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Soil microbial functions are affected by organic matter removal in temperate deciduous forest

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Abstract

A growing demand for renewable carbon (C) has led to intensified forest management resulting in the use of forest residues (e.g. canopy, bark or litter layer) as energy sources with potential modifications of soil properties and tree productivity. Because microbes mediate the recycling of C and nutrients sequestered in organic matter, we investigated the effects of organic matter (OM) removal on soil properties, root surfaces, microbial functions and abundance using a new observational forest network. We compared leaf litter and logging residue removal plots to reference plots in six beech forests located in the northern half of France. After three consecutive years of OM removal, C and nitrogen (N) pools were not affected, but OM exportation decreased the cation exchange capacity and available phosphorus (P) pool by respectively 12% and 30% in the topsoil (0-5 cm depth). Fine root surface area significantly increased by 21% in the subsoil (5-10 cm depth) in response to OM removal. Enzymatic activities involved in N and P mobilisation decreased from 12 to 38% with the manipulation of OM. Community-level profiling (CLPP) based on BIOLOG approach revealed that the metabolic potential of the microbial community strongly decreased in response to OM removal. Our results indicated that intensive forestry could affect microbial functions implicated in nutrients mobilisation. We demonstrated that soil organic matter (SOM) content is a predictor of microbial functions resistance to forest residue removal. We recommend that intensive forestry should be reduced or limited in beech forests characterised by low soil OM contents.

Keywords

Organic matter removal, soil microbial functions, enzyme activity, CLPP, microbial biomass.

Highlights

- Soil microbial functions change in the short term in response to organic matter removal
- Organic matter removal leads to a decrease in the microbial metabolic potential and enzyme activities involved in nitrogen and phosphorus mobilisation
- The initial soil organic matter content is a predictor of microbial function sensitivity to organic matter removal

INTRODUCTION

Forests are major components of the carbon (C) cycle on Earth, sequestering large quantities of C (Lal, 2005; Pan et al., 2011; Bar-On et al., 2018). Consequently, managing forest C is a key challenge for mitigating climate change (Bonan, 2008; Canadell and Raupach, 2008). One of the proposed strategies to mitigate climate change consists of using more renewable energy sources in place of oil and coal. However, old-growth forests are defined as important C sinks, and this C, even soil C, could return to the atmosphere if these forests are disturbed (Luyssaert et al., 2008; Bellassen and Luyssaert, 2014). Furthermore, microorganisms participate in the dynamics of C and nutrient cycles in forests through processes that breakdown plant OM in a form that trees can assimilate (van der Heijden et al., 2008; Baskaran et al., 2017; Kvaschenko et al., 2017). Although this remains controversial in some countries, the application of forest management guidelines toward more intensive management practices is progressively observed (Achat et al., 2015a). Indeed, there is a demand to maximise post-harvest residue removal from forests and to divert this previously unused fresh plant organic matter (OM) into biofuel and energy production. This intensification of forest management practices (e.g. shorter rotation, stronger thinning, higher forest residue harvesting) may have negative impacts on soil properties and tree productivity as suggested by two recent meta-analyses (Achat et al., 2015a, 2015b). However, our understanding of how intensive forestry impacts soil microbial diversity and functions remains incomplete.

The effects of logging operations on soil microbial communities were mainly investigated in two different experimental networks in temperate and boreal forests. The Long-Term Soil Productivity (LTSP) study was established in 1989 to analyse the effects of OM manipulation in a network of coniferous forests (Powers et al., 2005). Investigations of these LTSP sites demonstrated that bacterial and fungal community compositions changed in response to OM manipulation (Hartmann et al., 2009; Hartmann et al., 2012; Wilhelm et al., 2017). Additionally, Cardenas et al. (2015) used a metagenomic approach to reveal a decrease in the OM degradation gene potential in response to OM manipulation. The Detritus Input and Removal Treatments (DIRT) is an experimental network including eight sites in temperate deciduous forests (Nadelhoffer et al., 2004; Lajtha et al., 2005;). Kotroczo et al. (2014) and Veres et al. (2015) reported that the soil enzymes involved in organic C, N and P decreased in

response to OM removal. The Kotroczo et al. (2014) and Veres et al. (2015) studies were focused at one site of the DIRT network. Other studies conducted independently of these two experimental networks highlighted the impact of OM removal on soil microbial functions (Hassett and Zak, 2005; Busse et al., 2006; Tan et al., 2008) and were also focused at one or two sites. Moreover, Achat et al. (2015b) indicated that long term impact of intensive forestry remains poorly examined and notably on soil microbial compartment.

In the present study, we measured soil parameters and associated microbial functions in six temperate beech forest sites of a new long-term observational French network, comparing OM removal treatment and conventional forest management (Akroume et al., 2016). This large-scale investigation aims to mimic the effect of middle / long-term OM removal thanks artificial and annual exportation of woody debris and litter layer (Stone, 2001). In addition to measurements of soil chemical parameters and fine root surface areas, we monitored the soil enzymatic activities and the community level profiling (CLPP) of the microbial communities in the topsoil (0-5 cm) and subsoil (5-10 cm) layers. Additionally, bacterial and fungal abundances were also assessed using quantitative PCR (qPCR). After three years of OM removal, we hypothesised decline of microbial abundance and associated functions implicated in OM degradation depending of the initial edaphic parameters of each studied site.

MATERIALS AND METHODS

Study site, experimental design and soil sampling

The study was conducted in six OM manipulation sites belonging to the “Matières Organiques des Sols réseau” (MOS network) in France (Table 1). All the sites are dominated by *Fagus sylvatica*. Each site contained 12 plots (40 m × 40 m), which corresponded to four OM manipulation treatments repeated three times as described by Akroume et al. (2016). In this study, we focused on two treatments: conventional French forest management (reference plot: REF) and yearly residue and litter removal (OM removal: OMR). The OMR treatment consisted of manually removing every late autumn all coarse woody debris and leaf litter using a power-driven sidewalk sweeper. When we collected the soil samples, the OM removal treatment had been applied for three years. In each forest site, we selected the pair plots that had the most similar soil characteristics before harvest residue removal, based on the soil data published by Akroume et al. (2016). In September 2016, we sampled on both sides of the boundary separating adjacent REF and OMR plots at each site nine soil cores (15 cm diameter) for each of the two treatments (n=108 soils cores) as shown in Figure 1. Sampling was conducted when the soil moisture was relatively elevated and within the period of high soil microbial activity (Boerner et al., 2005; Kang et al., 2009). The separation into the topsoil (0-5 cm) and lower layer (5-10 cm) was performed in the field for each biological replicate (n=216 soils sampled). In the

laboratory, the soil samples were sieved (2 mm) and homogenised before the enzymatic analyses and CLPP assay. An aliquot of each soil sample was freeze-dried for DNA extraction. Composites of three successive sampling points per depth, per treatment and per site were also made (n=72 composites). These composite soils were used for the Biolog® Ecoplate (Biolog Inc., Hayward CA., USA) approach and soil properties analysis.

Soil properties and the fine root surface area

Soil analyses were carried out by the Laboratoire d'Analyse des Sols d'Arras (INRA, France) on the composite soil samples (n=72). The cation exchange capacity (CEC) was determined according to the cobalt hexamine method. The pH was determined by the water method using a soil/water ratio of 1:5 (w/v). Total soil organic C and total N contents were measured using a CHN analyser, while the available P content was determined according to Duchaufour and Bonneau (1959). Beech tree roots were collected in each soil sample at the sieving step, and root length and total root surface were measured with the WinRHIZO system according to manufacturer's manual (Regent Instruments, Québec, Canada) (Wang and Zhang, 2009). Fine root surface areas defined as diameters less than 2 mm (Liu et al., 2018) were expressed as a projected area per soil core.

Bacterial and fungal abundance

Total nucleic acids were extracted from 0.5 g of sieved soil (n=216 soil samples) using the FastDNA SPIN Kit for soil (MP Biomedicals, Solon, OH, USA) with the addition of 20 mg of Polyvinylpyrrolidone (Sigma-Aldrich, France). The bacterial and fungal abundances were measured as defined by Cébron et al. (2008) and Chemidlin Prévost-Bouré et al. (2011). Amplification reactions were carried out on 1 µL of 1:10 diluted template DNA, standard plasmids (10^9 to 10^3 gene copies/µL) or water (negative control), in a 20 µL volume using iQ SYBR green Supermix (Bio-Rad). Amplification temperature profiles consisted of 5 min at 98°C, followed by four steps of 40 cycles, 30 s at 98°C, 30 s at the primer-specific annealing temperatures (56°C and 50°C for 16S and 18S rRNA, respectively) and 10 s at 80°C to dissociate the primer dimers and capture the fluorescence intensity of the SYBR green. At the end, a melting curve analysis was performed from 70°C to 95°C, with a temperature increase of 0.4°C. Data are expressed as numbers of 16S or 18S copies per g of dry soil.

Enzyme activities

The first approach we used to characterize microbial functions was a soil enzyme activities assessment. We chose to measure eight hydrolytic and oxidative extracellular enzymes: beta-glucosidase, cellobiohydrolase, xylosidase, glucuronidase, laccase, N-acetylglucosaminidase, L-leucine-aminopeptidase and acid phosphatase. Briefly, 2 g of fresh soil (n=216 soil samples) was

weighed and added to 40 ml of acetate buffer at pH 5.0 (methylumbelliferone fluorochrome and laccase assay) and pH 8.0 (methylcoumarine fluorochrome) to give a final concentration of 1:20 w/v. Diluted solutions were incubated at 4°C for one night under agitation. We applied the incubation times and substrate concentrations defined by Pritsch et al. (2011). For the laccase test, a solution of 2 mM 2,9-azinobis-3-ethylbenzothiazoline-6-sulfonate (ABTS) was prepared in sodium acetate buffer at pH 5. In each well of a clear flat-bottom 96-well plate (Sarstedt, Newton, NC, USA), 200 µl of the diluted soil solution was added to 50 µl of 2 mM ABTS solution. After incubation at 25°C the plate was centrifuged at 4,500 g for 5 minutes, and 100 µl of the clear solution was transferred into a new, clear flat-bottom 96-well plate, and measurements were performed at 415 nm (Bio-Rad model 550). For the microplate assays with the methylumbelliferone or methylcoumarine fluorochromes, in each well of a clear flat-bottom 96-well plate (Sarstedt, Newton, NC, USA), 200 µl of the diluted soil solution was added to 50 µl of the substrate solutions. After incubation at 25°C, the plate was centrifuged at 4,500 g for 5 minutes, and 50 µl of the clear solution was transferred into a black opaque 96-well microplate (Perkin-Elmer, Life Sciences) containing 50 µl of stopping buffer (Tris 2.5 M pH 10–11) in each well. Measurements were carried out using a Victor3 microplate reader (Wallac Perkin-Elmer Life Sciences, Villebon-sur-Yvette, France) with an excitation wavelength of 360 nm and an emission wavelength of 450 nm. Control measurements were made for each enzyme assay. Enzymatic activity (EA) was expressed in pmol of substrates released per g of dry soil per min. All of the chemicals were purchased from Sigma-Aldrich (France).

