15.8 (±1.1)
Alkyl C %	21.0 (±0.1) [a]	26.5 (±0.7) [b]	23.4 (±0.7) [ab]	24.0 (±0.5) [ab]	21.9 (±1.6) [ab]	24.5 (±2.9) [ab]
O-Alkyl C %	45.4 (±5.1)	41.8 (±4.0)	46.3 (±1.7)	50.3 (±0.9)	48.7 (±2.8)	45.1 (±1.7)
Carboxyl C %	10.0 (±2.6)	7.4 (±1.2)	10.3 (±1.1)	6.9 (±0.4)	7.3 (±1.6)	11.7 (±3.4)
Aromatic C %	23.4 (±5.5)	24.1 (±5.2)	19.8 (±1.3)	18.7 (±1.2)	21.8 (±5.5)	18.6 (±2.4)
$CaCO_3$	3.55 (±1.17)	2.33 (±1.31)	0.37 (±0.53)	1.50 (±1.48)	0.84 (±1.01)	1.09 (±1.89)
pH	7.41 (±0.22) [b]	7.02 (±0.19) [b]	6.96 (±0.33) [b]	6.73 (±0.57) [b]	6.96 (±0.11) [b]	5.20 (±0.79) [a]

Table 2: Effect of the chemical properties of W and TL residues (considering N, C, C to N ratio, aromatic C, alkyl C O-alkyl C, carboxyl C, Ar, HR1, HR2 and pH) on CLPP, EEA patterns, and bacterial and fungal community structure (finger prints). Significance of the effects were determined by ANOVA on RDA model with(significance levels are indicated as follow: P<0.05: *; P<0.01: **).

Dataset	**CLPP**		**EEA**		**Fungal community structure**		**Bacterial community structure**	
Residues	**W**	**TL**	**W**	**TL**	**W**	**TL**	**W**	**TL**
% Var	41.06	31.55	25.41	20.52	36.26	34.85	24.06	31.47
F	1.6721	1.1063	0.8174	0.6198	1.3652	1.2884	0.7605	1.1023
P	0.002**	0.37	0.6	0.828	0.019*	0.049 *	0.877	0.338

Table 3: Correlation coefficients (r) between soil chemical and microbiological parameters as determined by Pearson's Test. (significance levels are indicated as follow: *P <0.05. **P <0.01 and ***P <0.001, n=54).

	Total N (%)	Organic C (%)	C/N	pH H_2O	Carboxyl C	Alkyl C
Catabolic Diversity (H')	ns	ns	ns	ns	-0.41*	ns
Protease Activity	0.45*	ns	ns	ns	ns	ns
Cellulase Activity	0.47**	0.70***	ns	ns	ns	ns
Urease Activity	0.59***	0.59**	ns	ns	ns	ns
Tyrosinase Activity	ns	0.53**	0.42*	ns	ns	ns
Active Microbial Biomass	0.79***	0.73***	ns	-0.42*	ns	ns
Bacterial Biomass (16S copy number)		0.71***	ns	ns	ns	ns
Fungal Biomass (ITS copy number)	ns	0.66***	ns	ns	ns	ns
Fungi to Bacteria ratio	-0.53**	ns	0.56**	ns	-0.55**	ns
Fungal composition (H')	ns	ns	ns	ns	ns	0.42*
Fungal Richness (number of ITS amplicons)	ns	ns	ns	ns	0.46*	ns
Bacterial composition (H')	ns	ns	ns	ns	ns	ns
Bacterial Richness (number of T-RFs)	ns	ns	ns	ns	ns	ns

Declaration of interests

 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.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: