



**HAL**  
open science

## Does forest stand density affect soil microbial communities?

Marine Fernandez, Gaëlle Vincent, Erica Dorr, Souleyman Bakker, Thomas Lerch, Julie Leloup, Nathalie Korboulewsky, Stéphane Bazot

### ► To cite this version:

Marine Fernandez, Gaëlle Vincent, Erica Dorr, Souleyman Bakker, Thomas Lerch, et al.. Does forest stand density affect soil microbial communities?. *Applied Soil Ecology*, 2024, 195, pp.105244. 10.1016/j.apsoil.2023.105244 . hal-04451951

**HAL Id: hal-04451951**

**<https://hal.inrae.fr/hal-04451951v1>**

Submitted on 12 Feb 2024

**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

# Does forest stand density affect soil microbial communities?

Marine Fernandez\*<sup>1,2</sup>, Gaëlle Vincent<sup>3</sup>, Erica Dorr<sup>3</sup>, Souleyman Bakker<sup>3</sup>, Thomas Z. Lerch<sup>4</sup>,  
Julie Leloup<sup>5</sup>, Nathalie Korboulewsky<sup>1</sup>, Stéphane Bazot<sup>3</sup>

1. Institut National de Recherche pour l'Agriculture, l'Alimentation et Environnement (INRAE), UR EFNO - Centre de recherche Val de Loire, 45290 Nogent-Sur-Vernisson, France

2. Département des sciences biologiques, Université du Québec à Montréal (UQAM), C.P. 8888, Succ. Centre-ville, Montréal, Québec, Canada, H3C 3P8

3. Ecologie Systematique Evolution, University of Paris-Sud, CNRS, AgroParisTech, Université Paris-Saclay, 91400 Orsay, France

4. Institute of Ecology and Environmental Sciences - Paris, UMR 7618 (Sorbonne Université, UPEC, CNRS, IRD, INRA), 94010 Créteil, France

5. Institute of Ecology and Environmental Sciences - Paris, UMR 7618 (Sorbonne Université, UPEC, CNRS, IRD, INRA), 75005 Paris, France

\*Corresponding author: [Fernandez.marine@courrier.uqam.ca](mailto:Fernandez.marine@courrier.uqam.ca)

Département des sciences biologiques, Université du Québec à Montréal  
141, Président-Kennedy, Montreal, QC, H2X 1Y4 – Canada

23 **Abstract**

1  
2 24 Forest management aims to maintain sustainable production of quality wood while limiting  
3  
4 25 increased competition between trees for light, water, and nutrients. Thinning is a widely used  
5  
6 26 silvicultural practice to reduce plants competition for resources while still exploiting the  
7  
8  
9 27 wood. The investigation of the effects of forest management on stand functioning typically  
10  
11 28 centers on the above-ground compartment, overlooking the alterations and influences exerted  
12  
13  
14 29 on below-ground biotic factors. Within the soil matrix, biological mechanisms are mainly  
15  
16 30 governed by microbial communities. Many studies have focused on the effects of thinning on  
17  
18  
19 31 soil microbial communities (SMC), evidencing contrasted effects. Conversely, stand density  
20  
21 32 effects on SMC are less documented. The aim of this study is therefore to focus on the effects  
22  
23  
24 33 of stand density (SD) on SMC biomass, gene abundance, functional diversity, and activity,  
25  
26 34 according two silvicultural practices: dynamic (low SD) and conservative (medium SD) in a  
27  
28  
29 35 temperate *Quercus petraea* Stand (QS) in Europe Forest. We hypothesized that dynamic  
30  
31 36 silviculture (low-SD) could promote soil SMC biomass, abundance, functional diversity, and  
32  
33  
34 37 activity. Our results showed that dynamic silvicultural practices in oak forests reduced the  
35  
36 38 abundances of bacteria, archaea and fungi were reduced by 43%, 29% and 34%, respectively.  
37  
38  
39 39 SMC functional diversity was reduced by 10% in dynamic forestry stands. On the contrary,  
40  
41 40 dynamic silvicultural practices increased soil microbial activity by 13 to 47%, depending on  
42  
43 41 the carbon source added, compared with conservative silviculture. Our results were  
44  
45 42 incremented with an extensive number of biotic and abiotic environmental variables that had  
46  
47  
48 43 contrasting effects on SMC, and there is no single factor, which alone can explain all the  
49  
50 44 SMC responses. Our results seem to advocate dynamic silvicultural practices in oak forests to  
51  
52  
53 45 promote soil microbial activity. However, it remains to be seen what the long-term effects  
54  
55 46 will be of the reduced abundance and functional diversity of SMCs observed jointly in low-  
56  
57  
58 47 SD.  
59  
60  
61  
62  
63  
64  
65

48 **Key words:** forest stand density, soil microbes, biomass, abundance, functional diversity,  
1  
2 49 activity, environmental factors  
3  
4

5  
6 50  
7

8 **51 Abbreviations**  
9

10  
11 52 *QS* *Quercus* Stand  
12  
13

14  
15 53 L-SD Low Stand Relative Density  
16  
17

18 54 M-SD Medium Stand Relative Density  
19  
20

21 55 MSIR Multiple Substrates Induced Respiration  
22  
23

24 56 SMB Soil Microbial Biomass  
25  
26

27 57 SMC Soil Microbial Community  
28  
29

30 58 SOM Soil Organic Matter  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

59           **1. Introduction**

60           Forests provide large ecosystem services, *i.e.* providing timber, food, habitat for  
61 biodiversity, regulating water resources, allowing recreational opportunities (Ding *et al.*,  
62 2011) and have a fundamental role in carbon sequestration (Duncker *et al.* 2012; Huang *et al.*  
63 2020). Facing the continuous increase of atmospheric CO<sub>2</sub>, research has focused on how  
64 forests can limit the CO<sub>2</sub> level on Earth's surface, through photosynthetic activity of trees and  
65 soil ability to store carbon (Peng *et al.* 2008). Nowadays, one of the major challenges of forest  
66 management is the balance trade-off between wood production and carbon sequestration  
67 potential (Favero *et al.* 2020). Increase in tree biomass has produced more litter, leading to soil  
68 accumulated carbon (Bolte *et al.* 2019), and estimations suggest that it will increase further in  
69 the coming years to the point that soil carbon storage may become more important than tree  
70 carbon storage, which appears to be the case already in the aging Central European forests  
71 (Liski *et al.* 2002; Jonard *et al.* 2017). Consequently, promoting litter production from living  
72 trees through forest management could better regulate soil carbon stocks. The global mean  
73 soil-derived respiratory of CO<sub>2</sub> emissions to the atmosphere overshadows by tenfold the  
74 annual CO<sub>2</sub> emissions from fossil fuel emissions (Oertel *et al.* 2016). The significance of soil  
75 lies in the fact that it is considered the most complex biomaterial and, at the same time, the  
76 most diverse and important ecosystem on Earth. On average, within a fertile soil, there will be  
77 more individual organisms than the total number of human beings who have ever lived: 1  
78 trillion bacteria, 10,000 protozoa, 10,000 nematodes, 25 kilometers of fungi, and countless  
79 other species (Young and Crawford 2004). While most studies focus on forest management's  
80 impact on aboveground compartments for increased soil carbon sequestration potential, some  
81 research emphasizes the urgent need of understanding soil microbial ecology's role in carbon  
82 exchange between land and the atmosphere within the framework of climate change (Bardgett  
83 *et al.* 2008). Maximizing multiple benefits such as carbon sequestration from forest ecosystem

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

84 services requires better knowledge of the dynamics of biological soil functioning (Noormets *et al.* 2015). In the context of rapid global change, it is therefore essential to understand the influence of forest management on soil microbial communities, that contribute to the mineralization of organic matter (Ontl *et al.* 2020; Wang and Huang 2020; Dinca *et al.* 2021).

88 Reducing forest stand density, through thinning, is one of the main management strategies in temperate forest allowing to enhance wood production (Gauthier *et al.* 2015), and increase forest resilience to environmental disturbances such as drought (Sohn *et al.* 2016; Bastida *et al.* 2019). Lower stand density also increases stand sustainability through reduction of tree competition, and thus controls the maintenance of carbon storage in soil (Jandl *et al.* 2007). Canopy gap caused by thinning leads to changes in the microclimate with higher radiation that directly impact soil functioning, and indirectly through the development of understory vegetation. It is now well established that soil functioning will be mainly impacted by changes in abiotic parameters such as water content (Wang *et al.* 2018), C:N ratio (Masyagina *et al.* 2010), pH, organic carbon (Wu *et al.* 2019), fine root density and nutrient balances (Dang *et al.* 2018; Wang *et al.* 2019; Trentini *et al.* 2020; S Liu *et al.* 2021) but also biotic parameters: the soil microbial communities (SMC), which are responsible for a large part of the belowground activities. It is estimated that 80 to 90% of soil biological activity is carried out by bacteria and fungi on the topsoil (Gupta *et al.* 1997). By mineralizing most of the soil organic matter (SOM, mostly derived from residuals plant tissues and rhizodeposition, Chaparro *et al.*, 2013), they contribute to the maintenance of soil functioning and regulate the nutrients cycling (Tefs and Gleixner 2012; Adeleke *et al.* 2016; Lladó *et al.* 2018). Moreover, beyond influencing these key ecological processes, SMC interact reciprocally with plants to the point of being an integral part of their functioning as resource acquisition strategy (Fernandez *et al.* 2022; Han *et al.* 2023). More than just a tool, the SMC has thus become a relevant component to be integrated to optimize forest management strategies like thinning (Staddon *et al.* 1999; Lladó

109 and Baldrian 2017), in the context of climate change. It is well established that tree species  
1  
2 110 impact SMC, notably by releasing specific chemical composition of carbon substrates (Jiang *et*  
3  
4 111 *al.* 2012; Prescott and Grayston 2013; Gartzia-Bengoetxea *et al.* 2016; Khelifa *et al.* 2017), but effect  
5  
6  
7 112 of thinning on microbial biomass, abundance, respiration, and catabolic profiling is less  
8  
9  
10 113 understood (Dang *et al.* 2018; Kim *et al.* 2019). A short review of the literature on forest  
11  
12 114 management reveals that thinning could have contradictory impacts on the microbial  
13  
14 115 community. **Table 1** presents the response (increase, decrease or no significant effect) to  
15  
16  
17 116 thinning of four SMC parameters such as (i) total microbial biomass C and N, (ii) microbial  
18  
19 117 abundance, (iii) diversity and (iv) activity. The table clearly illustrates that the responses of  
20  
21  
22 118 the SMC to thinning are most often variable within the same study. Responses to thinning  
23  
24 119 also vary according to the SMC domain considered: bacteria, archaea and fungi. Concerning  
25  
26  
27 120 soil basal respiration, variable responses to thinning were measured: increase (Zhang *et al.*,  
28  
29 121 2018: in broadleaves and mixed forest), decrease or stable (Zhang *et al.*, 2018: in coniferous  
30  
31 122 forest). Several additional factors have been recognized as influencing SMC, including  
32  
33  
34 123 precipitation, temperature, season, forest site exposure and litter amount, composition, and  
35  
36 124 decomposition stage (Nave *et al.* 2010; Jonard *et al.* 2017; Lladó and Baldrian 2017; Richter *et al.*  
37  
38  
39 125 2018; Xiao *et al.* 2018). It is commonly understood that soil microbial biomass, abundance,  
40  
41 126 diversity, and activities depend on environmental variations (Bolat 2014; Yang *et al.* 2017).  
42  
43  
44 127 Moreover, as thinning induced a decrease of the stand density, this led to changes of the  
45  
46 128 microclimate in the understory and in the soil. Trees, through their species or age for instance,  
47  
48  
49 129 can also introduce changes to both physicochemical and biological soil characteristics.  
50  
51 130 Therefore, changes of SMC parameters can be attributed to change in soil pH, water content,  
52  
53  
54 131 organic matter, moisture, nutrient availability, temperature, litter characteristics, understory  
55  
56 132 plants, radiation, microclimate, tree roots traits and rhizodeposits etc. (Lladó *et al.* 2018; Wu *et*  
57  
58 133 *al.* 2019). Contribution of environmental factors can have different importance on SMC, for  
59  
60  
61  
62  
63  
64  
65

134 instance, Chodak and Niklińska (2010) showed that soil texture had more effect than planted  
135 vegetation on SMC parameters.

136 The diverse effects of thinning on SMC result from the interplay between biotic and  
137 abiotic factors that shape forest soil microbial structure and activity (Mabuhay *et al.* 2006;  
138 Griffiths and Philippot 2013; Simonin and Richaume 2015). This complex assemblage makes it  
139 challenging to understand how silvicultural practices impact SMC.

140 Many studies, including those referenced in **Table 1**, primarily examine the immediate  
141 impact of forest thinning intensity, while the longer-term influence of stand density is less  
142 explored and documented. Regarding the stand density, Wang et al., (2021) wrote that “no  
143 comprehensive analysis of soil enzyme activities and microbial compositions, nor any  
144 detailed observations of correlations between biological and physicochemical properties, have  
145 been performed”. The overarching goal of this study was therefore to move beyond the  
146 examination of thinning as a transient disturbance, and to focus on the effect of stand relative  
147 density (SD) on SMC. We compared the effects of two silvicultural practices: dynamic *i.e.*,  
148 low stand relative density (L-SD) and conservative *i.e.*, medium stand relative density (M-SD)  
149 on microbial community after one year since the last tree cut. We measured the effect of these  
150 two SD on (i) soil basal respiration, (ii) the soil microbial biomass carbon (SMB-C) and  
151 nitrogen (SMB-N), (iii) the bacterial, archaeal, and fungal gene abundance (by quantitative  
152 PCR), and (iv) the SMC functional diversity and activity. The project was conducted on an  
153 experimental device (OPTMix), for which abiotic environmental data (rainfall, water table  
154 depth, temperature, etc.) and biotic data (vegetation cover of understory species) were  
155 measured. We hypothesized that dynamic silviculture (low-SD) could promote soil SMC  
156 biomass, abundance, functional diversity, and activity. We also expected biotic and abiotic  
157 environmental factors, such as precipitations, would influence SMC, but to a lesser extent  
158 compared to SD. Lastly, we expected the results to highlight the significance of extending



159 analysis beyond the short-term effects of thinning. We aimed to emphasize the enduring  
160 impact of SD on SMC, particularly in perennial ecosystems like forests. This perspective  
161 could play a pivotal role in advancing our understanding of soil biological processes.

## 162 2. Materials and methods

### 163 2.1. Study area

164 Sampling and measurements were done at the OPTMix (Oak Pine Tree Mixture) experimental  
165 site in the Forêt d'Orléans, France (47.82717°N, 2.45313°E, **Figure 1**). OPTMix consists of a  
166 network of even-aged adult forest plots (33 plots over 40 ha) that have been managed by the  
167 INRAE Forest Ecosystems Research Unit (Nogent-sur-Vernisson, France) to isolate and study  
168 the effects of various forest management strategies (tree densities, pure and mixed stands,  
169 presence/absence of large wild ungulates thanks to enclosures that exclude deer and wild  
170 boar) on ecosystem functioning. Each plot is about 0.5 ha and tree populations are 60-80 years  
171 old. Soils are composed of a sandy loam top layer (0-50 cm depth) with an increasing gradient  
172 of clay below and are classified as planosols (Lamotte et al., 1988, **Table 2**). Common  
173 understory vegetation includes purple moor grasses (*Molinia caerulea* (L.) Moench), ferns  
174 (*Pteridium aquilinum* (L.) Kuhn) and heath (*Calluna vulgaris* (L.) Hull). We focused on 3  
175 mono-specific oak (*Quercus petraea* (Matt.) Liebl), one of the most widespread tree species  
176 in France) stands, each composed of 2 plots with different stand density. Stand density index  
177 measures the density of a stand of trees based on the number of trees per unit area and  
178 diameter at breast height (DBH) of the tree of average basal area (Reineke 1933). For each  
179 *Quercus* Stand (QS), there are one plot in low stand density (L-SD) and another in medium  
180 stand density (M-SD). Plots density was evaluated using Relative Density Index (SD): 0.4 for  
181 L-SD and 0.7 for M-SD that were achieved by thinning between 2012 and 2017. The mean  
182 oak diameter in the 6 plots was 24.4 cm. The intensity of thinning varies according to the

183 plots insofar as they aimed to achieve a specific SD. The plot characteristics were presented in

184 **Table 3.**

185 2.2.Sampling design

186 Soil samples were taken from the 6 forest plots (3 QS x 2 SD) in June 2018. In each plot, a  
187 total of ten soil cores of the top 10 cm of soil (litter layer excluded) were randomly collected  
188 within the plot and pooled together to form a single composite sample. We therefore had one  
189 soil sample per plot, for a total of six soils. Each of the six composite soil samples was then  
190 sieved in a 2 mm sieve to homogenize and remove roots and rock fragments. After this step,  
191 each of the six samples was split into 4 subsamples for technical replicates, for a total of 24  
192 soils. For these 24 soil samples, a portion of each sample was flash-frozen for molecular  
193 biology experiments to avoid DNA damaging. Another portion of each sample was used for  
194 microbial biomass C and N extraction and water content estimation. The remaining soil was  
195 stored in a freezer at -20°C for two years for MicroResp analyses.

196 2.3.Soil basal respiration

197 Soil basal respiration was measured *in situ* by a closed dynamic system, composed from a  
198 portable infrared gas analyser (EGM4, PPsystems, Hitchin, UK), connected to a soil  
199 respiration chamber (SRC1, PPsystems, Hitchin, UK). The chamber (100 mm diameter, 150  
200 mm high) was set up directly on soil for measurement. In each plot, 30 measures were  
201 conducted in June 2019.

202 2.4.Soil microbial biomass C and N

203 Soil microbial biomass C (SMB-C), N (SMB-N) and microbial C:N ratio were estimated by  
204 determining and comparing the carbon and nitrogen contents in unaltered and treated samples  
205 by fumigation with chloroform. Fumigation method is presented in **Appendix 1.**

## 206 2.5. Microbial gene abundance

1  
2  
3 207 The gene abundance of total bacterial, archeal and fungal microbial communities were  
4  
5 208 estimated by quantitative PCR (qPCR) assays ( $n=4$  technical replicates for each plot). Total  
6  
7  
8 209 bacterial and archaeal communities were targeted using 16S rDNA genes and fungal  
9  
10 210 communities by using 18S rDNA genes (**Table 4**). DNA extraction and gene amplification  
11  
12  
13 211 methods are presented in **Appendix 2**.

14  
15 212 Microbial gene abundances were expressed as gene copy numbers per gram of dry soil.  
16  
17

## 18 213 2.6. Microbial functional diversity and activity

19  
20  
21 214 Activity was measured by assessing the Multiple Substrate-Induced Respiration. MSIR was  
22  
23  
24 215 determined with the MicroResp™ method using the functional capacities of carbon sources  
25  
26 216 mineralization (Campbell *et al.* 2003). Soil samples were first incubated in a 96 deep-well plate  
27  
28  
29 217 for 2 weeks at 25°C to stabilize the microbial communities (Lerch *et al.* 2011) before substrate  
30  
31 218 addition. Fifteen different substrates belonging to 3 different molecular families were  
32  
33  
34 219 selected: 5 sugars (D-fructose, D-glucose, D-galactose, L-arabinose and D-(+)-trehalose  
35  
36 220 dehydrate); 6 amino acids (L-alanine, N-acetylglucosamine, L-lysine-HCl, L-proline, L-  
37  
38 221 cysteine-HCl monohydrate and  $\gamma$ -aminobutyric acid), and 4 carboxylic acids (citric acid,  
39  
40  
41 222 ascorbic acid, L-malic acid, and  $\alpha$ -ketoglutaric acid). Final substrates concentration was 30  
42  
43 223 mg C mL<sup>-1</sup> and substrates addition brought the water content to 60 % of water holding  
44  
45  
46 224 capacity. Thereafter, the soils were incubated for 6 h at 25 °C and the absorbance of each well  
47  
48 225 was measured at a wavelength of 570 nm using a microplate reader (BioTek Eon™).  
49  
50  
51 226 After conversion of absorbance into CO<sub>2</sub> flux, MSIR was calculated for each substrate by  
52  
53 227 subtracting the respiration of the control (without substrate) to that of the total respiration.  
54  
55  
56 228 Total substrate mineralization was calculated as the sum of CO<sub>2</sub> evolved for each substrate  
57  
58 229 and the functional diversity of SMC based on MSIR was estimated using the Shannon index  
59  
60 230 calculated as followed:  
61  
62  
63  
64  
65

231 
$$H = -\sum p_i \times \ln p_i$$

1  
2  
3 232 where  $p_i$  is the respiration response to the substrate  $i$  as a proportion of total substrate  
4  
5 233 mineralization. We then search among the different biochemical classes of substrate  
6  
7  
8 234 (carbohydrates, amino acids, and organic acids), through an analysis of variance, if one or  
9  
10 235 more of these classes are more specifically used by SMC).

## 13 236 2.7.Environmental parameters

16 237 The environmental parameters in each plot, were obtained from the OPTMix dataset. A total  
17  
18  
19 238 of 24 environmental parameters were tested but only those with the highest number of  
20  
21 239 significant correlations ( $n > 8$ ,  $r > |0.3|$  and  $p < 0.05$ ) with the SMC parameters were presented in  
22  
23  
24 240 the results. The 24 environmental parameters have been divided into 5 categories: (i) stand  
25  
26 241 characteristics (*SD*, *final volume of standing trees* in the plots after thinning and *total volume*  
27  
28 242 *of cutting trees*, representing thinning intensity), (ii) water properties (sum of *precipitation*  
29  
30 243 under the tree canopy, *soil water content*, *depth of the water table during the last 30 days*  
31  
32 244 *before the soil harvest* and *depth of the water table on the day of the soil harvest*), (iii) the  
33  
34  
35 245 physicochemical properties of the soil (soil texture including *clay*, *sand* and *silt*, percentage  
36  
37 246 of *organic matter*, *organic carbon*, *nitrogen*, *calcium*, *potassium*, *magnesium*, *pH*, *thickness*  
38  
39 247 *of the organic horizon* (OH), *cation exchange capacity*, *average soil temperature during the*  
40  
41 248 *last 30 days prior to soil harvest*), (iv) litter mass (*average of leaf litter mass during the last*  
42  
43 249 *30 days prior to soil harvest*) and (v) the average vegetation cover of understory species  
44  
45  
46 250 (*Calluna vulgaris*, *Molinia caerulea* and *Rubus fructosa*) within each of the six plots. The  
47  
48  
49 251 different methodologies used to obtain the different environmental parameters in OPTMix  
50  
51  
52 252 forest are detailed in Bello et al., 2019, Korboulewsky et al., 2015 and Perot et al., 2019.

## 56 253 2.8.Statistical analysis

57  
58  
59  
60  
61  
62  
63  
64  
65

254 Statistical analyses were performed using R software (Version 3.4.1.). The MSIR data were  
1  
2 255 log-transformed before the statistical analyses for the normalization. The data were tested for  
3  
4 256 normality and homoscedasticity using Shapiro-Wilk test and using Levene test, respectively.  
5  
6  
7 257 ANalysis Of VAriance (ANOVA,  $\alpha = 0.05$ ) was performed to assess the effects of thinning on  
8  
9 258 SMB-C, SMB-N, microbial gene abundance, functional diversity of SMC, MSIR and soil  
10  
11 259 basal respiration. For each SD, there were 3 biological replicates. Correlation coefficient with  
12  
13 260 environmental factors and associate p-value were performed using the correlation function  
14  
15 261 from the easystats {correlation} package. Pearson correlations between microbial and  
16  
17 262 environmental parameters were considered significant at  $p\text{-value} \leq 0.05$  and non-significant  
18  
19 263 data were identified as “*ns*”. We logically did not compare environmental data measured  
20  
21 264 exclusively in 2018 with soil basal respiration data measured in 2019.  
22  
23  
24  
25  
26

### 27 265 3. Results

#### 28 266 3.1. Soil basal respiration

29  
30  
31 267 The soil basal respiration was not significantly impacted by forest SD (**Figure 2**), with a value  
32  
33 268 of  $3.64 \pm 0.17$  and  $3.54 \pm 0.15 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$  in L-SD and M-SD, respectively. Correlation  
34  
35 269 analysis also shows no relationship between SD and soil basal respiration (**Table 5 – SD**  
36  
37 270 column).  
38  
39  
40  
41  
42  
43

#### 44 271 3.2. Soil microbial biomass C and N

45  
46  
47 272 SMB-C mean tended to be higher in L-SD (1.3 times) compared to M-SD ( $p = 0.07$ ,  
48  
49 273 **Figure 3.a**), with a value of  $858.47 \pm 105.78$  and  $644.41 \pm 36.23 \text{ mg.kg}^{-1}$  in L-SD and M-SD,  
50  
51 274 respectively. However, although Anova's analysis showed a trend, no correlation was found  
52  
53 275 between SMB-C and SD (Pearson analysis) (**Table 5 – SD** column). SMB-N was not  
54  
55 276 significantly affected by SD, with a value of  $58.86 \pm 9.64$  and  $50.81 \pm 5.06 \text{ mg.kg}^{-1}$  in L-SD  
56  
57 277 and M-SD, respectively (**Figure 3.b**). Similarly, microbial C:N ratio was not significantly  
58  
59  
60  
61  
62  
63  
64  
65

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

278 affected by SD, with a value of  $16.57 \pm 1.30$  and  $13.80 \pm 1.19$  mg.kg<sup>-1</sup> in L-SD and M-SD,  
279 respectively (**Figure 3.c**). Biomass C tended to be higher in L-SD (ANOVA analysis).

### 280 3.3. Soil microbial gene abundance

281 Regarding the microbial gene abundance, SD showed significant impact on the three  
282 SMC domains. M-SD led to a higher gene abundance compared to L-SD for each group:  
283 bacteria ( $p = 0.005$ ), archaea ( $p = 0.04$ ) and fungi ( $p = 0.007$ ), corresponding to an increase of  
284 43%, 29% and 34% respectively (**Figure 4.a, b and c**). The Archaea:Bacteria ratio (A:B  
285 ratio) was significantly higher ( $p = 0.02$ ) under L-SD compared to M-SD, but there was no  
286 effect of SD on the F:B ratio (**Figure 4.e and f**). According to correlation analysis, there was  
287 no strong effect of SD on SMC gene abundance (positive correlation coefficients are lower  
288 than 0.5, **Table 5** – SD column).

### 289 3.4. Microbial functional diversity and MSIR

290 Functional diversity of SMC was significantly lower (1.1 times) in L-SD than in M-SD ( $p <$   
291  $0.001$ ) evidencing clear differences in the microbial functional diversity composition between  
292 L-SD and M-SD (**Figure 5**).

293 The CO<sub>2</sub> rate was significantly lower in M-SD for all the substrates ( $p < 0.02$ ) excepted  
294 galactose ( $p = 0.15$ , **Figure 6**). Ketoglutaric acid stood out with the highest increase between  
295 L-SD and M-SD (47%). For the other substrates, the increase varied between 13% (water) and  
296 33% (alanine). The substrates nature also influenced respiration as carboxylic acid led to  
297 higher CO<sub>2</sub> rate, especially ascorbic, malic and ketoglutaric acid ( $> 0.19$  μg C-CO<sub>2</sub> g<sup>-1</sup> h<sup>-1</sup>)  
298 while amino acids cause the lower, especially lysine, proline, and cysteine ( $< 0.09$  μg C-CO<sub>2</sub>  
299 g<sup>-1</sup> h<sup>-1</sup>). Water caused the lowest functional activity ( $< 0.05$  μg C-CO<sub>2</sub> g<sup>-1</sup> h<sup>-1</sup>).

### 300 3.5. Correlations between environmental factors and microbial parameters

301 **Table 5** shows the significant correlation coefficients between the 26 SMC parameters  
1  
2 302 (rows in the table) presented previously and the environmental parameters (columns in the  
3  
4 303 table) that showed the highest number of correlations ( $n \geq 8$ ,  $r \geq |0.3|$  and  $p < 0.05$ ). Of the 24  
5  
6  
7 304 environmental parameters studied, 15 corresponded to the above criteria. The correlations  
8  
9  
10 305 were variable depending on the SMC parameters (biomass, gene abundance, functional  
11  
12 306 diversity and activity).

13  
14  
15 307 *Stand characteristics*

16  
17  
18 308 SD was positively correlated with the functional diversity of SMC ( $r = 0.57$  and  $p <$   
19  
20 309  $0.001$ ) and negatively with the respiration induced by  $\alpha$ -ketoglutaric acid ( $r = -0.5$  and  $p <$   
21  
22 310  $0.001$ ) and citric acid ( $r = -0.51$  and  $p < 0.001$ ). The correlation between SD and respiration  
23  
24 311 induced by other substrates was globally negative and moderate ( $-0.5 < r < -0.3$  and  $p < 0.05$ ).  
25  
26  
27 312 The final volume of standing trees per hectare presented correlation coefficients logically  
28  
29 313 similar to those of the SD with globally higher values. The total volume of cut trees per  
30  
31 314 hectare had a significant positive correlation with microbial C:N ratio ( $r = 0.59$  and  $p < 0.001$ )  
32  
33 315 and negative correlations with bacterial ( $r = -0.62$  and  $p < 0.001$ ), archaeal ( $r = -0.58$  and  $p <$   
34  
35 316  $0.001$ ), and fungal ( $r = -0.62$  and  $p < 0.001$ ) gene abundances. However, the total volume of  
36  
37 317 cutting trees did not exhibit any correlation with the SMC (except for  $\alpha$ -ketoglutaric acid,  $r =$   
38  
39 318  $0.79$  and  $p < 0.001$ ), unlike the SD.

40  
41  
42  
43  
44  
45 319 *Hydric properties*

46  
47  
48  
49 320 The environmental parameter that exhibited the highest number of correlations with  
50  
51 321 the different microbial parameters was the average precipitation of the last 30 days before soil  
52  
53 322 harvesting. A total of 17 microbial parameters had a significant correlation greater than  $|0.5|$   
54  
55 323 with precipitation. Precipitation had significant positive correlations with microbial biomass C  
56  
57 324 ( $r = 0.71$  and  $p < 0.001$ ) and N ( $r = 0.84$  and  $p < 0.001$ ) but negative with microbial C:N ratio  
58  
59  
60  
61  
62  
63  
64  
65

325 (r = -0.74 and p < 0.001). Precipitations also had significant positive correlations with the  
326 gene abundance, especially with archaea (r = 0.54 and p < 0.001), and MSIR (except  $\alpha$ -  
327 ketoglutaric-induced respiration for which the correlation coefficient was negative, r = -0.77  
328 and p < 0.001, and there was no correlation with citric acid). Contrary to the precipitations,  
329 soil water content of the last 30 days before soil harvesting showed a significant negative  
330 correlation with each bacterial (r = -0.63 and p < 0.001), archaeal (r = -0.61 and p < 0.001),  
331 and fungal (r = -0.65 and p < 0.001) gene abundance and did not have correlation with MSIR  
332 (except  $\alpha$ -ketoglutaric-induced respiration, r = 0.82 and p < 0.001). The average water table  
333 depth during the 30 days prior to soil harvesting had a significant (p < 0.001) negative  
334 correlation with microbial C:N ratio (r = -0.71) and positive correlations with each bacterial (r  
335 = 0.63), archaeal (r = 0.64), and fungal (r = 0.67) gene abundance. On the contrary, the  
336 average depth on the day of harvesting did not exhibit correlation with microbial gene  
337 abundance and had negative correlation with microbial biomass C (r = -0.53 and p < 0.001).  
338 The average depth on the day of harvesting had positive correlation with functional diversity  
339 of SMC (r = 0.62 and p < 0.001) and negative correlations with MSIR.

#### 340 *Soil physicochemical properties*

341 Soil organic matter had negative correlations with soil basal respiration (r = -0.70 and  
342 p < 0.001) and the microbial C:N ratio (r = -0.83 and p < 0.001). Conversely, it had positive  
343 correlations with microbial biomass C (r = 0.57 and p < 0.001) and N (r = 0.85 and p < 0.001)  
344 and each bacterial (r = 0.58 and p < 0.001), archaeal (r = 0.71 and p < 0.001), and fungal (r =  
345 0.56 and p < 0.001) gene abundance. The correlation between SOM and respiration induced  
346 by substrates was globally positive and moderate (0.3 < r < 0.7 and p < 0.01, except with  $\alpha$ -  
347 ketoglutaric acid which exhibited negative correlation, r = -0.67 and p < 0.001, and there was  
348 no correlation with citric acid). Cation exchange capacity correlation was positive with soil  
349 basal respiration (r = 0.59 and p < 0.001) and was globally negative and moderate with



350 microbial biomass C and N, and MSIR ( $-0.6 < r < -0.4$  and  $p < 0.001$ , except with  $\alpha$ -  
351 ketoglutaric acid which exhibited positive correlation,  $r = 0.75$  and  $p < 0.001$ , and there was  
352 no correlation with citric acid). Soil temperature mean the 30 days prior to soil harvest had  
353 negative and moderate correlation with SMC gene abundance ( $-0.5 < r < -0.3$  and  $p < 0.05$ )  
354 and with functional diversity of SMC ( $r = -0.55$  and  $p < 0.001$ ). On the contrary, it had  
355 globally positive and moderate correlation with MSIR ( $0.3 < r < 0.6$  and  $p < 0.05$ ).

#### 356 *Litter mass*

357 Leaf litter mass had negative correlations with microbial biomass C ( $r = -0.66$  and  $p <$   
358  $0.001$ ) and N ( $r = -0.54$  and  $p < 0.001$ ), and MSIR (except  $\alpha$ -ketoglutaric-induced respiration  
359 for which the correlation coefficient was positive,  $r = 0.54$  and  $p < 0.001$ , and there was no  
360 correlation with citric acid).

#### 361 *Understory species cover*

362 *Calluna vulgaris* cover in the understory had a negative correlation with functional  
363 diversity of SMC ( $r = -0.6$  and  $p < 0.001$ ) while it exhibited a positive correlation positive  
364 with MSIR ( $0.5 < r < 0.9$  and  $p < 0.001$ ). *Molinia caerulea* cover had positive correlations  
365 with soil basal respiration ( $r = 0.5$  and  $p < 0.001$ ), microbial C:N ratio ( $r = 0.62$  and  $p < 0.001$ )  
366 and moderate with MSIR. On the contrary, it had negative correlation with each bacterial ( $r =$   
367  $-0.52$  and  $p < 0.001$ ), archaeal ( $r = -0.48$  and  $p < 0.001$ ), and fungal ( $r = -0.54$  and  $p < 0.001$ )  
368 gene abundance. Among the understory species, *Rubus fructosus* cover had the greatest  
369 number of coefficient correlation with microbial parameters. The correlations between *R.*  
370 *fructosus* and soil basal respiration, microbial biomass and gene abundance were similar to  
371 those obtained with *M. caerulea*. In contrast, there was a global negative correlation between  
372 *R. fructosus* and MSIR ( $-0.6 < r < -0.4$  and  $p < 0.001$ , except  $\alpha$ -ketoglutaric-induced

1  
2 374 respiration for which the correlation coefficient was positive,  $r = 0.79$  and  $p < 0.001$ , and there  
3 was no correlation with citric acid).

#### 4 375 **4. Discussion**

5  
6  
7  
8 376 In line with the short review of forest thinning research (**Table 1**), our results highlight the  
9 diverse influence on soil microbial communities, depending on the specific stand  
10 characteristics and environmental factors considered.

##### 11 377 4.1. Soil basal respiration

12  
13  
14  
15  
16 379 In our study, soil basal respiration was not influence by stands characteristics, but we  
17 noted a moderate positive correlation with thinning intensity (*i.e.* total volume of cutting trees  
18 per hectare). Soil basal respiration can exhibit differing trends based on the specific study  
19 conditions. For instance, consistently with our finding, thinning intensity has been linked to  
20 an increase in soil respiration (Lee *et al.* 2023). However, Liu et al., (2021) showed that soil  
21 respiration was higher in stands with medium density when compared to those with low  
22 density, but it also depends on the age of the stand. These outcomes suggest that soil  
23 respiration is likely influenced by stand characteristics, including temporary disturbances  
24 (such as thinning) and SD. Impacts on soil basal respiration are thus diverse and seem to arise  
25 from a combination of multiple factors.

##### 26 380 4.2. Soil microbial biomass C and N

27  
28  
29 381 Global mean of microbial biomass in this study was similar to those measured in  
30 *Quercus sessiflora* Morvan forest (Lejon *et al.* 2005) and in *Quercus petraea* forest in Turkey  
31 (Bolat and Şensoy 2019). Our results showed that SMB-C and SMB-N were not significantly  
32 impacted by SD, but we did observe that biomass C tended to be higher in low-density stands.  
33 However, neither SD nor thinning (total volume of cutting trees per hectare) showed any  
34 correlation with SMC, contrary to studies on *Quercus* forests (Kim *et al.* 2018, 2019) and *Pinus*

397 forests (Bolat 2014; Wu *et al.* 2019), that observed an increase with thinning intensity. Through  
398 global meta-analysis, Zhou et al., (2020) demonstrated that thinning does not affect microbial  
399 biomass, highlighting a discordance regarding the effects of thinning on the SMC biomass. Is  
400 our study, we can conclude that SD and, more broadly, the characteristics of the forest stand,  
401 have not significant impact on SMB-C and SMB-N.

#### 402 4.3. Soil microbial gene abundance

403 The effect of SD on microbial gene abundance can be interpreted differently depending on  
404 whether ANOVA or Pearson correlation analysis is considered. According to the ANOVA,  
405 microbial gene copies number was lower in L-SD for each SMC domains, but there was no  
406 effect through correlation analysis. The ANOVA clearly showed that microbial gene  
407 abundance was higher in medium-density stands than in low-density stands. Pearson's  
408 correlation coefficients indicate moderate positive correlations between SD and microbial  
409 gene abundance, except for archaea where there is no correlation. Yet, the correlation matrix  
410 showed a negative correlation between thinning intensity (total volume of trees cut per  
411 hectare) and microbial gene abundance, reinforcing the idea that decreasing tree density in the  
412 forest stand induced a decrease in microbial gene abundance. Cai et al., (2020) and Wu et al.,  
413 (2019) results revealed that effect of thinning on relative abundance of the soil dominant  
414 bacterial taxa varied according to thinning intensity. Medium-intensity thinning tended to  
415 increase of some bacterial taxa (*e.g.* Gram-positive and Gram-negative) relative abundance.  
416 On the contrary, low-intensity thinning, which leads to higher stand density than medium-  
417 intensity thinning, caused a decrease of bacterial taxa relative abundance (*e.g.*  
418 *Gemmatimonadetes* and *Nitrospirae*), which was the opposite of our results. At this point, we  
419 can hypothesize that both thinning and SD affect SMC abundance. Nevertheless, the direction  
420 of the effect (positive or negative) varies according to the studies, which supports the idea that

1  
2 421 the response of SMC is subject to a combination of factors, including the distinct influences  
3  
4 422 of SD and thinning.

#### 5 423 4.4. Microbial functional diversity and MSIR

6  
7  
8 424 Two key findings stand out from the MicroResp™ analyses: (i) the higher microbial  
9  
10 425 functional diversity and (ii) the lower microbial respiration, in M-SD compared to L-SD.  
11  
12 426 SMC were thus more efficient for mineralize all C-substrates in plots with dynamic  
13  
14 427 silviculture (L-SD) despite there was less functional diversity.  
15  
16  
17

18 428 Carboxylic acids induced the highest CO<sub>2</sub> rate whatever the SD, while amino acid had led  
19  
20 429 to a lower CO<sub>2</sub> rate, which is commonly observed in studies (Banning *et al.* 2012; Gartzia-  
21  
22 430 Bengoetxea *et al.* 2016; Xu *et al.* 2019). Exudates and decomposition of plant tissues contain a  
23  
24 431 significant portion of low molecular weight carboxylic acids (Strobel 2001; Macias-Benitez *et al.*  
25  
26 432 2020) that constitutes an important source of labile C for SMC (Van Hees and Clercx 2003; Fujii  
27  
28 433 *et al.* 2010). Klimek *et al.*, (2016) demonstrated that carboxylic acids contributed the most to  
29  
30 434 differences in SMC functional diversity between forest types, underlying that forest soil  
31  
32 435 bacteria preferentially use this substrate category. Interestingly, the analysis of correlations  
33  
34 436 between microbial functional activity and environmental parameters highlights similar  
35  
36 437 coefficients, except for citric acid and ketoglutaric acid. Specifically, microbial respiration  
37  
38 438 induced by  $\alpha$ -ketoglutaric was strongly and positively correlated with total volume of cutting  
39  
40 439 trees per hectare and soil water content, but negatively with precipitations, contrary to other  
41  
42 440 substrates that were positively correlated with precipitations (except respiration induced by  
43  
44 441 citric acid that was not correlated). Regarding stand density, the coefficients were notably  
45  
46 442 most negative with citric acid and ketoglutaric acid. This finding demonstrated that dynamic  
47  
48 443 silvicultural practices enhance microbial activity. Ritz *et al.*, (2006) also showed that citric  
49  
50 444 acid and  $\alpha$ -ketoglutaric acid were the substrates that allowed to establish differences in SIR  
51  
52 445 between coniferous woodland soils and the others, including deciduous woodland. Thus, our  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

446 results corroborate studies that emphasize citric acid and ketoglutaric acid as the primary  
1  
2 447 substrates for identifying functional differences in SMC, considering various factors studied,  
3  
4 448 such as stand density and vegetation type. Overall, it has been frequently observed that the  
5  
6  
7 449 types of microbial carbon sources utilized vary among thinning treatments, with a  
8  
9  
10 450 significantly increased of some enzyme activities with thinning intensities (Tan *et al.* 2008; Wu  
11  
12 451 *et al.* 2019; Zhou *et al.* 2020). Conversely, Kim *et al.*, (2018) demonstrated that thinning had no  
13  
14 452 significant effect on activities of all enzymes although microbial biomass was generally  
15  
16  
17 453 higher with thinning, again highlighting the variability of SMC response to forest harvesting.  
18  
19  
20 454 It clearly appears from both combined ANOVA and correlation analyses that SD alone is  
21  
22 455 insufficient as an explanatory factor to describe the soil microbial community. Therefore, it is  
23  
24  
25 456 necessary to consider other environmental factors and forest management parameters.  
26

#### 27 28 457 4.5. Multifactorial responses of soil microbial community 29 30

31 458 Overall, the results of our study showed that SD impacts SMC differently depending  
32  
33  
34 459 on the parameter considered (*i.e.*, SMC biomass, gene abundance, functional diversity or  
35  
36 460 activity). A dynamic silviculture (L-SD) led to a lower functional diversity of SMC but tend  
37  
38  
39 461 to favor soil microbial mineralization than a conservative silviculture (M-SD). The  
40  
41 462 correlation analysis further underscored the significance of various environmental factors in  
42  
43 463 influencing SMC.  
44

45  
46 464 Depending on the stand characteristic parameters studied, although they are partly  
47  
48  
49 465 linked, the correlation coefficients with the microbial parameters were different. To our  
50  
51 466 knowledge, there is limited existing research that comparatively examines the impact of  
52  
53  
54 467 thinning and the SD on SMC. Most of the available literature primarily focuses on the  
55  
56 468 influence of thinning practices on soil functioning and the associated microbial communities.  
57  
58  
59 469 Thinning is a one-off forest management method which provides presumably temporary  
60  
61  
62  
63  
64  
65

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

470 information on the SMC while the SD could provide a more lasting representation of the  
471 structure and functioning of the SMC. These hypotheses could be supported by providing  
472 more study on the effects in stand density on SMC (Wang *et al.* 2021). Nevertheless, it is worth  
473 noting that none of the three stand characteristic parameters exhibited a correlation with all of  
474 the microbial parameters when considered individually.

475           Incorporating additional environmental factors, such as hydric properties, revealed that  
476 precipitation stood out as the primary factor exhibiting the highest number of robust and  
477 statistically significant correlations with microbial parameters. Zhao *et al.*, (2016) also  
478 demonstrated a positive correlation between precipitations and microbial biomass. Overall,  
479 shifts in microbial community composition could be largely attributed to changes in soil water  
480 and nutrient availability (Ma *et al.* 2012), but surprisingly, only negative correlation was found  
481 in soil water content with the microbial gene abundance, and one positive correlation was  
482 found with microbial respiration for  $\alpha$ -ketoglutaric acid. Difference of precipitation and soil  
483 water content effects on SMC can be attributed to the fact that soil water content is not only  
484 dependent on precipitation but is a result of interactions including also soil texture, litter and  
485 understory species (Dodd and Lauenroth 1997; Cubera and Moreno 2007; Xiong *et al.* 2008). The  
486 analysis of perched water table depth at two different time scales, long term (mean over the 30  
487 days prior to soil harvest) and short term (day of harvest) shows contrasting effects on SMC.  
488 Logically, soil water content and perched water table depth mean the 30 days prior to soil  
489 harvest have opposite effects on SMC. On the other hand, on the day of harvest, we observed  
490 that the higher the water table, the more active the SMC was, corroborating the correlations  
491 with precipitations. The results of our study support the widely supported consensus that soil  
492 water properties and SMC are closely interacting.

493 Regarding soil physicochemical properties, we found that temperature was negatively  
494 correlated with functional diversity of SMC suggesting that increase in temperature decrease

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

495 SMC functional diversity, but this does not necessarily affect its activity. Chen et al., (2015)  
496 described soil temperature as one of the major factors affecting the functional diversity of the  
497 SMC, underlying the need to analyze effect on soil surface temperature on microbial biomass,  
498 abundance, and activities (Mateos-Rivera *et al.* 2016). Soil temperature and moisture also  
499 depend on the quantity of fresh litter, which play a crucial role in shaping the temporal  
500 variation in the microbial community on a month to season scale (Chemidlin Prevost-Boure *et*  
501 *al.* 2011). Numerous studies have also shown that litter properties and SMC are closely linked.  
502 We found that leaf litter mass average the 30 prior to harvest was mostly negatively correlated  
503 with SMC parameters which was not in line with Q. Wang et al., (2014) that demonstrating a  
504 positive effect of leaf litter addition on soil organic carbon mineralization. The multiplicity of  
505 litter properties directly or indirectly influencing SMC (*e.g.* changes in soil temperature) may  
506 explain differences in effects between studies. For instance, Tan et al., (2008) demonstrated  
507 that a numerous SMC response, including biomass, respiration, or mineralization, clearly  
508 depends on the stage of litter decomposition. The role of litter and particularly its stage of  
509 decomposition may also be one reason why the effects of thinning on SMC may differ from  
510 one study to another. Our results also showed that O horizon thickness increase led to  
511 decrease in microbial functional diversity that does not corroborate Cartwright et al., (2016).  
512 However, a thick O horizon was observed to enhance microbial activity, aligning with the  
513 common findings in the existing literature (Hellwig *et al.* 2018).

514 Regarding the biotic factors, few studies described crucial role of understory species on SMC  
515 in forest ecosystems. Understory removal could significantly reduce soil microbial biomass C  
516 (Xiong *et al.* 2008) and change microbial community composition (Wu *et al.* 2011), leading to  
517 decreased respiration. To our knowledge, no study described effect of the presence of *R.*  
518 *fructosus* and *C. vulgaris* on soil microbial community. Our study shows the importance of  
519 considering understory vegetation, especially *C. vulgaris* and *R. fructosus*, in the analysis of

1 520 microbial communities and especially their activity. Further studies on these understory  
2 521 species should be conducted to understand the extent to which their rhizodeposits influence  
3  
4 522 the soil microbial community, as is the case with different forest species (Philippot *et al.* 2013;  
5  
6  
7 523 Fu *et al.* 2015; Yang *et al.* 2018).  
8  
9

10 524 Finally, precipitations, and to a lesser extent soil organic matter and H horizon thickness, are  
11  
12 525 the three factors that favor both SMC biomass, gene abundance, and MSIR. In contrast,  
13  
14 526 functional diversity appears to be positively influenced by higher stand density and greater  
15  
16  
17 527 standing tree volume. Perched water table depth, leaf litter mass and *R. fructosus* cover were  
18  
19 528 the environmental parameters that were overall negatively correlated with microbial biomass,  
20  
21 529 gene abundance, functional diversity and/or activity. Other environmental parameters,  
22  
23  
24 530 including SD, had contrasting correlations with microbial parameters.  
25  
26

27  
28 531 Our study reflects the great complexity of interactions between abiotic and biotic factors in  
29  
30 532 the soil ecosystem. In addition to considering a multitude of hydrological, chemical, and  
31  
32 533 physical factors, it appears that the time frame over which these factors are assessed holds  
33  
34 534 paramount significance. Indeed, we note that within the same forest, the differences between  
35  
36  
37 535 the environmental factors measured can be significant although stands are separated of a  
38  
39  
40 536 maximum of 30 km. The stand QS3, particularly the L-SD plot, is clearly different from the  
41  
42 537 others regarding the studied factors (**Figure A.1**). A probable reason for such a difference is  
43  
44 538 that the thinning intensity was on average 1.8 and 2 times higher in plots QS1 L-SD and QS2  
45  
46  
47 539 L-SD, respectively. However, thinning intensity is not a sufficient explanatory factor either,  
48  
49  
50 540 as shown by the correlation analysis. Besides, variation of precipitations under the canopy  
51  
52 541 were observed between the 6 plots (**Table A.6**). Such differences could thus be explained by  
53  
54 542 (i) geographical distance, (ii) the position of the rain gauges in the plots, and/or (iii) the  
55  
56  
57 543 canopy density of each stand. Grayston and Rennenberg, (2006) study demonstrated that  
58  
59 544 forest stand fine local characteristics (*e.g.* geographical exposure) could have strong effects on  
60  
61  
62  
63  
64  
65



545 SMC and interfered with thinning effect. For instance, soil microbial activity was  
1 significantly higher in the plots of the northeast-facing compared with the site southwest-  
2 facing and was significantly reduced by heavy thinning only on the northeast-facing site.  
3  
4  
5  
6  
7 548 Furthermore, Liu et al., (2019) study focusing on fungal community, established that  
8  
9  
10 549 geographic location was a determining factor for differential fungal diversity patterns.  
11  
12 550 Previous studies also observed an altitudinal, latitudinal, and longitudinal gradient of  
13  
14 551 microbial biomass responses but this has rarely been observed on such a small scale (Van Horn  
15  
16  
17 552 *et al.* 2013; Ren *et al.* 2018; Xu *et al.* 2018; Liu *et al.* 2019). The fact that *QS3* is situated more to  
18  
19 553 the northwest than the other two stations could lead to differences in certain abiotic variables  
20  
21 554 (*e.g.*, soil history, wind, etc.). None of the data from our studied database allows us to support  
22  
23  
24 555 this hypothesis or establish a particular factor to explain the uniqueness of the results obtained  
25  
26  
27 556 in *QS3* compared to *QS1* and *QS2*.

28  
29  
30 557 It is therefore important to maximize the number of technical and biological replicates  
31  
32 558 to characterize with more precision the environmental properties of each forest plot. We also  
33  
34 559 wish to emphasize the importance of the forest metric data (*e.g.* stand characteristics  
35  
36  
37 560 parameters) as well as the duration (*e.g.* point parameters such as thinning, or longer term  
38  
39 561 parameters such as stand density) considered in the study of factors impacting SMC. These  
40  
41  
42 562 considerations are in line with recent studies highlighting the need to improve current  
43  
44 563 practices in hypothesis generation, modeling, and visual representation of interactions in  
45  
46  
47 564 ecology (Spake *et al.* 2023).

## 50 565 **5. Conclusions and outcomes**

51  
52  
53 566 Microbial biomass and gene abundance seem to depend more on forest local environmental  
54  
55 567 characteristics than forest plot density or even thinning, contrary to our initial expectations.  
56  
57  
58 568 An important consideration is that although the forest stands were supposed to be similar  
59  
60 569 (same pedological station, trees age, size and composition, and understory characteristics) and  
61  
62  
63  
64  
65

1 within 30 km of each other, the differences observed in abiotic factors (*e.g.* precipitation  
2 571 under canopy, soil properties) explained better the microbial biomass and gene abundance  
3  
4 572 than forest relative density index. Conversely, it appears that SD exerts a more significant  
5  
6  
7 573 influence on the functional diversity and activity of SMC. A dynamic silvicultural practice  
8  
9 574 negatively affected SMC functional diversity but favored their activity, partly validating our  
10  
11 575 initial hypotheses. A noteworthy aspect of our study is that our primary focus was to analyze  
12  
13 576 the impact of SD, whereas many other studies typically investigate the effects of thinning  
14  
15  
16 577 intensity. Thinning represents a temporary disturbance, yet our findings emphasize the  
17  
18  
19 578 importance of considering thinning post-effects, taking into account in particular the stand  
20  
21 579 SD, which provides a good indicator of SMC in the longer term. Recent studies also support  
22  
23  
24 580 the idea that understanding these effects in the context of a longer timeframe is crucial (*Lee et*  
25  
26 581 *al.* 2023), especially in perennial ecosystems like forests. Thus, longer-term studies should be  
27  
28  
29 582 conducted to characterize the effect of forest plot density on soil microbial community. An  
30  
31 583 acceptable conclusion which is in line with Bolat (2014) is that the influence of forest  
32  
33  
34 584 thinning on the SMC parameters result in the combination of multiple biotic and abiotic  
35  
36 585 factors including soil properties, understory species and environmental conditions, one  
37  
38  
39 586 influencing the other. Additional research efforts should be directed towards investigating  
40  
41 587 various environmental parameters across forest stands, with a particular emphasis on stand  
42  
43  
44 588 density, which has received comparatively less attention than thinning in previous studies.  
45  
46 589 Furthermore, a thorough environmental characterization should be carried out, involving the  
47  
48  
49 590 interactions between different these factors, to provide a more holistic understanding of the  
50  
51 591 soil microbial communities. This could also help to fill an important gap in our understanding  
52  
53 592 of forest soil ecosystem dynamics.  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

593 **Acknowledgement**

1  
2  
3 594 The OPTMix experimental site (<https://optmix.inrae.fr/>) where our study took place was  
4  
5 595 installed and equipped by INRAE EFNO thanks to the Centre Val-de-Loire region, the Loiret  
6  
7  
8 596 and the French National Forest Office.  
9

10  
11 597 The site belongs to the French national research infrastructure, ANAEE-F (<http://www.anaee->  
12  
13 598 [france.fr/fr/](http://www.anaee-france.fr/fr/)), and is included in the SOERE TEMPO (<https://tempo.pheno.fr/>). The site is also  
14  
15  
16 599 in the framework of the ZAL (LTSER Zone Atelier Loire) and the GIS Coop network  
17  
18 600 (<https://www6.inra.fr/giscoop/>), which is supported by the French Ministry for Agriculture  
19  
20  
21 601 and Food.  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

- 602 **Adeleke R, Nwangburuka C, Oboirien B. 2016.** Origins, roles and fate of organic acids in soils: A  
1 603 review. *South African Journal of Botany* **108**: 393–406.  
2
- 3 604 **Banning NC, Lalor BM, Cookson WR, Grigg AH, Murphy D V. 2012.** Analysis of soil microbial  
4 605 community level physiological profiles in native and post-mining rehabilitation forest: Which  
5 606 substrates discriminate? *Applied Soil Ecology* **56**: 27–34.  
6
- 7 607 **Bardgett RD, Freeman C, Ostle NJ. 2008.** Microbial contributions to climate change through carbon  
8 608 cycle feedbacks. *The ISME Journal* **2**: 805–814.  
9
- 10 609 **Bastida F, López-Mondéjar R, Baldrian P, et al. 2019.** When drought meets forest management:  
11 610 effects on the soil microbial community of a Holm oak forest ecosystem. *Science of the Total*  
12 611 *Environment* **662**: 276–286.  
13
- 14 612 **Bello J, Hasselquist NJ, Vallet P, Kahmen A, Perot T, Korboulewsky N. 2019.** Complementary  
15 613 water uptake depth of *Quercus petraea* and *Pinus sylvestris* in mixed stands during an extreme  
16 614 drought. *Plant and Soil* **437**: 93–115.  
17
- 18 615 **Bolat I. 2014.** The effect of thinning on microbial biomass C, N and basal respiration in black pine  
19 616 forest soils in Mudurnu, Turkey. *European Journal of Forest Research* **133**: 131–139.  
20
- 21 617 **Bolat İ, Şensoy H. 2019.** Microbial biomass soil content and activity under black alder and sessile oak  
22 618 in the western black sea region of Turkey. *International Journal of Environmental Research* **13**: 781–  
23 619 791.  
24
- 25 620 **Bolte Andreas, Block J, Eichhorn J, Sanders TGM, Wellbrock Nicole. 2019.** Sustainable use and  
26 621 development of forests and forest soils: a resume In: Wellbrock N., Bolte A., eds. *Status and*  
27 622 *Dynamics of Forests in Germany Results of the National Forest Monitoring*. Ecological Studies: New  
28 623 York, NY, USA, 2019, 355–374.  
29
- 30 624 **Cai M, Peng X, Cheng X, et al. 2020.** Soil element stoichiometry drives bacterial community  
31 625 composition following thinning in a *Larix* Plantation in the subalpine regions of Northern China.  
32 626 *Forests* **11**: 1–14.  
33
- 34 627 **Campbell CD, Chapman SJ, Cameron CM, Davidson MS, Potts JM. 2003.** A rapid microtiter  
35 628 plate method to measure carbon dioxide evolved from carbon substrate amendments so as to determine  
36 629 the physiological profiles of soil microbial communities by using whole soil. *Applied and*  
37 630 *Environmental Microbiology* **69**: 3593–3599.  
38
- 39 631 **Cartwright J, Dzantor EK, Momen B. 2016.** Soil microbial community profiles and functional  
40 632 diversity in limestone cedar glades. *Catena* **147**: 216–224.  
41
- 42 633 **Chaparro JM, Badri D V., Bakker MG, Sugiyama A, Manter DK, Vivanco JM. 2013.** Root  
43 634 exudation of phytochemicals in *Arabidopsis* follows specific patterns that are developmentally  
44 635 programmed and correlate with soil microbial functions. *PLoS ONE* **8**.  
45
- 46 636 **Chemidlin Prevost-Boure N, Maron P-A, Ranjard L, et al. 2011.** Seasonal dynamics of the  
47 637 bacterial community in forest soils under different quantities of leaf litter. *Applied Soil Ecology* **47**:  
48 638 14–23.  
49
- 50 639 **Chen X-L, Wang D, Chen Xin, et al. 2015.** Soil microbial functional diversity and biomass as  
51 640 affected by different thinning intensities in a Chinese fir plantation. *Applied Soil Ecology* **92**: 35–44.  
52
- 53 641 **Chodak M, Niklińska M. 2010.** Effect of texture and tree species on microbial properties of mine  
54 642 soils. *Applied Soil Ecology* **46**: 268–275.  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

- 643 **Cubera E, Moreno G. 2007.** Effect of land-use on soil water dynamic in dehesas of Central-Western  
1 644 Spain. *Catena* **71**: 298–308.
- 2  
3 645 **Dang P, Gao Y, Liu J, Yu S, Zhao Z. 2018.** Effects of thinning intensity on understory vegetation  
4 646 and soil microbial communities of a mature Chinese pine plantation in the Loess Plateau. *The Science  
5 647 of the total environment* **630**: 171–180.
- 6  
7 648 **Dinca L, Onet A, Samuel AD, et al. 2021.** Microbial soil biodiversity in beech forests of European  
8 649 mountains. *Canadian Journal of Forest Research* **51**: 1833–1845.
- 9  
10 650 **Dodd MB, Lauenroth WK. 1997.** The influence of soil texture on the soil water dynamics and  
11 651 vegetation structure of a shortgrass steppe ecosystem. *Plant Ecology* **133**: 13–28.
- 12  
13 652 **Duncker PS, Raulund-Rasmussen K, Gundersen P, et al. 2012.** How forest management affects  
14 653 ecosystem services, including timber production and economic return: Synergies and trade-offs.  
15 654 *Ecology and Society* **17**: 50.
- 16  
17 655 **Favero A, Daigneault A, Sohngen B. 2020.** Forests: carbon sequestration, biomass energy, or both?  
18 656 *Science Advances* **6**: 1–14.
- 19  
20 657 **Fernandez M, Vernay A, Henneron L, Adamik L, Malagoli P, Balandier P. 2022.** Plant N  
21 658 economics and the extended phenotype: Integrating the functional traits of plants and associated soil  
22 659 biota into plant- plant interactions. *Journal of Ecology* **00**: 1–18.
- 23  
24 660 **Fu X, Yang F, Wang J, et al. 2015.** Understory vegetation leads to changes in soil acidity and in  
25 661 microbial communities 27years after reforestation. *Science of the Total Environment* **502**: 280–286.
- 26  
27 662 **Fujii K, Hayakawa C, Van Hees PAW, Funakawa S, Kosaki T. 2010.** Biodegradation of low  
28 663 molecular weight organic compounds and their contribution to heterotrophic soil respiration in three  
29 664 Japanese forest soils. *Plant and Soil* **334**: 475–489.
- 30  
31 665 **Gartzia-Bengoetxea N, Kandeler E, Martínez de Arano I, Arias-González A. 2016.** Soil microbial  
32 666 functional activity is governed by a combination of tree species composition and soil properties in  
33 667 temperate forests. *Applied Soil Ecology* **100**: 57–64.
- 34  
35 668 **Gauthier MM, Barrette M, Tremblay S. 2015.** Commercial thinning to meet wood production  
36 669 objectives and develop structural heterogeneity: A case study in the spruce-fir forest, Quebec, Canada.  
37 670 *Forests* **6**: 510–532.
- 38  
39 671 **Grayston SJ, Rennenberg H. 2006.** Assessing effects of forest management on microbial community  
40 672 structure in a central European beech forest. *Canadian Journal of Forest Research* **36**: 2595–2604.
- 41  
42 673 **Griffiths BS, Philippot L. 2013.** Insights into the resistance and resilience of the soil microbial  
43 674 community. *FEMS Microbiology Reviews* **37**: 112–129.
- 44  
45 675 **Gupta VVSR, Neate SM, Leonard E. 1997.** *Life in the soil: the relationship between agriculture and  
46 676 soil organisms.*
- 47  
48 677 **Han M, Chen Y, Sun L, et al. 2023.** Linking rhizosphere soil microbial activity and plant resource  
49 678 acquisition strategy. *Journal of Ecology* **111**: 875–888.
- 50  
51 679 **Van Hees AFM, Clerckx APPM. 2003.** Shading and root-shoot relations in saplings of silver birch,  
52 680 pedunculate oak and beech. *Forest Ecology and Management* **176**: 439–448.
- 53  
54 681 **Hellwig N, Gómez-Brandón M, Ascher-Jenull J, et al. 2018.** Humus forms and soil microbiological  
55 682 parameters in a mountain forest: Upscaling to the slope scale. *Soil Systems* **2**: 1–22.
- 56  
57  
58  
59  
60  
61  
62  
63  
64  
65

- 683 **Van Horn DJ, Van Horn ML, Barrett JE, et al. 2013.** Factors controlling soil microbial biomass  
1 684 and bacterial diversity and community composition in a cold desert ecosystem: role of geographic  
2 685 scale. *PLoS ONE* **8**: 1–12.  
3
- 4 686 **Huang L, Zhou M, Lv J, Chen K. 2020.** Trends in global research in forest carbon sequestration: A  
5 687 bibliometric analysis. *Journal of Cleaner Production* **252**: 1–17.  
6
- 7 688 **Jandl R, Lindner M, Vesterdal L, et al. 2007.** How strongly can forest management influence soil  
8 689 carbon sequestration? *Geoderma* **137**: 253–268.  
9
- 10 690 **Jenkinson DS, Brookes PC, Powlson DS. 2004.** Measuring soil microbial biomass. *Soil Biology and*  
11 691 *Biochemistry* **36**: 5–7.  
12
- 13 692 **Jiang Y, Chen C, Xu Z, Liu Y. 2012.** Effects of single and mixed species forest ecosystems on  
14 693 diversity and function of soil microbial community in subtropical China. *Journal of Soils and*  
15 694 *Sediments* **12**: 228–240.  
16
- 17 695 **Jonard M, Nicolas M, Coomes DA, Caignet I, Saenger A, Ponette Q. 2017.** Forest soils in France  
18 696 are sequestering substantial amounts of carbon. *Science of the Total Environment* **574**: 616–628.  
19
- 20 697 **Khelifa R, Paquette A, Messier C, Reich PB, Munson AD. 2017.** Do temperate tree species diversity  
21 698 and identity influence soil microbial community function and composition? *Ecology and Evolution* **7**:  
22 699 7965–7974.  
23
- 24 700 **Kim S, Li G, Han SH, et al. 2018.** Thinning affects microbial biomass without changing enzyme  
25 701 activity in the soil of *Pinus densiflora* Sieb. et Zucc. forests after 7 years. *Annals of Forest Science* **75**:  
26 702 1–10.  
27
- 28 703 **Kim S, Li G, Han SH, Kim C, Lee ST, Son Y. 2019.** Microbial biomass and enzymatic responses to  
29 704 temperate oak and larch forest thinning: Influential factors for the site-specific changes. *Science of the*  
30 705 *Total Environment* **651**: 2068–2079.  
31
- 32 706 **Klimek B, Chodak M, Jaźwa M, Solak A, Tarasek A, Niklińska M. 2016.** The relationship  
33 707 between soil bacteria substrate utilisation patterns and the vegetation structure in temperate forests.  
34 708 *European Journal of Forest Research* **135**: 179–189.  
35
- 36 709 **Korboulewsky N, Pérot T, Balandier P, et al. 2015.** Dispositif expérimental de suivi à long terme du  
37 710 fonctionnement de la forêt mélangée. *RDV technique ONF* **47**: 60–70.  
38
- 39 711 **Lamotte M, Bruand A, Duval O, Humbel FX. 1988.** Un système planosol-sol hydromorphe en forêt  
40 712 d'Orléans. *Science du sol* **26**: 139–155.  
41
- 42 713 **Lee JG, Lee DH, Jung JY, et al. 2023.** The effects of stand density control on carbon cycle in  
43 714 *Chamaecyparis obtusa* (Siebold and Zucc.) Endl. forests. *Forests* **14**: 1–12.  
44
- 45 715 **Lejon DPH, Chaussod R, Ranger J, Ranjard L. 2005.** Microbial community structure and density  
46 716 under different tree species in an acid forest soil (Morvan, France). *Microbial Ecology* **50**: 614–625.  
47
- 48 717 **Lerch TZ, Nunan N, Dignac MF, Chenu C, Mariotti A. 2011.** Variations in microbial isotopic  
49 718 fractionation during soil organic matter decomposition. *Biogeochemistry* **106**: 5–21.  
50
- 51 719 **Liski J, Perruchoud D, Karjalainen T. 2002.** Increasing carbon stocks in the forest soils of western  
52 720 Europe. *Forest Ecology and Management* **169**: 159–175.  
53
- 54 721 **Liu T, Peng D, Tan Z, Guo J, Zhang Y. 2021.** Effects of stand density on soil respiration and labile  
55 722 organic carbon in different aged *Larix principis-rupprechtii* plantations. *Ecological Processes* **10**: 1–  
56 723 15.  
57  
58  
59  
60  
61  
62  
63  
64  
65

- 724 **Liu D, Wang H, An S, Bhole P, Davlatbekov F. 2019.** Geographic distance and soil microbial  
1 725 biomass carbon drive biogeographical distribution of fungal communities in Chinese Loess Plateau  
2 726 soils. *Science of the Total Environment* **660**: 1058–1069.  
3
- 4 727 **Liu S, Yin H, Li Xiangjun, et al. 2021.** Short-term thinning influences the rhizosphere fungal  
5 728 community assembly of *Pinus massoniana* by altering the understory vegetation diversity. *Frontiers in*  
6 729 *Microbiology* **12**: 1–14.  
7
- 8 730 **Lladó S, Baldrian P. 2017.** Community-level physiological profiling analyses show potential to  
9 731 identify the copiotrophic bacteria present in soil environments. *PLoS ONE* **12**: 1–15.  
10
- 11 732 **Lladó S, López-Mondéjar R, Baldrian P. 2018.** Drivers of microbial community structure in forest  
12 733 soils. *Applied Microbiology and Biotechnology* **102**: 4331–4338.  
13
- 14 734 **Ma L, Huang W, Guo C, Wang R, Xiao C. 2012.** Soil microbial properties and plant growth  
15 735 responses to carbon and water addition in a temperate steppe: The importance of nutrient availability.  
16 736 *PLoS ONE* **7**: 1–12.  
17
- 18 737 **Mabuhay JA, Nakagoshi N, Isagi Y. 2006.** Soil microbial biomass, abundance, and diversity in a  
19 738 Japanese red pine forest: First year after fire. *Journal of Forest Research* **11**: 165–173.  
20
- 21 739 **Macias-Benitez S, Garcia-Martinez AM, Caballero Jimenez P, Gonzalez JM, Tejada Moral M,**  
22 740 **Parrado Rubio J. 2020.** Rhizospheric organic acids as biostimulants: monitoring feedbacks on soil  
23 741 microorganisms and biochemical properties. *Frontiers in Plant Science* **11**: 1–16.  
24
- 25 742 **Masyagina O V., Prokushkin SG, Koike T. 2010.** The influence of thinning on the ecological  
26 743 conditions and soil respiration in a larch forest on Hokkaido Island. *Eurasian Soil Science* **43**: 693–  
27 744 700.  
28
- 29 745 **Mateos-Rivera A, Yde JC, Wilson B, Finster KW, Reigstad LJ, Øvreås L. 2016.** The effect of  
30 746 temperature change on the microbial diversity and community structure along the chronosequence of  
31 747 the sub-arctic glacier forefield of Styggegdalsbreen (Norway). *FEMS Microbiology Ecology* **92**: 1–13.  
32
- 33 748 **Nave LE, Vance ED, Swanston CW, Curtis PS. 2010.** Harvest impacts on soil carbon storage in  
34 749 temperate forests. *Forest Ecology and Management* **259**: 857–866.  
35
- 36 750 **Noormets A, Epron D, Domec JC, et al. 2015.** Effects of forest management on productivity and  
37 751 carbon sequestration: A review and hypothesis. *Forest Ecology and Management* **355**: 124–140.  
38
- 39 752 **Oertel C, Matschullat J, Zurba K, Zimmermann F, Erasmi S. 2016.** Greenhouse gas emissions  
40 753 from soils - A review. *Chemie der Erde* **76**: 327–352.  
41
- 42 754 **Ontl TA, Janowiak MK, Swanston CW, et al. 2020.** Forest management for carbon sequestration  
43 755 and climate adaptation. *Journal of Forestry* **118**: 86–101.  
44
- 45 756 **Peng Y, Thomas SC, Tian D. 2008.** Forest management and soil respiration: Implications for carbon  
46 757 sequestration. *Environmental Reviews* **16**: 93–111.  
47
- 48 758 **Perot T, Balandier P, Couteau C, Perret S, Seigner V, Korboulewsky N. 2019.** Transmitted light  
49 759 as a tool to monitor tree leaf phenology and development applied to *Quercus petraea*. *Agricultural and*  
50 760 *Forest Meteorology* **275**: 37–46.  
51
- 52 761 **Philippot L, Raaijmakers JM, Lemanceau P, Van Der Putten WH. 2013.** Going back to the roots:  
53 762 The microbial ecology of the rhizosphere. *Nature Reviews Microbiology* **11**: 789–799.  
54
- 55 763 **Prescott CE, Grayston SJ. 2013.** Tree species influence on microbial communities in litter and soil:  
56 764 Current knowledge and research needs. *Forest Ecology and Management* **309**: 19–27.  
57  
58  
59  
60  
61  
62  
63  
64  
65

- 765 **Reineke LH. 1933.** Perfecting a stand-density index for even-aged forests. *Journal of Agricultural*  
1 766 *Research* **46**: 627–638.
- 2  
3 767 **Ren C, Zhang W, Zhong ZK, et al. 2018.** Differential responses of soil microbial biomass, diversity,  
4 768 and compositions to altitudinal gradients depend on plant and soil characteristics. *Science of the Total*  
5 769 *Environment* **610–611**: 750–758.
- 6  
7 770 **Richter A, Schöning I, Kahl T, Bauhus J, Ruess L. 2018.** Regional environmental conditions shape  
8 771 microbial community structure stronger than local forest management intensity. *Forest Ecology and*  
9 772 *Management* **409**: 250–259.
- 10  
11 773 **Ritz K, Harris J a., Pawlett M, Stone D. 2006.** *Catabolic profiles as an indicator of soil microbial*  
12 774 *functional diversity*. Environment Agency: Rio House, Waterside Drive, Aztec West, Almondsbury,  
13 775 Bristol, BS32 4UD.
- 14  
15 776 **Simonin M, Richaume A. 2015.** Impact of engineered nanoparticles on the activity, abundance, and  
16 777 diversity of soil microbial communities: a review. *Environmental Science and Pollution Research* **22**:  
17 778 13710–13723.
- 18  
19 779 **Sohn JA, Saha S, Bauhus J. 2016.** Potential of forest thinning to mitigate drought stress: A meta-  
20 780 analysis. *Forest Ecology and Management* **380**: 261–273.
- 21  
22 781 **Spake R, Bowler DE, Callaghan CT, et al. 2023.** Understanding ‘it depends’ in ecology: a guide to  
23 782 hypothesising, visualising and interpreting statistical interactions. *Biological Reviews* **98**: 983–1002.
- 24  
25 783 **Staddon WJ, Duchesne LC, Trevors JT. 1999.** The role of microbial indicators of soil quality in  
26 784 ecological forest management. *The forestry chronicle* **75**: 81–86.
- 27  
28 785 **Strobel BW. 2001.** Influence of vegetation on low-molecular-weight carboxylic acids in soil solution  
29 786 - A review. *Geoderma* **99**: 169–198.
- 30  
31 787 **Tan X, Chang SX, Comeau PG, Wang Y. 2008.** Thinning effects on microbial biomass, N  
32 788 mineralization, and tree growth in a mid-rotation fire-origin lodgepole pine stand in the lower  
33 789 Foothills of Alberta, Canada. *Forest Science* **54**: 465–474.
- 34  
35 790 **Tefs C, Gleixner G. 2012.** Importance of root derived carbon for soil organic matter storage in a  
36 791 temperate old-growth beech forest - Evidence from C, N and <sup>14</sup>C content. *Forest Ecology and*  
37 792 *Management* **263**: 131–137.
- 38  
39 793 **Trentini CP, Campanello PI, Villagra M, Ferreras J, Hartmann M. 2020.** Thinning partially  
40 794 mitigates the impact of atlantic forest replacement by pine monocultures on the soil microbiome.  
41 795 *Frontiers in Microbiology* **11**: 1–18.
- 42  
43 796 **Vance ED, Brookes PC, Jenkinson DS. 1987.** An extraction method for measuring soil microbial  
44 797 biomass C. *Soil Biology and Biochemistry* **19**: 703–707.
- 45  
46 798 **Wang Z, He Q, Hu B, Pang X, Bao W. 2018.** Gap thinning improves soil water content, changes the  
47 799 vertical water distribution and decreases the fluctuation Correspondence to. *Canadian Journal of*  
48 800 *Forest Research* **48**: 1042–1048.
- 49  
50 801 **Wang S, Huang Y. 2020.** Determinants of soil organic carbon sequestration and its contribution to  
51 802 ecosystem carbon sinks of planted forests. *Global Change Biology* **26**: 3163–3173.
- 52  
53 803 **Wang D, Olatunji OA, Xiao J. 2019.** Thinning increased fine root production, biomass, turnover rate  
54 804 and understory vegetation yield in a Chinese fir plantation. *Forest Ecology and Management* **440**: 92–  
55 805 100.
- 56  
57  
58  
59  
60  
61  
62  
63  
64  
65