CLPP approach

We used the Biolog® Ecoplate as the second approach to explore microbial functions. For this experiment, 5 g of composite soils (n=72 soil samples) was diluted in 45 ml of sterile distilled water; after a brief agitation, it was diluted again to 1:20 w/v; and 100 µl of the 1:20 soil solution was used to inoculate the Biolog® Ecoplate microplate. Then the plates were incubated at 25°C, and colour development was measured at 590 nm (Bio-Rad model 550) after 72 h of incubation. Metabolism of the different substrates in the wells resulted in the reduction of tetrazolium, which changed from colourless to purple formazan. The OD 590 nm of the reference well was subtracted from the other wells containing carbon sources. These corrected absorbances were used for the subsequent analysis. The substrates were divided into five classes: carbohydrates, carboxylic and acetic acids, amines, amino acids and polymers as defined by Frac et al. (2012). We also calculated the substrate utilisation richness (Garland, 1997), which represents the number of carbon substrates metabolised (corrected absorbance values > 0.25).

Data analysis and statistics

Statistical analyses and data representations were performed using R software (R Core Team 2016). Three-way analyses of variance (ANOVA) were used to test the following effects: site, depth and treatment impacts on soil properties, root surface, bacterial and fungal abundance, enzyme activities and CLPP data. Then, we used a mixed linear model with the lme4 package (Bates et al., 2015) with site as a random factor to evaluate the impact of the treatment on soil properties, root surface, bacterial and fungal abundance, enzyme activities and CLPP data in the function of the two studied soil layers (0-5 and 5-10 cm). From the Vegan package (Oksanen et al., 2013), we calculated Euclidean dissimilarity indices between the enzymatic activities (n=8 enzymes), and the CLPP assay (n=31 substrates) measured in the OMR and REF treatments within each site for both soil layers (0-5 and 5-10 cm). The Corrplot package (Taiyun Wei and Viliam Simko., 2017) was used to identify and represent the Pearson correlation between the soil properties of the REF plots and the impact of OM removal on the root surface (a log 2-fold change OMR/REF), bacterial and fungal abundance (a log 2-fold change OMR/REF), enzyme activities (the Euclidean dissimilarity index between OMR and REF) and CLPP results of the Euclidean dissimilarity between OMR and REF). The levels of significance obtained are indicated in each figure and table legend.

RESULTS

Plant organic matter removal effect on soil properties

The total soil N, soil organic C, soil organic matter (SOM) and pH were not impacted by OM removal (Table 2). In contrast, OM removal significantly affected the other soil parameters measured in the 0-5 cm soil layer (Figure 2), leading to an average decrease of available P concentration (-30%), CEC (-12%) and C/N (-8%) in the OMR plots compared with the REF treatments. Significant responses of these soil properties to OM manipulation were restricted to the top soil layer (0-5 cm). Decreases in CEC and C/N were observed for the six studied sites, while P decreases occurred in the following sites: Gaillefontaine, Saint-Quirin and Verrières-du-Grosbois (Supplementary Figure 1).

Plant organic matter removal effect on bacterial and fungal abundances and fine root surface areas

OM removal significantly impacted bacterial abundance (Table 3). Indeed, the bacterial abundances significantly increased (+20%) in the OM removal plots, notably in the 0-5-cm layer (Figure 2). Although fungal abundance was not significantly affected by OM removal, it tended to increase in the OM removal plots. In addition, the fine root surface areas increased significantly in response to OM removal by 21% in the 5-10 cm soil layer of the OM removal plots. This increase was measured at all

six studied sites (Supplementary Figure 2). Although the effect was not significant, this increase in the fine root surface area was also observed in the topsoil (0-5 cm).

Organic matter removal effects on enzyme activities

Beta-glucosidase, cellobiohydrolase, laccase and xylosidase activities were not significantly affected by OM removal (Table 4). Glucuronidase, N-acetylglucosaminidase, leucine aminopeptidase and acid phosphatase were affected by OM removal with significant decreases of 40%, 38%, 28% and 14%, respectively, in the 0-5-cm soil layer (Figure 2). Leucine aminopeptidase was the only enzyme significantly impacted by OM removal in the 5-10 cm soil layer, with a significant decrease of 29% in the OM removal plots.

Organic matter removal effects on microbial metabolic profiles

OM manipulation caused substantial shifts in the soil microbial metabolic profiles. The CLPP approach revealed that amines, amino acids, carboxylic acids and carbohydrates substrates were affected by OM removal (Table 5). OM removal also impacted the total metabolic capacities and substrate metabolic richness of the microbial community. OM manipulation induced a significant decrease in amine (-62%), amino acid (-22%), carboxylic and ketonic acid (-28%), carbohydrate (-39%) and polymer (-25%) metabolic capacities in the topsoil layer (0-5 cm). The carbohydrate and amine substrates classes also significantly decreased in the 5-10-cm soil layer (-30% and -55%). The total metabolic capacities and substrate metabolic richness significantly decreased by 32% and 24%, respectively, in the 0-5 cm soil layer.

Correlation between soil properties and tree and microbial factors

With the goal of identifying the possible drivers and regulators of microbial functions and associated site sensitivity to OM removal, we explored the relationships between the local soil properties and OM removal impacts on the root surface, microbial abundance and microbial functions. Pearson correlation analyses were conducted between the soil parameters of each reference plot and the OM removal impact on bacterial and fungal abundances, fine root surface areas, enzymatic activities and microbial metabolic capacities for the two soil layers studied (0-5 and 5-10 cm). Euclidean dissimilarity indices calculated on the basis of the CLPP results between the REF and OMR plots for each studied site were significantly negatively ($P \leq 0.05$, $r = -0.81$) correlated with the SOM content of the REF plots (Figure 3). The Euclidean dissimilarity index calculated on the basis of the enzymatic activities results

between the REF and OMR plots for each studied site were also significantly negatively ($P \leq 0.05$, $r = -0.90$) correlated with the SOM content of the REF plots. No other significant correlation was highlighted.

DISCUSSION

After three years of experimental OM removal, no significant changes in the soil C pool were measured. It is however worth noting the slight trend of a C decrease, especially for the Gaillefontaine and Ban Harol sites in the topsoil layer. These findings are close to those obtained in some other forest sites (Johnson and Curtis, 2001; Barthès et al. 2008). Indeed, it has been shown that experimentally or due to intensive forestry, OM removal does not strongly impact soil organic C pool (Nadelhoffer et al., 2004; Sayer, 2006; Fekete et al., 2011; Lajtha et al., 2014). Although Slesak et al. (2017) reported a overall decrease in SOM twenty years after forest floor removal on sites belonging to the LTSP network, their results demonstrated that the effect of this OM removal on soil properties was site-specific. This result is in agreement with our observations, highlighting that the impact of OM removal on the C cycle could strongly depend on the site local parameters, particularly C reservoir. Otherwise, we did not measure the effect of OM removal on the N pools. Changes in the N soil pools in response to OM removal commonly follow a similar tendency to those observed for soil C pools (Lajtha et al., 2014; Slesak et al., 2017). A slight but significant decrease in the CEC in response to OM removal was measured in the topsoil layer. The impact of OM manipulation on the soil P content was reported in other OM manipulation experiments (Wilhelm et al., 2013; Vadeboncoeur et al., 2014; Slesak et al., 2017). Due to the low level of P pools in forest soil, the decrease in available P concentration at all six sites may suggest future soil P limitation, which is a serious concern for tree productivity (Goswami et al., 2018).

The impact of OM removal in temperate forests on fine root surface areas remains poorly explored. The increase of fine root surface areas in the subsoil layer in the six studied sites seems to be a rapid reaction of beech trees to OM removal. Similarly, litter fall removal significantly increased fine root production by 14% in 20-year-old Masson pine (*Pinus resinosa*) plantations (Da-Lun et al. 2010). Because the fine roots of beech are partly located in the upper organic horizons and in the fragmented litter, this increase could result directly from a vertical shift of the roots following the removal of litter. Furthermore, this increase of fine root surface areas could result also from a decrease of soil fertility in response to the OM removal. Indeed, Persson and Ahlström (1990) reported that N fertilization in Sweden Scots pine stands had negative effects on the development of fine roots, resulting in a reduction in the amount of fine roots to about 50% of that in the control. These fertilisation effects on the fine root biomass in forests have been reported in other studies (Cleveland and Townsend, 2006; Phillips and Fahey, 2007). Conversely, fertility reduction due to OM removal (e.g. decrease in CEC and P concentrations in our study) could lead to stimulation of root growth. However, this

phenomenon could have important impacts on soil functioning. Increase of fine root surface areas could lead to an increase of C and nutrients derived from root exudates and dead roots in the organo-mineral layers of OM removal plots due to the growth and rapid turnover of this tree organ (Kuzakov, 2010). We can also suggest that a more important root exudation in these soil compartments could provide a priming effect at midterm and an increase in the degradation of recalcitrant C in the subsoil layers in the OMR plots compared to the REF plots (Dijkstra et al., 2013; Wang et al., 2016). This increase in fine root surface areas could also contribute to a change in the microbial community and their associated functions, including the ectomycorrhizal fungi that express different functional profiles related to their ecological niches (Buée et al., 2007).

In opposition to our hypothesis, bacterial and fungal abundances tended to increase in response to OM removal in this study. Some authors observed a decrease in microbial biomass as a response to OM removal (Li et al., 2004; Feng et al., 2009; Leff et al., 2012), while Fisk and Fahey (2001) did not observe any changes after OM removal. Smolander et al. (2010), who studied wood exportation scenarios in a boreal forest, revealed a site-specific effect with either an increase or a decrease of soil microbial biomass. Phospholipid fatty acid content, another indicator of microbial biomass, also increased in response to whole-tree harvesting and forest floor removal compared to a control plot (Ponder and Tadros, 2002). Observed increases of bacterial and fungal abundance could be linked to the measured increase of fine root surface area potentially increasing the root exudations well known as C energy source for microorganisms (Kaiser et al., 2015).

After three years of OM removal, enzyme activities involved in the mineralization of cellulose and hemicellulose (i.e., beta-glucosidase, cellobiohydrolase, and xylosidase) were not significantly impacted by the treatment. Our results are not consistent with the observations made by Kotroczo et al. (2014) and Veres et al (2015), who report a decrease of beta-glucosidase activity in response to leaf litter removal in temperate forests. This difference may be explained by the fact that they measured OM removal effects after 6 and 10 years whereas our sampling occurred just after 3 years of OM removal. Using an isotope labelling approach, Leung et al. (2016) revealed that OM removal has a small impact on the hemicellulolytic degradation capacity of the microbial community. However, we hypothesize that the enzymes involved in plant cell wall degradation and C mobilisation similarly will decrease at our experimental sites in the coming years. The enzymes involved in P (acid phosphatase) and N (leucine aminopeptidase and N-acetylglucosaminidase) mobilisation were negatively impacted by OM removal in the topsoil layer and in the subsoil layer for N mobilisation. In our experiment, decreased acid phosphatase activity was correlated with decreased available P content in the soil. In contrast, this link was not confirmed for the enzymes involved in N mobilisation. Indeed, soil N concentration had not yet decreased in response to OM removal, but the enzymes involved in N degradation had sharply decreased. Here, the total N does not allow estimates of the labile N pool that

could regulate specific organic N mobilisation enzymes, such as chitinases or proteases. Similar to our results, many studies reported the sensitivity of N and P microbial mobilisation capacities in response to OM removal using mineralisation and enzymatic approaches (Perez-Batallon et al., 2001; Hassett and Zak, 2005; Tan et al., 2008; Smolander et al., 2010). These variations in the activities of the enzymes that degrade SOM could be linked to shifts in decomposition rates, indicating a reduction in the decomposition process in response to OM removal. Moreover, plant litter is rich in secondary compounds as tannins, which would contribute to microbial enzyme inhibition, as an important mechanism controlling some forest ecosystem processes (Harrison, 1971; Kraus et al., 2003; Joannis et al., 2007). Consequently, litter exportation could directly change tannins availability, and microbial enzyme regulation. In addition, tannins and phenolics in fresh and decomposed beech litter are much more concentrated than other tree species, such as oak, Scots pine and black cherry (Lorenz et al., 2004). Indeed, Adamczyk et al. (2017) showed that tannin-enzyme interaction led to decreases but also increases of enzymatic activity due to protein conformation changes or tannin-enzyme precipitates. Variations in the microbial composition and their associated functions in response to OM removal may be a further explanation (Hartmann et al., 2012; Hartmann et al., 2009). Here, we confirmed that soil enzyme activities are more sensitive indicators of changes in response to OM removal than soil parameters, as observed by Caldwell and Gri (1999) and Tan et al. (2008). The availability of soil nutrients depends on the microbial enzyme activities that provide balanced resources for both microorganisms and the trees. In our study, the relative increase in microbial abundance coupled with the decrease in N and P mobilisation enzymes in response to OM removal could modify the balance of these elements between microbes and trees, and increase the nutritional stress of trees in the OM removal plots.

An analysis of the Biolog® data only for the carbon source utilisation pattern demonstrated that OM removal had a significant impact on the microbial functional diversity and overall metabolic activity. Similar to the enzyme activities, these changes are mainly marked by a decrease in metabolic capacity and metabolic richness in response to OM removal in the topsoil layer. Nevertheless, in contrast to the enzymatic approach, the CLPP results in response to OM manipulation are scarce in the literature. In a critical review, Preston-Mafham et al. (2002) explained that the reduction of substrate degradation due to Biolog® Ecoplate could result from the decreased bacterial inoculum size. Using qPCR, we did not observe a decrease in the bacterial abundance and thus rejected this hypothesis. Because nutrient-poor forest soils are mainly dominated by slow-growing oligotrophic bacteria (Jones et al., 2009; Jeanbille et al., 2016; Žifčáková et al., 2016), we suggest that the removal of OM could increase the proportion of these oligotrophic bacteria in the manipulated plots, thus decreasing the relative abundance of copiotrophic bacteria. As the CLPP approach based on Biolog® Ecoplate is mainly driven by copiotrophic bacteria (Lladó and Baldrian, 2017), we propose that the differences in microbial

functional profiles between the REF and OMR plots resulted from a change in the ratio between oligotrophic and copiotrophic bacteria.

We identified the general trends of the soil properties, fine root surface areas, microbial abundances and microbial function patterns to OM removal across the six studied sites. Nevertheless, we also observed that some sites appeared to be more susceptible to this disturbance simulating intensive forestry. These results reveal that certain sites are more sensitive to OM removal than others. Similarly, it was observed that the OM removal effects on soil properties and tree productivity depend on the initial nutrient pool, including organic C and, as a result, the high fertility mitigating OM removal's negative impacts (Egnell and Leijon, 1999; Ponder and Tadros, 2002; Powers et al., 2005; O'Hehir and Nambiar, 2010; Smolander et al., 2010). Here, we revealed that soil microbial functions followed the same trends observed for soil properties and tree productivity as those reported in the literature. We showed that for both the microbial functional diversity characterisations (CLPP and enzyme activities), site sensitivity was predicted by the initial SOM content. Thus, sites presenting the more important changes in soil microbial functions, with several declines in enzymatic activities and metabolic capacities, are the sites with the lower initial SOM levels in the topsoil.

CONCLUSION

Our results demonstrate that soil C and N pools in temperate forests were resistant to intensive OM removal while available P concentration and CEC decreased. However, OM removal leads to decreases in N and P mobilisation enzymes. The metabolic capacity of the microbial community also declines in response to OM removal. These declines of enzymatic activities and metabolic profiles are not explained by a decrease in microbial abundance. In addition, the initial SOM content in the topsoil layer is a predictor of the soil microbial functions' resistance in response to OM removal. We recommend that forest practices limit intensive OM harvests, particularly in forests characterised by lower soil OM contents to ensure the maintenance of the soil microbes' role in tree nutrition and sustainable forest productivity. Relevant knowledge should thus be rapidly produced and efficiently transferred to decision makers to tackle this main challenge that forestry will have to face. As microbial functions appear to be sensitive to OM removal, it would be useful to develop microbial soil-based indicators for sustainable forest management.

Table

TABLE 1 Description of organic matter manipulation experimental sites.

TABLE 2 Three-way ANOVA of the influence of site, depth and treatment and their interactions in the soil properties (n=3). Values in the table represent the F-ratios and the levels of significance

(*P≤0.05; **P≤0.01; ***P≤0.001). Abbreviations: N, total nitrogen; C, organic carbon; SOM, soil organic matter; P, available phosphorus.

TABLE 3 Three-way ANOVA of the influence of site, depth and treatment and their interactions in the bacterial and fungal abundance and fine root surface areas (n=9). Enzymatic activities were expressed in pmol of substrates released per g of dry soil per min. Values in the table represent the F-ratios and the levels of significance (*P≤0.05; **P≤0.01; ***P≤0.001).

TABLE 4 Three-way ANOVA of the influence of site, depth and treatment and their interactions on soil enzymatic activities (n=9). Values in the table represent the F-ratios and the levels of significance (*P≤0.05; **P≤0.01; ***P≤0.001).

TABLE 5 Three-way ANOVA of the influence of site, depth and treatment and their interactions, in the soil CLPP (n=3). Values in the table represent the F-ratios and the levels of significance (*P≤0.05; **P≤0.01; ***P≤0.001).

Figure captions

FIGURE 1 Schematic representation of the experimental design, sampling procedure and representative pictures of the REF and OMR plots of the Ban-Harol experimental site.

FIGURE 2 Differential analysis of the impact of OM removal (percentage change in the OMR plots calculated with REF plots as denominator) on soil parameters (pink), fine root surface areas (blue), microbial abundances (green), enzymatic activities (brown) and metabolic capacities (orange) of the soil microbial communities (in %) for the six studied sites in both soil layers (0-5 cm and 5-10 cm). Abbreviations: N, total nitrogen; C, organic carbon; SOM, soil organic matter; and P, available phosphorus. Levels of significance are as follows: *P≤0.05; **P≤0.01; ***P≤0.001.

FIGURE 3 A. Pearson correlations between the soil properties of the REF plots and the following measurements: i) OM removal treatment on fine root surface areas, ii) bacterial and fungal abundance calculated for each site (log 2-fold change OMR/REF) and iii) dissimilarity index of enzymatic activities and the CLPP approach between the OMR and REF plots; for all sites (n=6) in both soil layers (0-5 cm and 5-10 cm). Colour of the circle depends on the direction of the correlation (blue = positive; red = negative). Size of the circle is proportional to the r value. The value in the circle represents the P-value of the Pearson correlation. P-values in white are significant (*P≤0.05; **P≤0.01). Abbreviations: N, total nitrogen; SOM, soil organic matter; P, available phosphorus; Dis enzyme: Euclidean dissimilarity index of enzyme activities calculated for each site; Dis CLPP,

Euclidean dissimilarity index of the CLPP results calculated for each site. B. Representation of the Pearson correlation between the SOM content and Euclidean dissimilarity index of the enzyme activities and CLPP results calculated for each site for the 0-5 cm soil layer.

Supplementary data

SUPPLEMENTARY FIGURE 1 Soil parameters in the different sites in the treatment function (OMR and REF) and depth (0-5 cm and 5-10 cm). Abbreviations: N, total nitrogen; C, organic carbon; SOM, soil organic matter; P, available phosphorus.

SUPPLEMENTARY FIGURE 2 Bacterial and fungal abundances and fine root surface areas in the different sites in the treatment function (OMR and REF) and depth (0-5 cm and 5-10 cm).

SUPPLEMENTARY FIGURE 3 Soil enzyme activities in the different sites in the treatment function (OMR and REF) and depth (0-5 cm and 5-10 cm). Enzymatic activities were expressed in pmol of substrates released per g of dry soil per min.

SUPPLEMENTARY FIGURE 4 CLPP results in the different sites in the treatment function (OMR and REF) and depth (0-5 cm and 5-10 cm).

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Conflict of interest

We state that there is no conflict of interest.

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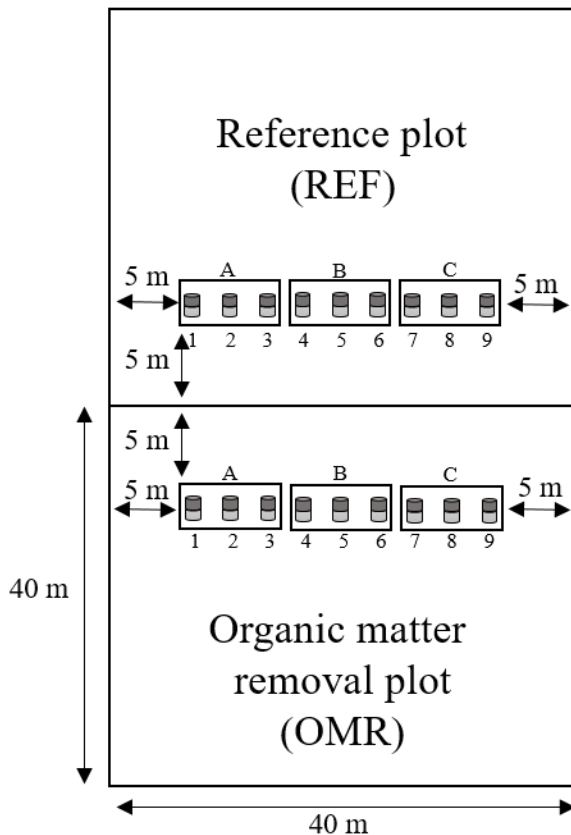
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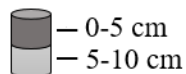
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665



Soil core



Individual soil core (n=216)

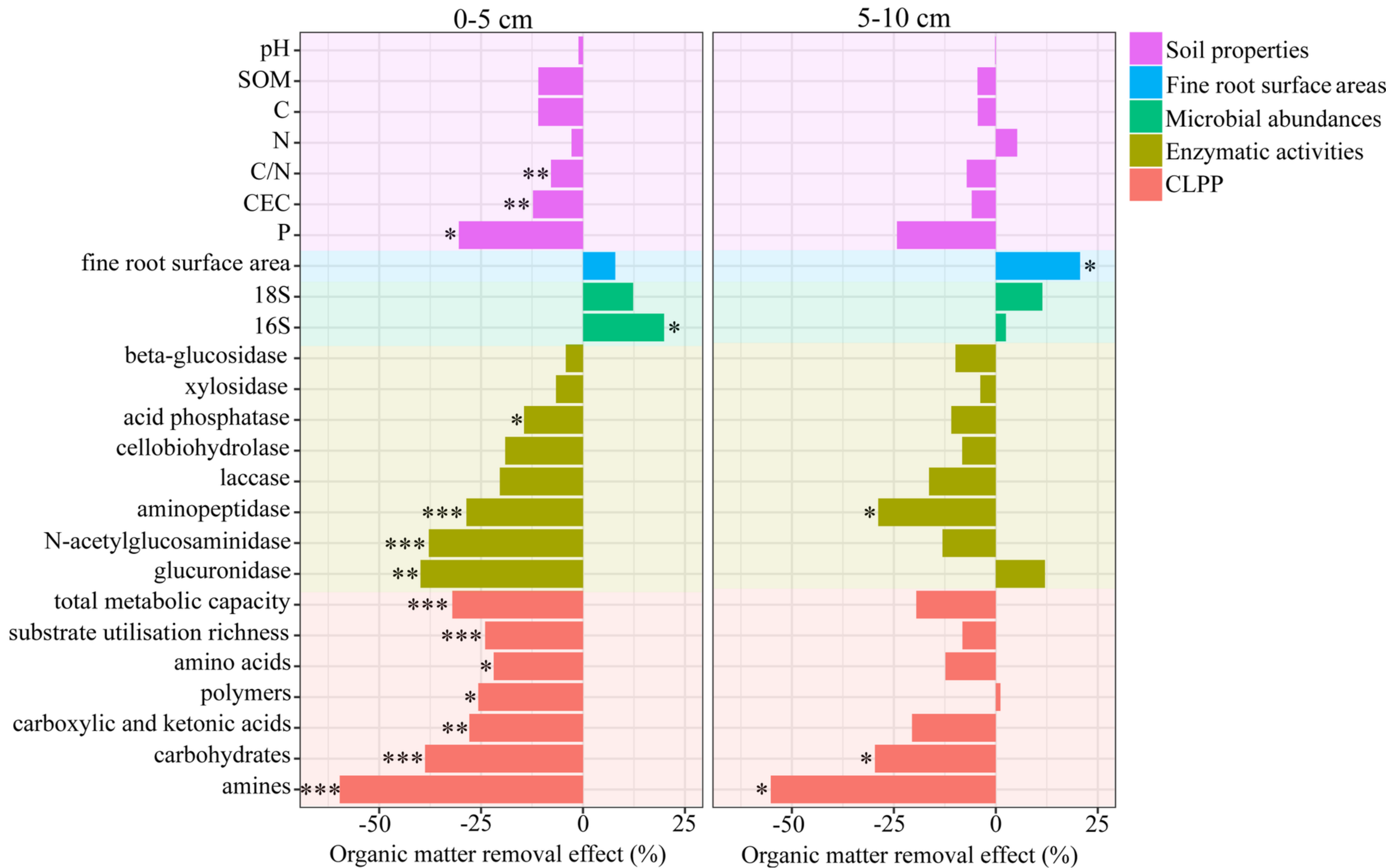


- Fine root surface areas
- Enzymatic activities
- Microbial abundance



Composite soil (n=72)

