



HAL
open science

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

Francois Maillard, Valentin Leduc, Cyrille Bach, Arnaud Reichard, Laure Fauchery, Laurent Saint-André, Bernhard Zeller, Marc Buée

► To cite this version:

Francois Maillard, Valentin Leduc, Cyrille Bach, Arnaud Reichard, Laure Fauchery, et al.. Soil microbial functions are affected by organic matter removal in temperate deciduous forest. *Soil Biology and Biochemistry*, 2019, 133, pp.28-36. 10.1016/j.soilbio.2019.02.015 . hal-02912755

HAL Id: hal-02912755

<https://hal.univ-lorraine.fr/hal-02912755v1>

Submitted on 6 Aug 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial - NoDerivatives 4.0 International License

1 Soil microbial functions are affected by organic matter removal in temperate deciduous forest
2 François Maillard¹, Valentin Leduc¹, Cyrille Bach¹, Arnaud Reichard², Laure Fauchery¹, Laurent
3 Saint-André², Bernhard Zeller² and Marc Buée¹.

4 ¹ Université de Lorraine, Inra, IAM, F-54000 Nancy, France

5 ² INRA, UR 1138 BEF, F-54280 Champenoux, France

6 Corresponding author's E-mail addresses:

7 Marc Buée, marc.buee@inra.fr

8 François Maillard, francois.maillard2@gmail.com

9

10 **Abstract**

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

28

29

30 **Keywords**

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

32

33 **Highlights**

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

39

40 **INTRODUCTION**

41 Forests are major components of the carbon (C) cycle on Earth, sequestering large quantities of C (

42 Lal, 2005; Pan et al., 2011; Bar-On et al., 2018). Consequently, managing forest C is a key challenge

43 for mitigating climate change (Bonan, 2008; Canadell and Raupach, 2008). One of the proposed

44 strategies to mitigate climate change consists of using more renewable energy sources in place of oil

45 and coal. However, old-growth forests are defined as important C sinks, and this C, even soil C, could

46 return to the atmosphere if these forests are disturbed (Luyssaert et al., 2008; Bellassen and Luyssaert,

47 2014). Furthermore, microorganisms participate in the dynamics of C and nutrient cycles in forests

48 through processes that breakdown plant OM in a form that trees can assimilate (van der Heijden et al.,

49 2008; Baskaran et al., 2017; Kyaschenko et al., 2017). Although this remains controversial in some

50 countries, the application of forest management guidelines toward more intensive management

51 practices is progressively observed (Achat et al., 2015a). Indeed, there is a demand to maximise post-

52 harvest residue removal from forests and to divert this previously unused fresh plant organic matter

53 (OM) into biofuel and energy production. This intensification of forest management practices (e.g.

54 shorter rotation, stronger thinning, higher forest residue harvesting) may have negative impacts on soil

55 properties and tree productivity as suggested by two recent meta-analyses (Achat et al., 2015a, 2015b).

56 However, our understanding of how intensive forestry impacts soil microbial diversity and functions

57 remains incomplete.

58 The effects of logging operations on soil microbial communities were mainly investigated in two

59 different experimental networks in temperate and boreal forests. The Long-Term Soil Productivity

60 (LTSP) study was established in 1989 to analyse the effects of OM manipulation in a network of

61 coniferous forests (Powers et al., 2005). Investigations of these LTSP sites demonstrated that bacterial

62 and fungal community compositions changed in response to OM manipulation (Hartmann et al., 2009;

63 Hartmann et al., 2012; Wilhelm et al., 2017). Additionally, Cardenas et al. (2015) used a metagenomic

64 approach to reveal a decrease in the OM degradation gene potential in response to OM manipulation.

65 The Detritus Input and Removal Treatments (DIRT) is an experimental network including eight sites

66 in temperate deciduous forests (Nadelhoffer et al., 2004; Lajtha et al., 2005;). Kotroczo et al. (2014)

67 and Veres et al. (2015) reported that the soil enzymes involved in organic C, N and P decreased in

68 response to OM removal. The Kotroczó et al. (2014) and Veres et al. (2015) studies were focused at
69 one site of the DIRT network. Other studies conducted independently of these two experimental
70 networks highlighted the impact of OM removal on soil microbial functions (Hasset and Zak, 2005;
71 Busse et al., 2006; Tan et al., 2008) and were also focused at one or two sites. Moreover, Achat et al.
72 (2015b) indicated that long term impact of intensive forestry remains poorly examined and notably on
73 soil microbial compartment.

74

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

86

87 **MATERIALS AND METHODS**

88 **Study site, experimental design and soil sampling**

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

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

109

110 **Soil properties and the fine root surface area**

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

120

121 **Bacterial and fungal abundance**

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

133

134 **Enzyme activities**

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

139 weighed and added to 40 ml of acetate buffer at pH 5.0 (methylumbelliferone fluorochrome and
140 laccase assay) and pH 8.0 (methylcoumarine fluorochrome) to give a final concentration of 1:20 w/v.
141 Diluted solutions were incubated at 4°C for one night under agitation. We applied the incubation times
142 and substrate concentrations defined by Pritsch et al. (2011). For the laccase test, a solution of 2 mM
143 2,9-azinobis-3-ethylbenzothiazoline-6-sulfonate (ABTS) was prepared in sodium acetate buffer at pH
144 5. In each well of a clear flat-bottom 96-well plate (Sarstedt, Newton, NC, USA), 200 µl of the diluted
145 soil solution was added to 50 µl of 2 mM ABTS solution. After incubation at 25°C the plate was
146 centrifuged at 4,500 g for 5 minutes, and 100 µl of the clear solution was transferred into a new, clear
147 flat-bottom 96-well plate, and measurements were performed at 415 nm (Bio-Rad model 550). For the
148 microplate assays with the methylumbelliferone or methylcoumarine fluorochromes, in each well of a
149 clear flat-bottom 96-well plate (Sarstedt, Newton, NC, USA), 200 µl of the diluted soil solution was
150 added to 50 µl of the substrate solutions. After incubation at 25°C, the plate was centrifuged at 4,500 g
151 for 5 minutes, and 50 µl of the clear solution was transferred into a black opaque 96-well microplate
152 (Perkin-Elmer, Life Sciences) containing 50 µl of stopping buffer (Tris 2.5 M pH 10–11) in each well.
153 Measurements were carried out using a Victor3 microplate reader (Wallac Perkin-Elmer Life Sciences,
154 Villebon-sur-Yvette, France) with an excitation wavelength of 360 nm and an emission wavelength of
155 450 nm. Control measurements were made for each enzyme assay. Enzymatic activity (EA) was
156 expressed in pmol of substrates released per g of dry soil per min. All of the chemicals were purchased
157 from Sigma-Aldrich (France).

158

159 **CLPP approach**

160 We used the Biolog® Ecoplate as the second approach to explore microbial functions. For this
161 experiment, 5 g of composite soils (n=72 soil samples) was diluted in 45 ml of sterile distilled water;
162 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
163 inoculate the Biolog® Ecoplate microplate. Then the plates were incubated at 25°C, and colour
164 development was measured at 590 nm (Bio-Rad model 550) after 72 h of incubation. Metabolism of
165 the different substrates in the wells resulted in the reduction of tetrazolium, which changed from
166 colourless to purple formazan. The OD 590 nm of the reference well was subtracted from the other
167 wells containing carbon sources. These corrected absorbances were used for the subsequent analysis.
168 The substrates were divided into five classes: carbohydrates, carboxylic and acetic acids, amines,
169 amino acids and polymers as defined by Fraç et al. (2012). We also calculated the substrate utilisation
170 richness (Garland, 1997), which represents the number of carbon substrates metabolised (corrected
171 absorbance values > 0.25).

172

173 **Data analysis and statistics**

174 Statistical analyses and data representations were performed using R software (R Core Team 2016).
175 Three-way analyses of variance (ANOVA) were used to test the following effects: site, depth and
176 treatment impacts on soil properties, root surface, bacterial and fungal abundance, enzyme activities
177 and CLPP data. Then, we used a mixed linear model with the lme4 package (Bates et al., 2015) with
178 site as a random factor to evaluate the impact of the treatment on soil properties, root surface, bacterial
179 and fungal abundance, enzyme activities and CLPP data in the function of the two studied soil layers
180 (0-5 and 5-10 cm). From the Vegan package (Oksanen et al., 2013), we calculated Euclidean
181 dissimilarity indices between the enzymatic activities (n=8 enzymes), and the CLPP assay (n=31
182 substrates) measured in the OMR and REF treatments within each site for both soil layers (0-5 and 5-
183 10 cm). The Corrplot package (Taiyun Wei and Viliam Simko., 2017) was used to identify and
184 represent the Pearson correlation between the soil properties of the REF plots and the impact of OM
185 removal on the root surface (a log 2-fold change OMR/REF), bacterial and fungal abundance (a log 2-
186 fold change OMR/REF), enzyme activities (the Euclidean dissimilarity index between OMR and REF)
187 and CLPP results of the Euclidean dissimilarity between OMR and REF). The levels of significance
188 obtained are indicated in each figure and table legend.

189

190 **RESULTS**

191 **Plant organic matter removal effect on soil properties**

192 The total soil N, soil organic C, soil organic matter (SOM) and pH were not impacted by OM removal
193 (Table 2). In contrast, OM removal significantly affected the other soil parameters measured in the 0-5
194 cm soil layer (Figure 2), leading to an average decrease of available P concentration (-30%), CEC (-
195 12%) and C/N (-8%) in the OMR plots compared with the REF treatments. Significant responses of
196 these soil properties to OM manipulation were restricted to the top soil layer (0-5 cm). Decreases in
197 CEC and C/N were observed for the six studied sites, while P decreases occurred in the following
198 sites: Gaillefontaine, Saint-Quirin and Verrières-du-Grosbois (Supplementary Figure 1).

199

200 **Plant organic matter removal effect on bacterial and fungal abundances and fine root surface** 201 **areas**

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

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

209

210 **Organic matter removal effects on enzyme activities**

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

217

218 **Organic matter removal effects on microbial metabolic profiles**

219 OM manipulation caused substantial shifts in the soil microbial metabolic profiles. The CLPP
220 approach revealed that amines, amino acids, carboxylic acids and carbohydrates substrates were
221 affected by OM removal (Table 5). OM removal also impacted the total metabolic capacities and
222 substrate metabolic richness of the microbial community. OM manipulation induced a significant
223 decrease in amine (-62%), amino acid (-22%), carboxylic and ketonic acid (-28%), carbohydrate (-
224 39%) and polymer (-25%) metabolic capacities in the topsoil layer (0-5 cm). The carbohydrate and
225 amine substrates classes also significantly decreased in the 5-10-cm soil layer (-30% and -55%). The
226 total metabolic capacities and substrate metabolic richness significantly decreased by 32% and 24%,
227 respectively, in the 0-5 cm soil layer.

228

229 **Correlation between soil properties and tree and microbial factors**

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

239 between the REF and OMR plots for each studied site were also significantly negatively ($P \leq 0.05$, $r = -$
240 0.90) correlated with the SOM content of the REF plots. No other significant correlation was
241 highlighted.

242

243 **DISCUSSION**

244 After three years of experimental OM removal, no significant changes in the soil C pool were
245 measured. It is however worth noting the slight trend of a C decrease, especially for the Gaillefontaine
246 and Ban Harol sites in the topsoil layer. These findings are close to those obtained in some other forest
247 sites (Johnson and Curtis, 2001; Barthès et al. 2008). Indeed, it has been shown that experimentally or
248 due to intensive forestry, OM removal does not strongly impact soil organic C pool (Nadelhoffer et al.,
249 2004; Sayer, 2006; Fekete et al., 2011; Lajtha et al., 2014). Although Slesak et al. (2017) reported a
250 overall decrease in SOM twenty years after forest floor removal on sites belonging to the LTSP
251 network, their results demonstrated that the effect of this OM removal on soil properties was site-
252 specific. This result is in agreement with our observations, highlighting that the impact of OM removal
253 on the C cycle could strongly depend on the site local parameters, particularly C reservoir. Otherwise,
254 we did not measure the effect of OM removal on the N pools. Changes in the N soil pools in response
255 to OM removal commonly follow a similar tendency to those observed for soil C pools (Lajtha et al.,
256 2014; Slesak et al., 2017). A slight but significant decrease in the CEC in response to OM removal
257 was measured in the topsoil layer. The impact of OM manipulation on the soil P content was reported
258 in other OM manipulation experiments (Wilhelm et al., 2013; Vadeboncoeur et al., 2014; Slesak et al.,
259 2017). Due to the low level of P pools in forest soil, the decrease in available P concentration at all six
260 sites may suggest future soil P limitation, which is a serious concern for tree productivity (Goswami et
261 al., 2018).

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

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

284

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

295

296 After three years of OM removal, enzyme activities involved in the mineralization of cellulose and
297 hemicellulose (i.e., beta-glucosidase, cellobiohydrolase, and xylosidase) were not significantly
298 impacted by the treatment. Our results are not consistent with the observations made by Kotroczo et al.
299 (2014) and Veres et al (2015), who report a decrease of beta-glucosidase activity in response to leaf
300 litter removal in temperate forests. This difference may be explained by the fact that they measured
301 OM removal effects after 6 and 10 years whereas our sampling occurred just after 3 years of OM
302 removal. Using an isotope labelling approach, Leung et al. (2016) revealed that OM removal has a
303 small impact on the hemicellulolytic degradation capacity of the microbial community. However, we
304 hypothesize that the enzymes involved in plant cell wall degradation and C mobilisation similarly will
305 decrease at our experimental sites in the coming years. The enzymes involved in P (acid phosphatase)
306 and N (leucine aminopeptidase and N-acetylglucosaminidase) mobilisation were negatively impacted
307 by OM removal in the topsoil layer and in the subsoil layer for N mobilisation. In our experiment,
308 decreased acid phosphatase activity was correlated with decreased available P content in the soil. In
309 contrast, this link was not confirmed for the enzymes involved in N mobilisation. Indeed, soil N
310 concentration had not yet decreased in response to OM removal, but the enzymes involved in N
311 degradation had sharply decreased. Here, the total N does not allow estimates of the labile N pool that

312 could regulate specific organic N mobilisation enzymes, such as chitinases or proteases. Similar to our
313 results, many studies reported the sensitivity of N and P microbial mobilisation capacities in response
314 to OM removal using mineralisation and enzymatic approaches (Perez-Batallon et al., 2001; Hassett
315 and Zak, 2005; Tan et al., 2008; Smolander et al., 2010). These variations in the activities of the
316 enzymes that degrade SOM could be linked to shifts in decomposition rates, indicating a reduction in
317 the decomposition process in response to OM removal. Moreover, plant litter is rich in secondary
318 compounds as tannins, which would contribute to microbial enzyme inhibition, as an important
319 mechanism controlling some forest ecosystem processes (Harrison, 1971; Kraus et al., 2003; Joannis
320 et al., 2007). Consequently, litter exportation could directly change tannins availability, and microbial
321 enzyme regulation. In addition, tannins and phenolics in fresh and decomposed beech litter are much
322 more concentrated than other tree species, such as oak, Scots pine and black cherry (Lorenz et al.,
323 2004). Indeed, Adamczyk et al. (2017) showed that tannin-enzyme interaction led to decreases but also
324 increases of enzymatic activity due to protein conformation changes or tannin-enzyme precipitates.
325 Variations in the microbial composition and their associated functions in response to OM removal may
326 be a further explanation (Hartmann et al., 2012; Hartmann et al., 2009). Here, we confirmed that soil
327 enzyme activities are more sensitive indicators of changes in response to OM removal than soil
328 parameters, as observed by Caldwell and Gri (1999) and Tan et al. (2008). The availability of soil
329 nutrients depends on the microbial enzyme activities that provide balanced resources for both
330 microorganisms and the trees. In our study, the relative increase in microbial abundance coupled with
331 the decrease in N and P mobilisation enzymes in response to OM removal could modify the balance of
332 these elements between microbes and trees, and increase the nutritional stress of trees in the OM
333 removal plots.

334
335 An analysis of the Biolog® data only for the carbon source utilisation pattern demonstrated that OM
336 removal had a significant impact on the microbial functional diversity and overall metabolic activity.
337 Similar to the enzyme activities, these changes are mainly marked by a decrease in metabolic capacity
338 and metabolic richness in response to OM removal in the topsoil layer. Nevertheless, in contrast to the
339 enzymatic approach, the CLPP results in response to OM manipulation are scarce in the literature. In a
340 critical review, Preston-Mafham et al. (2002) explained that the reduction of substrate degradation due
341 to Biolog® Ecoplate could result from the decreased bacterial inoculum size. Using qPCR, we did not
342 observe a decrease in the bacterial abundance and thus rejected this hypothesis. Because nutrient-poor
343 forest soils are mainly dominated by slow-growing oligotrophic bacteria (Jones et al., 2009; Jeanbille
344 et al., 2016; Žifčáková et al., 2016), we suggest that the removal of OM could increase the proportion
345 of these oligotrophic bacteria in the manipulated plots, thus decreasing the relative abundance of
346 copiotrophic bacteria. As the CLPP approach based on Biolog® Ecoplate is mainly driven by
347 copiotrophic bacteria (Lladó and Baldrian, 2017), we propose that the differences in microbial

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

350

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

364

365 **CONCLUSION**

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

378

379

380 **Table**

381 TABLE 1 Description of organic matter manipulation experimental sites.

382

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

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

387

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

392

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

396

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

400

401 **Figure captions**

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

404

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

411

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

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

424

425 **Supplementary data**

426

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

430

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

433

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

437

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

440

441 **Acknowledgements**

442 We thank two anonymous reviewers for providing helpful comments to improve our manuscript. FM
443 holds a PhD fellowship awarded by the Région Lorraine and the Laboratory of Excellence ARBRE
444 (BRIDGE project). This work was supported by a grant overseen by the French National Research
445 Agency (ANR) as part of the ‘Investissements d’Avenir’ program (ANR-11-LABX-0002-01, Lab of
446 Excellence ARBRE). The OM removal experiment was supported by the mobile lab (M-
447 POETE) of ANAEE-France. ANAEE-France is an infrastructure of the French Investment for
448 the Future (*‘Investissements d’Avenir’*) program, overseen by the French National Research
449 Agency (ANR) (ANR-11-INBS-0001). The OM removal treatment was conducted in
450 collaboration with the UEAJ Nancy, UEAJ Besançon, UEAJ Montataire and colleagues from
451 the ECODIV laboratory of the University of Rouen.

452 **Conflict of interest**

453 We state that there is no conflict of interest.

454

455 **References**

- 456 Achat, D. L., Fortin, M., Landmann, G., Ringeval, B., and Augusto, L. (2015). Forest soil carbon is
457 threatened by intensive biomass harvesting. *Scientific Reports*, 5, 15991.
- 458 Achat, D.L., Deleuze, C., Landmann, G., Pousse, N., Ranger, J., and Augusto, L. (2015b). Quantifying
459 consequences of removing harvesting residues on forest soils and tree growth – A meta-analysis. *For.*
460 *Ecol. Manag.* 348, 124–141.
- 461 Adamczyk, B., Karonen, M., Adamczyk, S., Engström, M. T., Laakso, T., Saranpää, P., Kitunena, V.,
462 Aino, Smolander A., and Simon, J. (2017). Tannins can slow-down but also speed-up soil enzymatic
463 activity in boreal forest. *Soil Biology and Biochemistry*, 107, 60-67.
- 464 Akroume, E., Zeller, B., Buée, M., Santenoise, P., and Saint-André, L. (2016). Improving the design
465 of long-term monitoring experiments in forests: a new method for the assessment of local soil
466 variability by combining infrared spectroscopy and dendrometric data. *Ann. For. Sci.* 73, 1005–1013.
- 467 Bar-On, Y.M., Phillips, R., and Milo, R. (2018). The biomass distribution on Earth. *Proc. Natl. Acad.*
468 *Sci.* 201711842.
- 469 Barthès, B. G., Brunet, D., Hien, E., Enjalric, F., Conche, S., Freschet, G. T., et al. (2008).
470 Determining the distributions of soil carbon and nitrogen in particle size fractions using near-infrared
471 reflectance spectrum of bulk soil samples. *Soil Biology and Biochemistry*, 40: 1533-1537.
- 472 Baskaran, P., Hyvönen, R., Berglund, S.L., Clemmensen, K.E., Ågren, G.I., Lindahl, B.D., and
473 Manzoni, S. (2017). Modelling the influence of ectomycorrhizal decomposition on plant nutrition and
474 soil carbon sequestration in boreal forest ecosystems. *New Phytol.* 213, 1452–1465.
- 475 Bates, D., Maechler, M., Bolker B., and Walker, S. (2015). Fitting linear mixed-effects models using
476 lme4. *Journal of Statistical Software*, 67(1), 1-48.
- 477 Bellassen, V., and Luysaert, S. (2014). Carbon sequestration: Managing forests in uncertain times.
478 *Nature* 506, 153–155.
- 479 Boerner, R.E.J., Brinkman, J.A., and Smith, A. (2005). Seasonal variations in enzyme activity and
480 organic carbon in soil of a burned and unburned hardwood forest. *Soil Biol. Biochem.* 37, 1419–1426.
- 481 Bonan, G.B. (2008). Forests and Climate Change: Forcings, feedbacks, and the climate benefits of
482 forests. *Science* 320, 1444–1449.

483 Buée, M., Courty, P.E., Mignot, D., and Garbaye, J. (2007). Soil niche effect on species diversity and
484 catabolic activities in an ectomycorrhizal fungal community. *Soil Biol. Biochem.* 39, 1947–1955.

485 Buée, M., De Boer, W., Martin, F., van Overbeek, L., and Jurkevitch, E. (2009). The rhizosphere zoo:
486 An overview of plant-associated communities of microorganisms, including phages, bacteria, archaea,
487 and fungi, and of some of their structuring factors. *Plant Soil* 321, 189–212.

488 Busse, M.D., Beattie, S.E., Powers, R.F., Sanchez, F.G., and Tiarks, A.E. (2006). Microbial
489 community responses in forest mineral soil to compaction, organic matter removal, and vegetation
490 control. *Can. J. For. Res.* 36, 577–588.

491 Caldwell, B.A., Griffiths, R.P., Sollins, P., 1999. Soil enzyme response to vegetation disturbance in
492 two lowland Costa Rican soils. *Soil Biology and Biochemistry* 31, 1603–1608.

493
494 Canadell, J.G., and Raupach, M.R. (2008). Managing forests for climate change mitigation. *Science*
495 320, 1456–1457.

496 Cardenas, E., Kranabetter, J.M., Hope, G., Maas, K.R., Hallam, S., and Mohn, W.W. (2015). Forest
497 harvesting reduces the soil metagenomic potential for biomass decomposition. *ISME J.* 9, 2465–2476.

498 Cébron, A., Norini, M.-P., Beguiristain, T., and Leyval, C. (2008). Real-Time PCR quantification of
499 PAH-ring hydroxylating dioxygenase (PAH-RHD α) genes from Gram positive and Gram negative
500 bacteria in soil and sediment samples. *J. Microbiol. Methods* 73, 148–159.

501 Chemidlin Prévost-Bouré, N., Christen, R., Dequiedt, S., Mougél, C., Lelièvre, M., Jolivet, C.,
502 Shahbazkia, H.R., Guillou, L., Arrouays, D., and Ranjard, L. (2011). Validation and application of a
503 PCR primer set to quantify fungal communities in the soil environment by real-time quantitative PCR.
504 *PLoS ONE* 6, e24166.

505 Da-Lun, Tian., Yuan-Ying, Peng., Wen-De, Yan., Xi, Fang., Wen-Xing, Kang., Guang-Jun, Wang.,
506 and Xiao-Yong, Chen. (2010). Effects of thinning and litter fall removal on fine root production and
507 soil organic carbon content in Masson pine plantations. *Pedosphere*, 20: 486-493.

508 Dijkstra, F.A., Carrillo, Y., Pendall, E., and Morgan, J.A. (2013). Rhizosphere priming: a nutrient
509 perspective. *Front. Microbiol.* 4:216.

510 Duchaufour P, Bonneau M (1959) Une méthode nouvelle de dosage du phosphore assimilable dans les
511 sols forestiers. *Bul AFES*4:193–198.

512 Egnell, G., and Leijon, B. (1999). Survival and growth of planted seedlings of *Pinus sylvestris* and
513 *Picea abies* after different levels of biomass removal in clear-felling. *Scand. J. For. Res.* 14, 303–311.

514 Fahey, T.J., and Hughes, J.W. (1994). Fine root dynamics in a northern hardwood forest ecosystem,
515 Hubbard Brook Experimental Forest, NH. *J. Ecol.* 82, 533.

516 Fekete, I., Kotroczo, Z., Varga, C., Veres, Z., and Toth, J.A. (2011). The effects of detritus input on
517 soil organic matter content and carbon dioxide emission in a central European deciduous forest.
518 *Geoderma* 167-168, 15–21.

519 Feng, W., Zou, X., and Schaefer, D. (2009). Above and belowground carbon inputs affect seasonal
520 variations of soil microbial biomass in a subtropical monsoon forest of southwest China. *Soil Biol.*
521 *Biochem.* 41, 978–983.

522 Fraç, M., Oszust, K., and Lipiec, J. (2012). Community Level Physiological Profiles (CLPP),
523 Characterization and microbial activity of soil amended with dairy sewage sludge. *Sensors* 12, 3253–
524 3268.

525 Garland, J.L. (1997). Analysis and interpretation of community-level physiological profiles in
526 microbial ecology. *FEMS Microbiol. Ecol.* 12.

527 Goswami, S., Fisk, M.C., Vadeboncoeur, M.A., Garrison-Johnston, M., Yanai, R.D., and Fahey, T.J.
528 (2018). Phosphorus limitation of aboveground production in northern hardwood forests. *Ecology* 99,
529 438–449.

530 Harrison, A. F. (1971). The inhibitory effect of oak leaf litter tannins on the growth of fungi, in
531 relation to litter decomposition. *Soil Biology and Biochemistry*, 3: 167-172.

532 Hartmann, M., Howes, C.G., VanInsberghe, D., Yu, H., Bachar, D., Christen, R., Nilsson, R.H.,
533 Hallam, S.J., and Mohn, W.W. (2012). Significant and persistent impact of timber harvesting on soil
534 microbial communities in Northern coniferous forests. *The ISME Journal*, 6, 2199-2218.

535 Hartmann, M., Lee, S., Hallam, S.J., and Mohn, W.W. (2009). Bacterial, archaeal and eukaryal
536 community structures throughout soil horizons of harvested and naturally disturbed forest stands.
537 *Environ. Microbiol.* 11, 3045–3062.

538 Hassett, J.E., and Zak, D.R. (2005). Aspen harvest intensity decreases microbial biomass, extracellular
539 enzyme activity, and soil nitrogen cycling. *Soil Sci. Soc. Am. J.* 69, 227.

540 van der Heijden, M.G.A., Bardgett, R.D., and van Straalen, N.M. (2008). The unseen majority: soil
541 microbes as drivers of plant diversity and productivity in terrestrial ecosystems. *Ecol. Lett.* 11, 296–
542 310.

543 Jeanbille, M., Buée, M., Bach, C., Cébron, A., Frey-Klett, P., Turpault, M.P., and Uroz, S. (2016). Soil
544 parameters drive the structure, diversity and metabolic potentials of the bacterial communities across
545 temperate beech forest soil sequences. *Microb. Ecol.* 71, 482–493.

546 Joannis, G. D., Bradley, R. L., Preston, C. M., and Munson, A. D. (2007). Soil enzyme inhibition by
547 condensed litter tannins may drive ecosystem structure and processes: the case of *Kalmia angustifolia*.
548 *New Phytologist*, 175: 535-546.

549 Jones, R.T., Robeson, M.S., Lauber, C.L., Hamady, M., Knight, R., and Fierer, N. (2009). A
550 comprehensive survey of soil acidobacterial diversity using pyrosequencing and clone library
551 analyses. *ISME J.* 3, 442–453.

552 Johnson, D. W., and Curtis, P. S. (2001). Effects of forest management on soil C and N storage: meta
553 analysis. *Forest Ecology and Management*, 140: 227-238.

554 Kaiser C, Kilburn MR, Clode PL, Fuchslueger L, Koranda M, Cliff JB, Solaiman ZM, Murphy D.
555 2015. Exploring the transfer of recent plant photosynthates to soil microbes: mycorrhizal pathway vs
556 direct root exudation. *New Phytologist* 205: 1537–1551.

557 Kang, H., Kang, S., and Lee, D. (2009). Variations of soil enzyme activities in a temperate forest soil.
558 *Ecol. Res.* 24, 1137–1143.

559 Kotroczó, Z., Veres, Z., Fekete, I., Krakomperger, Z., Tóth, J.A., Lajtha, K., and Tóthmérész, B.
560 (2014). Soil enzyme activity in response to long-term organic matter manipulation. *Soil Biol.*
561 *Biochem.* 70, 237–243.

562 Kraus, T. E., Dahlgren, R. A., Zasoski, R. J. (2003). Tannins in nutrient dynamics of forest
563 ecosystems-a review. *Plant and Soil*, 256: 41-66.

564 Kuzyakov, Y. (2010). Priming effects: Interactions between living and dead organic matter. *Soil Biol.*
565 *Biochem.* 42, 1363–1371.

566 Kvaschenko, J., Clemmensen, K.E., Karlton, E., and Lindahl, B.D. (2017). Below-ground organic
567 matter accumulation along a boreal forest fertility gradient relates to guild interaction within fungal
568 communities. *Ecol. Lett.* 20, 1546–1555.

569 Lajtha, K., Crow, S.E., Yano, Y., Kaushal, S.S., Sulzman, E., Sollins, P., and Spears, J.D.H. (2005).
570 Detrital controls on soil solution N and dissolved organic matter in soils: A field experiment.
571 *Biogeochemistry* 76, 261–281.

572 Lajtha, K., Bowden, R.D., and Nadelhoffer, K. (2014). Litter and root manipulations provide insights
573 into soil organic matter dynamics and stability. *Soil Sci. Soc. Am. J.* 78, S261.

574 Lal, R. (2005). Forest soils and carbon sequestration. *For. Ecol. Manag.* 220, 242–258.

575 Leff, J.W., Wieder, W.R., Taylor, P.G., Townsend, A.R., Nemergut, D.R., Grandy, A.S., and
576 Cleveland, C.C. (2012). Experimental litterfall manipulation drives large and rapid changes in soil
577 carbon cycling in a wet tropical forest. *Glob. Change Biol.* 18, 2969–2979.

578 Leung, H.T.C., Maas, K.R., Wilhelm, R.C., and Mohn, W.W. (2016). Long-term effects of timber
579 harvesting on hemicellulolytic microbial populations in coniferous forest soils. *ISME J.* 10, 363–375.

580 Leuschner, C. (1998). Water extraction by tree fine roots in the forest floor of a temperate *Fagus-*
581 *Quercus* forest. *Ann. Sci. For.* 55, 141–157.

582 Li, Q., Lee Allen, H., and Wollum, A.G. (2004). Microbial biomass and bacterial functional diversity
583 in forest soils: effects of organic matter removal, compaction, and vegetation control. *Soil Biol.*
584 *Biochem.* 36, 571–579.

585 Liu, Y., Wang, G., Yu, K., Li, P., Xiao, L., and Liu, G. (2018). A new method to optimize root order
586 classification based on the diameter interval of fine root. *Sci. Rep.* 8.

587 Lladó, S., and Baldrian, P. (2017). Community-level physiological profiling analyses show potential to
588 identify the copiotrophic bacteria present in soil environments. *PLOS ONE* 12, e0171638.

589 Lorenz, K., Preston, C. M., Krumrei, S., and Feger, K. H. (2004). Decomposition of needle/leaf litter
590 from Scots pine, black cherry, common oak and European beech at a conurbation forest site. *European*
591 *Journal of Forest Research*, 123: 177-188.

592 Luysaert, S., Schulze, E.-D., Börner, A., Knohl, A., Hessenmöller, D., Law, B.E., Ciais, P., and
593 Grace, J. (2008). Old-growth forests as global carbon sinks. *Nature* 455, 213–215.

594 Melany C. Fisk, and Timothy J. Fahey (2001). Microbial biomass and nitrogen cycling responses to
595 fertilization and litter removal in young northern hardwood forests. *Biogeochemistry* 53, 201–223.

596 Nadelhoffer, K.J., Boone, R.D., Bowden, R.D., Canary, J.D., Kaye, J., Micks, P., Ricca, A.,
597 Aitkenhead, J.A., Lajtha, K., McDowell, W.H., 2004. The DIRT experiment: litter and root influences
598 on forest soil organic matter stocks and function. In: Foster, D., Aber, J. (Eds.), *Forests in Time: The*
599 *Environmental Consequences of 1000 Years of Change in New England*. Yale University Press, New
600 Haven, pp. 300–315.

601 O’Hehir, J.F., and Nambiar, E.K.S. (2010). Productivity of three successive rotations of *P. radiata*
602 plantations in South Australia over a century. *For. Ecol. Manag.* 259, 1857–1869.

603 Oksanen J, Blanchet FG, Kindt R, Legendre P, Minchin PR, O’Hara RB, et al. (2013) vegan:
604 Community Ecology Package, version 2.0–7. R package. Available: [http://CRAN.R-](http://CRAN.R-project.org/package=vegan)
605 [project.org/package=vegan](http://CRAN.R-project.org/package=vegan).

606

607 Pan, Y., Birdsey, R.A., Fang, J., Houghton, R., Kauppi, P.E., Kurz, W.A., Phillips, O.L., Shvidenko,
608 A., Lewis, S.L., Canadell, J.G., et al. (2011). A large and persistent carbon sink in the world’s forests.
609 *Science* 333, 988–993.

610 Perez-Batallon, P., Ouro, G., Macias, F., Merino, A., 2001. Initial mineralization of organic matter in a
611 forest plantation soil following different logging residue management techniques. *Ann. For. Sci.* 58,
612 807–818.

613 Ponder, F., and Tadros, M. (2002). Phospholipid fatty acids in forest soil four years after organic
614 matter removal and soil compaction. *Appl. Soil Ecol.* 19, 173–182.

615 Powers, R.F., Andrew Scott, D., Sanchez, F.G., Voldseth, R.A., Page-Dumroese, D., Elioff, J.D., and
616 Stone, D.M. (2005). The North American long-term soil productivity experiment: Findings from the
617 first decade of research. *For. Ecol. Manag.* 220, 31–50.

618 Preston-Mafham, J., Boddy, L., and Randerson, P.F. (2002). Analysis of microbial community
619 functional diversity using sole-carbon-source utilisation profiles – a critique. *FEMS Microbiol. Ecol.*
620 42, 1–14.

621 Pritsch, K., Courty, P.E., Churin, J.-L., Cloutier-Hurteau, B., Ali, M.A., Damon, C., Duchemin, M.,
622 Egli, S., Ernst, J., Fraissinet-Tachet, L., et al. (2011). Optimized assay and storage conditions for
623 enzyme activity profiling of ectomycorrhizae. *Mycorrhiza* 21, 589–600.

624 R Core Team (2016). R: A language and environment for statistical computing. R Foundation for
625 Statistical Computing, Vienna, Austria. URL <http://www.R-project.org/>.

626

627 Sayer, E.J. (2006). Using experimental manipulation to assess the roles of leaf litter in the functioning
628 of forest ecosystems. *Biol. Rev.* 81, 1-31.

629 Slesak, R.A., Palik, B.J., D’Amato, A.W., and Kurth, V.J. (2017). Changes in soil physical and
630 chemical properties following organic matter removal and compaction: 20-year response of the aspen
631 Lake-States Long Term Soil Productivity installations. *For. Ecol. Manag.* 392, 68–77.

632 Smolander, A., Kitunen, V., Tamminen, P., and Kukkola, M. (2010). Removal of logging residue in
633 Norway spruce thinning stands: Long-term changes in organic layer properties. *Soil Biol. Biochem.*
634 42, 1222–1228.

635 Stone, D.M. 2001. Sustaining aspen productivity in the Lake States. In *Sustaining Aspen in Western*
636 *Landscapes: Symposium Proceedings, Grand Junction, Colo., 13–15 June 2000*. Edited by W.D.
637 Shepperd, D. Binkley, D.L. Bartos, T.J. Stohlgren, and L.G. Eskew. USDA For. Serv. Proc. RMRS-P-
638 118. pp. 47–59.

639 Taiyun Wei and Viliam Simko (2017). R package "corrplot": Visualization of a Correlation Matrix
640 (Version 0.84). Available from <https://github.com/taiyun/corrplot>.

641 Tan, X., Chang, S.X., and Kabzems, R. (2008). Soil compaction and forest floor removal reduced
642 microbial biomass and enzyme activities in a boreal aspen forest soil. *Biol. Fertil. Soils* 44, 471–479.

643 Vadeboncoeur, M.A., Hamburg, S.P., Yanai, R.D., and Blum, J.D. (2014). Rates of sustainable forest
644 harvest depend on rotation length and weathering of soil minerals. *For. Ecol. Manag.* 318, 194–205.

645 Veres, Z., Kotroczó, Z., Fekete, I., Tóth, J.A., Lajtha, K., Townsend, K., and Tóthmérész, B. (2015).
646 Soil extracellular enzyme activities are sensitive indicators of detrital inputs and carbon availability.
647 *Appl. Soil Ecol.* 92, 18–23.

648 Wang, M.-B., and Zhang, Q. (2009). Issues in using the WinRHIZO system to determine physical
649 characteristics of plant fine roots. *Acta Ecol. Sin.* 29, 136–138.

650 Wang, X., Tang, C., Severi, J., Butterly, C.R., and Baldock, J.A. (2016). Rhizosphere priming effect
651 on soil organic carbon decomposition under plant species differing in soil acidification and root
652 exudation. *New Phytol.* 211, 864–873.

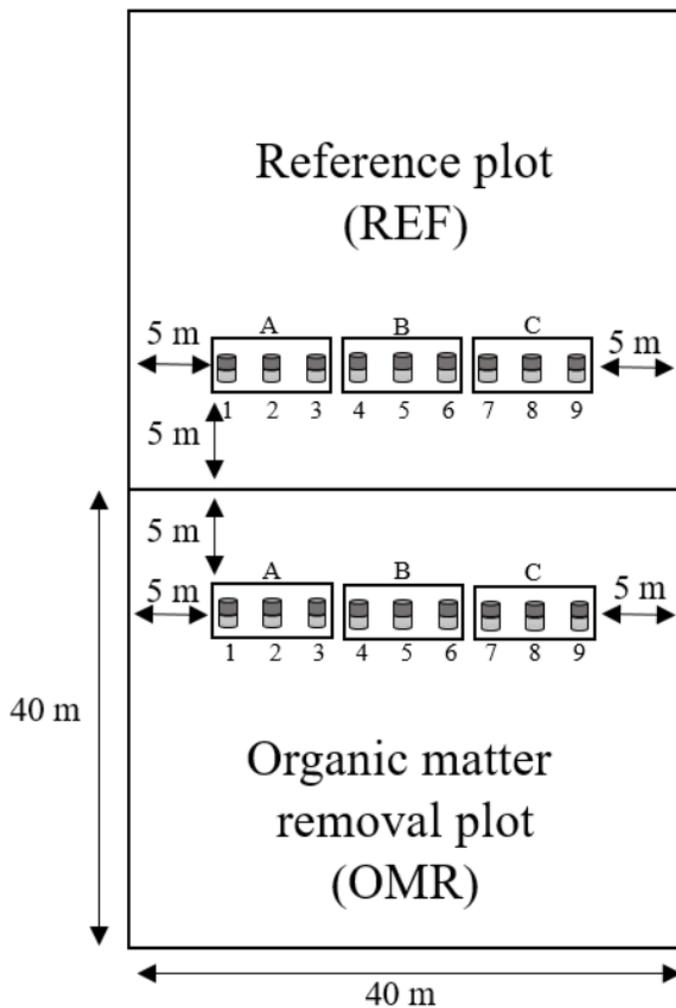
653 Wilhelm, K., Rathsack, B., and Bockheim, J. (2013). Effects of timber harvest intensity on
654 macronutrient cycling in oak-dominated stands on sandy soils of northwest Wisconsin. *For. Ecol.*
655 *Manag.* 291, 1–12.

656 Wilhelm, R.C., Cardenas, E., Maas, K.R., Leung, H., McNeil, L., Berch, S., Chapman, W., Hope, G.,
657 Kranabetter, J.M., Dubé, S., et al. (2017). Biogeography and organic matter removal shape long-term
658 effects of timber harvesting on forest soil microbial communities. *ISME J.* 11, 2552–2568.

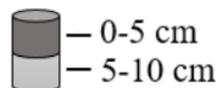
659 Zhang, B., Wang, H., Yao, S., Bi, L. (2013). Litter quantity confers soil functional resilience through
660 mediating soil biophysical habitat and microbial community structure on an eroded bare land restored
661 with mono *Pinus massoniana*. *Soil Biology and Biochemistry*, 57, 556–567.

662 Žifčáková, L., Větrovský, T., Howe, A., and Baldrian, P. (2016). Microbial activity in forest soil
663 reflects the changes in ecosystem properties between summer and winter: Seasonal dynamics of a soil
664 microbial community. *Environ. Microbiol.* 18, 288–301.

665



Soil core



Individual soil core (n=216)

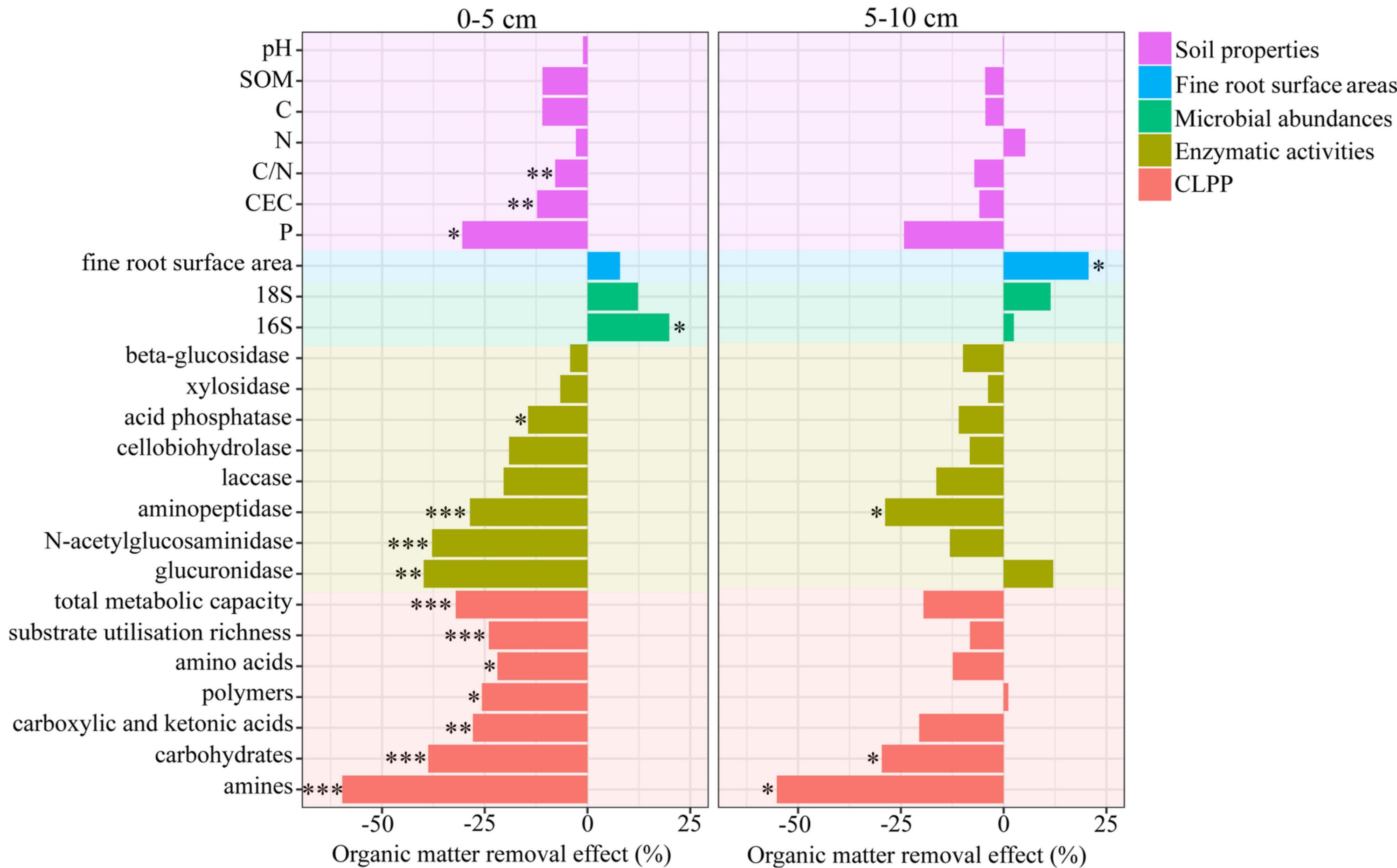


- Fine root surface areas
- Enzymatic activities
- Microbial abundance



Composite soil (n=72)

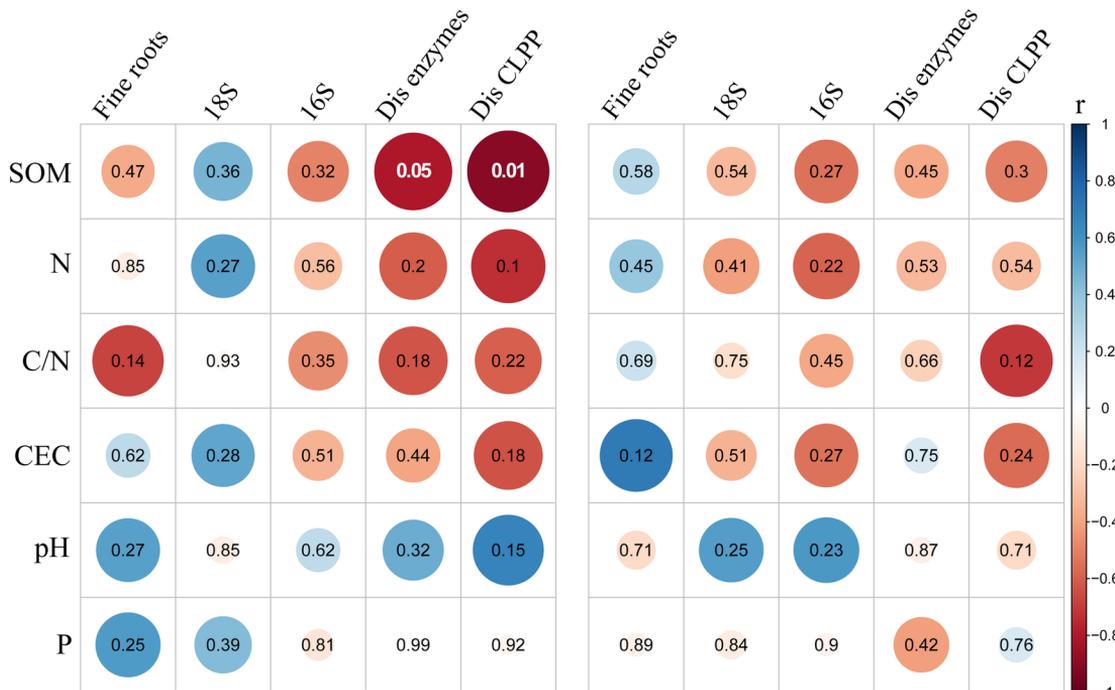
- Soil properties
- CLPP approach



A

0-5 cm

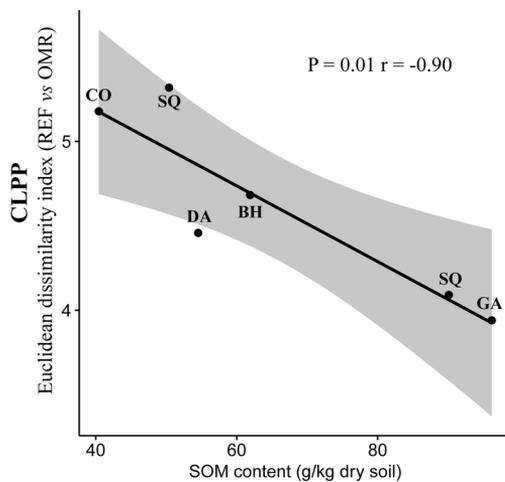
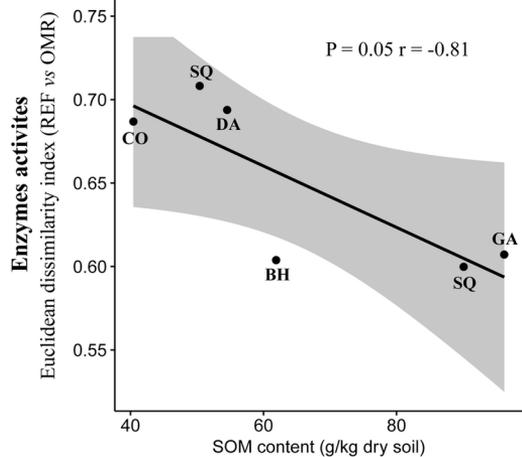
5-10 cm



B

0-5 cm

0-5 cm



Forest	Code	Species	First manipulation	Sampling date	Years	Coordinate dirN	GPS Lambert93 dirE	Elevation (m)	Humus type	Soil texture	Soil types (WRB 2014)
Verrière du Grosbois	VE	Beech	16/05/14	Sept. 2016	55	6683070.9	948588.4	585	Mesomull/oligomull	Silty clay loam	Dystric 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	Dystric 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 Dystric Cambisols
Saint-Quirin	SQ	Beech	27/09/14	Sept. 2016	35	6842790.239	996528.371	400	Mesomull/dysmull	Sandy-loam	Dystric 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.