- 806 **Wang Q, Wang S, He T, Liu L, Wu J. 2014.** Response of organic carbon mineralization and  
1 807 microbial community to leaf litter and nutrient additions in subtropical forest soils. *Soil Biology and*  
2 808 *Biochemistry* **71**: 13–20.
- 3  
4 809 **Wang C, Xue L, Dong Y, Jiao R. 2021.** Effects of stand density on soil microbial community  
5 810 composition and enzyme activities in subtropical *Cunninghamia lanceolata* (Lamb.) Hook plantations.  
6 811 *Forest Ecology and Management* **479**.
- 7  
8 812 **Wu R, Cheng X, Han H. 2019.** The effect of forest thinning on soil microbial community structure  
9 813 and function. *Forests* **10**: 352.
- 10  
11 814 **Wu J, Liu Z, Wang X, et al. 2011.** Effects of understory removal and tree girdling on soil microbial  
12 815 community composition and litter decomposition in two Eucalyptus plantations in South China.  
13 816 *Functional Ecology* **25**: 921–931.
- 14  
15 817 **Xiao W, Fei F, Diao J, Chen BJW, Guan Q. 2018.** Thinning intensity affects microbial functional  
16 818 diversity and enzymatic activities associated with litter decomposition in a Chinese fir plantation.  
17 819 *Journal of Forestry Research* **29**: 1337–1350.
- 18  
19 820 **Xiong Y, Xia H, Li Z, Cai X, Fu S. 2008.** Impacts of litter and understory removal on soil properties  
20 821 in a subtropical *Acacia mangium* plantation in China. *Plant and Soil* **304**: 179–188.
- 21  
22 822 **Xu Z, Yu G, Wang Q, et al. 2019.** Plant functional traits determine latitudinal variations in soil  
23 823 microbial function: evidence from forests in China. *Biogeosciences* **16**: 3333–3349.
- 24  
25 824 **Xu Z, Yu G, Zhang X, et al. 2018.** Biogeographical patterns of soil microbial community as  
26 825 influenced by soil characteristics and climate across Chinese forest biomes. *Applied Soil Ecology* **124**:  
27 826 298–305.
- 28  
29 827 **Yang Y, Geng Y, Zhou H, Zhao G, Wang L. 2017.** Effects of gaps in the forest canopy on soil  
30 828 microbial communities and enzyme activity in a Chinese pine forest. *Pedobiologia* **61**: 51–60.
- 31  
32 829 **Yang Y, Zhang X, Zhang C, et al. 2018.** Understory vegetation plays the key role in sustaining soil  
33 830 microbial biomass and extracellular enzyme activities. *Biogeosciences* **15**: 4481–4494.
- 34  
35 831 **Young IM, Crawford JW. 2004.** Interactions and self-organization in the soil-microbe complex.  
36 832 *Science* **304**: 1634–1637.
- 37  
38 833 **Zhang X, Guan D, Li W, et al. 2018.** The effects of forest thinning on soil carbon stocks and  
39 834 dynamics: A meta-analysis. *Forest Ecology and Management* **429**: 36–43.
- 40  
41 835 **Zhao C, Miao Y, Yu C, et al. 2016.** Soil microbial community composition and respiration along an  
42 836 experimental precipitation gradient in a semiarid steppe. *Scientific Reports* **6**: 1–9.
- 43  
44 837 **Zhou T, Wang C, Zhou Z. 2020.** Impacts of forest thinning on soil microbial community structure  
45 838 and extracellular enzyme activities: A global meta-analysis. *Soil Biology and Biochemistry* **149**:  
46 839 107915.

50 840

51 841

52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

842 **Figure 1.** Geographic map of the Orleans forest (France) showing the location of the 3 *Quercus* stands  
1  
2 843 (*QS*). The inset shows the Low (L-SD) and Medium SD (M-SD) plots of *QS*.  
3

4  
5 844 **Figure 2.** Soil basal respiration in June 2019 in L-SD (gray bar) and M-SD (dark bar) plots of the 3  
6  
7 845 *Quercus* stands. Values are reported as means  $\pm$  SE (n = 40).  
8  
9

10 846 **Figure 3.** Biomass C (a), biomass N (b) and microbial C:N ratio (c) in L-SD (gray bar) and M-SD  
11  
12 847 (dark bar) plots of the 3 *Quercus* stands. Values are reported as means  $\pm$  SE (n = 4). . corresponds to p  
13  
14 848 less than 0.1.  
15  
16

17 849 **Figure 4.** Microbial genes abundances (number of gene copies per gram of soil) of bacteria (a) of  
18  
19 850 archaea (b), fungi (c), (d) Archaea:Bacteria ratio, and (e) Fungi:Bacteria ratio in L-SD (gray bar) and  
20  
21 851 M-SD (dark bar) plots of the 3 *Quercus* stands. Values are reported as means  $\pm$  SE (n = 4). \* and \*\*  
22  
23 852 correspond to p less than 0.05, and 0.01, respectively.  
24  
25  
26

27 853 **Figure 5.** Functional diversity of SMC (calculated using Shannon index) in L-SD (gray bar) and  
28  
29 854 M-SD (dark bar) plots of the 3 *Quercus* stands. Values are reported as means  $\pm$  SE (n = 24). \*\*\*  
30  
31 855 corresponds to p less than 0,001.  
32  
33

34 856 **Figure 6.** CO<sub>2</sub> production (MSIR) in L-SD (gray bar) and M-SD (dark bar) plots of the 3 *Quercus*  
35  
36 857 stands for each substrate. Values are reported as means  $\pm$  SE (n = 24). \*, \*\*, \*\*\* corresponds to p less  
37  
38 858 than 0.1, 0.05, 0.01 and 0,001 respectively.  
39  
40  
41

42 859 **Table 5.** Correlation coefficients less than -0.5 (red shading according to correlation intensity) and  
43  
44 860 greater than 0.5 (blue shading) with p-value < 0.05 between microbial parameters (row) and  
45  
46 861 environmental parameters (column). The coefficients between -0.5 and 0.5 are uncolored. “*ns*”  
47  
48 862 indicate that there is no significative correlation between the 2-to-2 parameters. Given that soil basal  
49  
50 863 respiration was assessed in 2019, we do not present correlations with variable environmental  
51  
52 864 parameters measured in 2018.  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

865 **Figure S1.** Individual principal component analysis (PCA) of the SMC and environmental parameters  
1  
2 866 according to the plots (L-SD: empty symbols, M-SD: full symbols, QS1: circle, QS2: diamond, QS3 :  
3  
4 867 triangle).  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

**Table 1.** Short review of studies of forest thinning on the soil microbial communities, by measuring microbial biomass, microbial abundance, diversity, and activity. Studies are classified according to responses of microbial parameters (increase, decrease, no effect or variable effect) to thinning. In some cases, we specify which SMC domain (bacteria, archaea or fungi) was affected by forest thinning in the cited study.

Microbial parameters	Responses to thinning			
	Increase	Decrease	No effect	Variable effect
Biomass	(Lei et al., 2021)	(Geng et al., 2012)	(Tan et al., 2008); (Maassen et al., 2006); (Purahong et al., 2014): fungi	(Grady and Hart, 2006): no effect except a <b>decrease</b> in July for microbial C; (Chen et al., 2016): <b>increase</b> under high intensity thinning but no effect of light intensity; (Thibodeau et al., 2000): depends on horizon and C or N biomass; (Kim et al., 2018): <b>increase</b> in only one of the two sites; (Chen et al., 2015): depends on thinning intensity; (Grayston and Rennenberg, 2006): no effect or decrease, depends if forest site faces to North East or South West; (Lin et al., 2016): <b>increase</b> of fungi only in April, no effect for others months (Cai et al., 2020; Wu et al., 2019): <b>increase</b> or no effect, depends on thinning intensity; (Chen et al., 2015): <b>decrease</b> or not effect, depends on thinning intensity; (Dang et al., 2018); (Bastida et al., 2019; Purahong et al., 2014); <b>increase</b> in bacteria only in spring
Abundance				

Diversity	(Trentini et al., 2020) : bacteria and archaea	(Dang et al., 2018); (Trentini et al., 2020): fungi	(Wu et al., 2019): <b>increase</b> or no effect, depends on thinning intensity and season; (Collado et al., 2021): depends on fungal species
Activity		(Kim et al., 2018); (Tan et al., 2008); (Maassen et al., 2006); (Ntoko et al., 2018); (Purahong et al., 2014)	(Geng et al., 2012): depends on enzyme and soil depth; (Chen et al., 2016): <b>decrease</b> or not effect, depends enzyme; (Wu et al., 2019); <b>increase</b> or no effect, depends on date and thinning intensity; (Yang et al., 2017): depends on enzyme and thinning intensity; (Grayston and Rennenberg, 2006): depends on C-substrates, thinning intensity and exposure to North or South; (Bastida et al., 2019): <b>increase</b> or no effect, depends on enzyme and season; (Xiao et al., 2018): <b>increase</b> or no effect, depends on enzyme and litter decomposition phase

**Table 2.** Means of soil chemical properties for each *Quercus* stand (QS) according to the density; L-SD: Low Stand Density, M-SD: Medium Stand Density

Clay (%)	Sand (%)	Silt (%)	OM (%)	C (%)	N (%)	pH water	pH KCl	CEC meq.100g	Ca (mg.kg <sup>-1</sup> )	K (mg.kg <sup>-1</sup> )	Mg (mg.kg <sup>-1</sup> )
----------	----------	----------	--------	-------	-------	----------	--------	--------------	---------------------------	--------------------------	---------------------------

QS1	L-SD	9,1	59,5	29,4	2,09	1,22	0,06	4,95	4,45	2,00	20	50	21
	M-SD	11,6	61,5	24,9	2,01	1,17	0,05	4,86	4,36	2,17	80	46	25cv
QS2	L-SD	9,6	67,5	21,4	1,56	0,91	0,04	4,97	4,47	2,00	20	38	25
	M-SD	10,1	69,1	19,0	1,94	1,13	0,06	4,89	4,39	2,52	60	46	23
QS3	L-SD	13,7	63,6	21,8	0,93	0,54	0,04	4,95	4,45	4,19	260	57	86
	M-SD	8,2	76,1	15,0	0,74	0,43	0,03	5,17	4,67	2,00	60	26	14

**Table 3.** Plot characteristics at the end of the 2017 growing season after the last thinning. Density: plot density. BA tot.: total stand basal area, Dg: quadratic mean diameter, Ho: dominant height, BA exp. 2017: exported basal area (m<sup>3</sup>/ha) in the last thinning, BA exp. tot.: exported basal area (m<sup>3</sup>/ha) since 2012, V.cut tot.: exported volume (m<sup>3</sup>) since 2012, SD: Stand Density index after thinning.