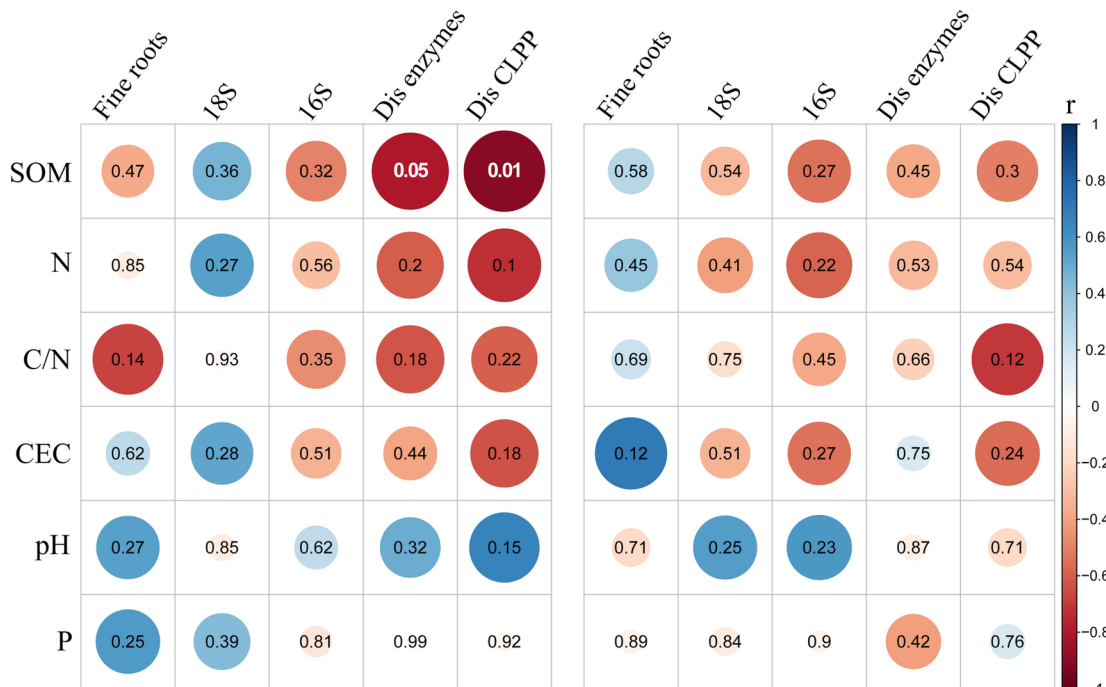
- Soil properties
- CLPP approach



A

0-5 cm

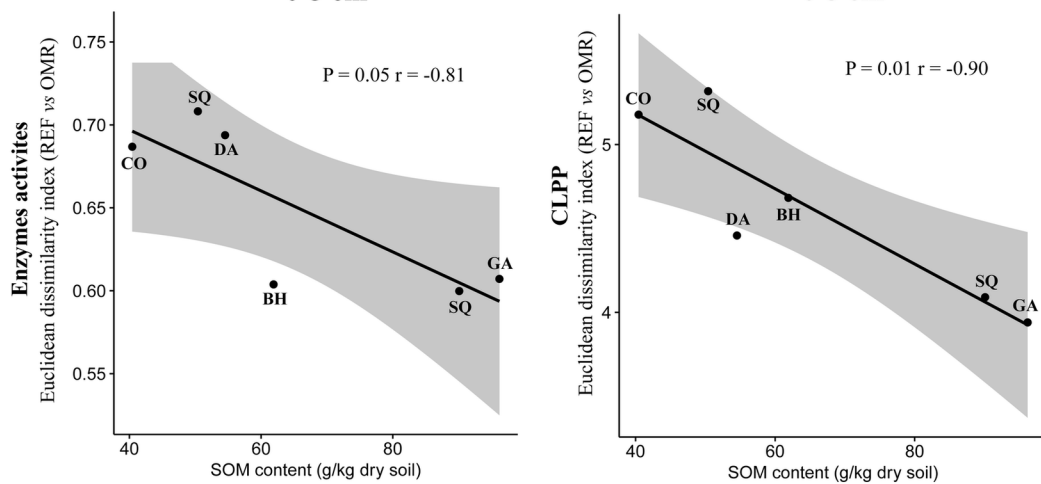
5-10 cm



B

0-5 cm

0-5 cm



Forest	Code	Species	First manipulation	Sampling date	Years	Coordinate	GPS Lambert93	Elevation (m)	Humus type	Soil texture	Soil types (WRB 2014)
						dirN	dirE				
Verrière du Grosbois	VE	Beech	16/05/14	Sept. 2016	55	6683070.9	948588.4	585	Mesomull/oligomull	Silty clay loam	Dystic Luvic Cambisols
Gaillefontaine	GA	Beech	17/10/14	Sept. 2016	40	6952055.8	602895.7	290	Eumull/mesomull	Silty-loam	Eutric Cambisols
Darney	DA	Beech	25/01/14	Sept. 2016	35	6779652.2	936520.6	500	Mesomull/oligomull	Sandy clay loam	Dystic Cambisols
Compiègne	CO	Beech	25/04/14	Sept. 2016	30	6914427.8	695219.1	185	Mesomull	Sandy-loam	Stagnic Luvisols
Ban d'Harol	BA	Beech	20/06/14	Sept. 2016	35	6783156.6	938890.7	350	Mesomull	Loam	Stagnic Dystic Cambisols
Saint-Quirin	SQ	Beech	27/09/14	Sept. 2016	35	6842790.239	996528.371	400	Mesomull/dysmull	Sandy-loam	Dystic Cambisols

Table 1.

	N	C/N	C	CEC	SOM	pH	P
Site	61.729 ***	29.947 ***	39.239 ***	29.874 ***	39.221 ***	23.096 ***	15.427 ***
Treatment	0.003	9.875 **	3.165	11.964 **	3.154	0.630	6.110 *
Depth	227.509 ***	4.350 *	70.586 ***	41.123 ***	70.730 ***	1.359	4.897 *
Site * treatment	3.668 **	3.852 **	0.884	0.619	0.882	2.603 *	1.421
Site * depth	12.699 ***	3.114 *	5.640 ***	2.178	5.627 ***	2.155	0.506
Treatment * depth	1.308	0.007	1.113	2.339	1.096	0.359	0.450
Site * depth * treatment	0.965	1.184	1.044	0.400	1.051	0.140	0.145

Table 2.

	18S (number of copies)	16S (number of copies)	Fine roots surfaces (projected area per soil core)
Site	15.689 ***	8.511 ***	2.560 *
Treatment	1.248	4.521 *	3.203
Depth	20.574 ***	75.774 ***	50.179 ***
Site * treatment	1.614	9.022 ***	1.063
Site * depth	1.940 *	5.591 ***	0.959
Treatment* depth	0.819	3.188	0.140
Site * depth * treatment	0.979	5.249 ***	1.208

Table 3.

	Cellobiohydrolase	Beta-glucosidase	Xylosidase	Glucuronidase	Aminopeptidase	N-acetyl- glucosaminidase	Acid phosphatase	Laccase
Site	5.516 ***	28.568 ***	10.887 ***	3.639 **	16.427 ***	1.474	20.578 ***	26.825 ***
Treatment	1.754	0.841	0.812	5.002 *	15.023 ***	11.121 **	8.654 **	3.624
Depth	37.850 ***	74.828 ***	46.645 ***	21.159 ***	15.228 ***	37.531 ***	124.429 ***	13.227 ***
Site * treatment	2.153	3.209 **	3.923**	2.006	1.811	2.609 *	1.840	2.077
Site * depth	1.568	1.214	1.463	2.297 *	0.710	1.045	3.092 *	0.277
Treatment *depth	0.872	0.013	0.176	8.318 **	0.406	6.022 *	1.387	0.351
Site * depth * treatment	1.170	0.253	0.222	1.329	0.645	0.939	1.506	0.160

Table 4.

	Amines (OD 590)	Amino acids (OD 590)	Carboxylic acids (OD 590)	Carbohydrates (OD 590)	Polymers (OD 590)	Total metabolic capacity (OD 590)	Substrate utilisation richness (n° of substrates metabolised)
Site	1.701	1.301	5.616 ***	8.985 ***	0.638	6.908 ***	5.661 ***
Treatment	18.548 ***	4.975 *	9.806 **	35.138 ***	1.742	27.127 ***	18.511 ***
Depth	14.639 ***	16.475 ***	62.083 ***	99.180 ***	1.598	84.133 ***	62.694 ***
Site * treatment	1.005	0.203	0.396	0.242	0.503	0.376	1.112
Site * depth	0.510	0.610	0.128	1.579	0.160	0.562	0.602
Treatment * depth	3.824	1.039	2.515	8.757 ***	1.997	7.430 **	7.750 **
Site * depth * treatment	0.775	0.233	1.251	2.235 *	0.396	1.354	0.472

Table 5.