Plot	Dimension (m)	Density	SD	V tot (m <sup>3</sup> /ha)	BA tot. (m <sup>2</sup> /ha)	Dg oak (cm)	Ho oak (m)	BA exp. tot.	V exp. tot.
QS1	50 x 100	L-SD	0.35	153.75	12.8	23.6	20.2	7.2	78.3
	70 x 70	M-SD	0.59	263.12	21.5	23.4	21.5	0	0
QS2	50 x 100	L-SD	0.35	145.97	12.8	24.1	18.6	7.4	68.6
	50 x 100	M-SD	0.53	218.67	19.9	20.5	18.6	0.6	0
QS3	60 x 80	L-SD	0.35	167.81	12.6	28.9	22.0	11.4	138.4
	60 x 80	M-SD	0.60	273.76	21.9	25.6	21.0	1.6	18.2

**Table 4. Primers, sequences of total bacterial, archeal and fungal communities using targets (16S rDNA or 18S rDNA primers) according to cited references.**

Primer	Sequence	Target	Reference
C341F:	5' CCT ACG GGA GGC AGC AG 3'	Bacterial 16S rRNA gene	López-Gutiérrez et al., 2004
C515R:	5' ATT ACC GCG GCT GCT GGC A 3'		López-Gutiérrez et al., 2004
ch519F	5'-CAG CCG CCG CGG TAA-3'	Archaeal 16S rRNA gene	Øvreås et al., 1997
c915R	5'-GTGCTCCCCCGC CAATTCCT-3'		Casamayor et al., 2000
FR14	5'-AIC-CAT- TCA-ATC-GGT-AIT-3'	Fungal 18S rRNA gene	Vainio and Hantula, 2000
F390	5'-CGA-TAA-CGA-ACG-AGA-CCT-3'		Vainio and Hantula, 2000

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65



## Appendix

**n<sub>bio</sub>** Number of biological replicates

**n<sub>tech</sub>** Number of technical replicates

### Appendix 1. Soil fumigation

Fumigation was done by exposing 5 g of fresh soil to chloroform vapors for 24 hours in a sealed vacuum. Chloroform vapors act as solvent extracting polar lipid molecules that compose microbial cell membranes, degrading the cell walls and releasing internal organic compounds into the soil (Vance *et al.* 1987). For both fumigated and non-fumigated samples, organic C and N were extracted from a 5 g soil sample into solution using 20 ml of a K<sub>2</sub>SO<sub>4</sub> buffer (0.5 M). Samples were placed on a shaking table at 250 rpm for 30 minutes to thoroughly mix the soil with the solvent and dissolve all organic C and N. The solution was passed through a Whatman GF/C glass microfiber filter into a Falcon tube to remove any soil particles and impurities, and the clear solution was frozen and sent to the INRAE Agronomy and Environment Lab in Nancy (France) for quantification of organic C and total N (TOC analyzer, (TOC-VCSH CSH/CNS, Shimadzu, Champs-sur-Marne, France) connected online to a N analyzer (TNM-1, Shimadzu)). The calculations of soil microbial biomass C and N were revised by a conversion factor of 2.22 (Jenkinson *et al.* 2004).

### Appendix 2. DNA extraction and PCR

Total DNA was extracted and purified from 500 mg of soil using the NucleoSpin Soil kit and the NucleoSpin gDNA clean-up kit (Macherey-Nagel, NucleoSpin Soil and NucleoSpin gDNA clean-up, 2017), according to manufacturer's instructions. The DNA quality was assessed by spectrophotometry (Biotek Eon spectrophotometer and Take3 plate), and DNA concentration was assessed by fluorimetry (QuBit dsDNA BR Assay Kit, Thermofisher).

Reactions were carried out in a Applied Biosystems Step One Plus qPCR System, with a 20 µL reaction volume containing 10 µl of 2X SsoAdvanced Universal SYBR Green Supermix (Biorad), 1 µL of each primer (at 10 µM for bacteria and archaea and 20 µM for fungi) 1.25 µl of BSA (2 mg ml<sup>-1</sup>), and 2 µl of template DNA at 0.2 ng/µL, so 0.4 ng of DNA. At least four independent runs were performed for each qPCR assay. Standard curves were obtained using serial dilutions of linearized plasmids containing the studied genes respectively amplified from *Pseudomonas fluorescens* and *Nitrososphaera viennensis* (16SDNA sequences), and *Trametes versicolor* (18SDNA sequence). PCR efficiency for the different assays ranged from 85 to 102% with R<sup>2</sup> > 0.9. No-template controls gave null or negligible values. The specificity of amplified products was verified by melting curves from 65 °C to 95 °C at 0.5 °C. Inhibition in qPCR assay was tested by using 10-fold serial dilutions of the DNA template, from 2ng to 0.02 ng.

**Table A.1 (Figure 2).** Means ± SE of soil respiration *in situ* (µmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>) in low stand density (L-SD) and medium stand density (M-SD) for each *Quercus* stand (QS). Test statistic (F-value and t-value), statistical significance (p-value), and degrees of freedom (DF) assessing the effect of density on the microbial parameters (ANOVA, α = 5%, n<sub>bio</sub> = 3, n<sub>tech</sub> = 4).

	Soil respiration	
	L-SD	M-SD
QS1	3.17 ± 0.24	3.14 ± 0.20
QS2	3.65 ± 0.22	3.75 ± 0.30
QS3	4.07 ± 0.37	3.70 ± 0.27
QS mean ± SE	3.64 ± 0.17	3.54 ± 0.15
F-value		0.28
p-value		0.6

**Table A.2 (Figure 3).** Means  $\pm$  SE of soil microbial carbon (SMB-C), nitrogen (SMB-N) biomass and microbial C:N ratio in low stand density (L-SD) and medium stand density (M-SD) for each *Quercus* stand (QS). Test statistic (F-value and t-value), statistical significance (p-value), and degrees of freedom (DF) assessing the effect of density on the microbial parameters (ANOVA,  $\alpha = 5\%$ ,  $n_{\text{bio}} = 3$ ,  $n_{\text{tech}} = 4$ ).

	SMB-C		SMB-N		Microbial C:N	
	L-SD	M-SD	L-SD	M-SD	L-SD	M-SD
QS1	1197.78 $\pm$ 172.02	772.34 $\pm$ 32.23	97.91 $\pm$ 5.63	66.11 $\pm$ 2.42	12.08 $\pm$ 1.18	11.71 $\pm$ 0.51
QS2	916.24 $\pm$ 28.63	603.35 $\pm$ 17.29	56.25 $\pm$ 5.55	57.19 $\pm$ 5.16	16.66 $\pm$ 1.30	10.79 $\pm$ 0.75
QS3	461.40 $\pm$ 24.94	557.54 $\pm$ 67.58	22.40 $\pm$ 2.24	29.30 $\pm$ 2.08	20.97 $\pm$ 1.53	18.89 $\pm$ 1.27
QS mean $\pm$ SE	858.47 $\pm$ 105.78	644.41 $\pm$ 36.23	58.86 $\pm$ 9.64	50.81 $\pm$ 5.06	10.60 $\pm$ 0.89	10.39 $\pm$ 0.66
F-value	3.67		0.55		0.03	
p-value	0.07 .		0.47		0.86	
DF	1		1		1	

**Table A.3 (Figure 4).** Means  $\pm$  SE of microbial abundance (gene copies) in low stand density (L-SD) and medium stand density (M-SD) for each *Quercus* stand (QS). Test statistic (F-value and t-value), statistical significance (p-value), and degrees of freedom (DF) assessing the effect of density on the microbial parameters

	Bacteria		Archaea		Fungi	
	L-SD	M-SD	L-SD	M-SD	L-SD	M-SD
QS1	2151501302 $\pm$ 199764267	3293263912 $\pm$ 311980198	1221883845 $\pm$ 69613400	1613868051 $\pm$ 102785864	45296161 $\pm$ 3765312	56889559 $\pm$ 7721492
QS2	1956832371 $\pm$ 331210533	4230976158 $\pm$ 933578277	1167612591 $\pm$ 153491846	1999268248 $\pm$ 230888870	41448723 $\pm$ 6177293	81238894 $\pm$ 6744637
QS3	1222727816 $\pm$ 195485152	1777285000 $\pm$ 153689256	746004078 $\pm$ 218229599	803024671 $\pm$ 74483305	32064082 $\pm$ 5977364	40676771 $\pm$ 1127581
QS mean $\pm$ SE	1777020496 $\pm$ 177803981	3100508357 $\pm$ 427947657	1045166838 $\pm$ 104997837	1472053657 $\pm$ 170020804	39602988 $\pm$ 3288411	59601742 $\pm$ 5910509
F-value	9.57		4.56		8.74	
p-value	0.005 **		0.04 *		0.007 **	
DF	1		1		1	

(ANOVA,  $\alpha = 5\%$ ,  $n_{\text{bio}} = 3$ ,  $n_{\text{tech}} = 4$ ).

	A:B ratio		F:B ratio	
	L-SD	M-SD	L-SD	M-SD
QS1	0.58 $\pm$ 0.05	0.50 $\pm$ 0.02	0.02 $\pm$ 0.0003	0.02 $\pm$ 0.0009
QS2	0.61 $\pm$ 0.03	0.52 $\pm$ 0.07	0.02 $\pm$ 0.0007	0.02 $\pm$ 0.005
QS3	0.58 $\pm$ 0.07	0.46 $\pm$ 0.04	0.03 $\pm$ 0.002	0.02 $\pm$ 0.003
QS mean $\pm$ SE	0.59 $\pm$ 0.03	0.49 $\pm$ 0.03	0.023 $\pm$ 0.001	0.021 $\pm$ 0.001
F-value	6.52		0.83	
p-value	0.02 *		0.3	
DF	1		1	

**Table A.4 (Figure 5).** Means  $\pm$  SE of functional diversity of SMC in low stand density (L-SD) and medium stand density (M-SD) for each *Quercus* stand (QS). Test statistic (F-value and t-value), statistical significance (p-value), and degrees of freedom (DF) assessing the effect of density on the microbial parameters (ANOVA,  $\alpha = 5\%$ ,  $n_{\text{bio}} = 3$ ,  $n_{\text{tech}} = 6$ ).

		Functional diversity of SMC (Shannon index)	
		L-SD	M-SD
	QS1	1.98 $\pm$ 0.05	2.39 $\pm$ 0.04
	QS2	1.78 $\pm$ 0.03	2.20 $\pm$ 0.05
	QS3	2.30 $\pm$ 0.05	2.45 $\pm$ 0.03
	QS mean $\pm$ SE	2.02 $\pm$ 0.04	2.34 $\pm$ 0.03
	F-value		52.11
	p-value		< 0.001 ***
	DF		1

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65



16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

**Table A.6.** Means  $\pm$  SE of environmental factors in low stand density (L-SD) and medium stand density (M-SD) for each *Quercus* stand (QS). Precipitation data showed the sum of rainfall for the last 30 days before harvest, not the mean. Test statistic (F-value and t-value), statistical significance (p-value), and degrees of freedom (DF) assessing

	Stand density						Hydric properties									
	SD		Final volume of standing trees.ha <sup>-1</sup>		Total volume of cutting trees.ha <sup>-1</sup>		Precipitations (-D30)		Soil water content (-D30)		Perched water table depth (D-30)		Perched water table depth (D0)			
	L-SD	M-SD	L-SD	M-SD	L-SD	M-SD	L-SD	M-SD	L-SD	M-SD	L-SD	M-SD	L-SD	M-SD		
QS1	0.35	0.59	153.75	263.12	78.27	0	274.0	268.6	31.09	27.38	60.37	60.4	44.21	44.35		
QS2	0.35	0.53	145.97	218.69	68.61	0	260.8	224.0	38.26	21.71	20.5	71.64	11.95	65.89		
QS3	0.35	0.60	167.81	273.76	138.38	18.23	73.8	152.8	56.51	29.03	37.99	36.3	51.46	71.27		
QS mean $\pm$ SE	0.35 $\pm$ 0.00	0.57 $\pm$ 0.00	156.07 $\pm$ 1.19	251.86 $\pm$ 2.83	96.90 $\pm$ 4.00	6.08 $\pm$ 1.02	195.21 $\pm$ 11.63	215.13 $\pm$ 5.66	43.12 $\pm$ 1.33	26.04 $\pm$ 0.37	37.39 $\pm$ 1.95	56.11 $\pm$ 1.75	34.97 $\pm$ 2.23	60.50 $\pm$ 1.38		
F-value	3860		903.9		530.2		2.52		167.4		51.38		98.73			
p-value	<0.001 ***		<0.001 ***		<0.001 ***		0.12		<0.001 ***		<0.001 ***		<0.001 ***			
DF	1		1		1		1		1		1		1			
	Soil physicochemical properties						Soil temperature (-D30)		Litter		Vegetation cover of understory species					
	Organic matter		OH thickness		Cation Exchange Capacity		Soil temperature (-D30)		Leaf litter mass (-D30)		<i>Calluna vulgaris</i>		<i>Molinia caerulea</i>		<i>Rubus fructose</i>	
	L-SD	M-SD	L-SD	M-SD	L-SD	M-SD	L-SD	M-SD	L-SD	M-SD	L-SD	M-SD	L-SD	M-SD	L-SD	M-SD
QS1	2.09	2.01	8.0	0.6	2.00	2.17	15.55	14.95	3.43	4.67	0.27	0.16	0.06	0.41	0.8	0.10
QS2	1.60	1.96	15.7	16.9	2.00	2.00	15.84	15.01	1.93	7.12	19.40	1.33	6.06	0.09	0.03	0.03
QS3	0.93	0.75	0	0	3.31	2.00	15.79	14.82	10.35	4.57	0.73	0.81	3.67	2.10	0.58	0.12
QS mean $\pm$ SE	1.48 $\pm$ 0.06	1.57 $\pm$ 0.07	7.89 $\pm$ 0.84	5.83 $\pm$ 0.93	2.48 $\pm$ 0.08	2.06 $\pm$ 0.01	15.75 $\pm$ 0.01	14.93 $\pm$ 0.009	5.43 $\pm$ 0.48	5.45 $\pm$ 0.14	7.50 $\pm$ 1.14	0.76 $\pm$ 0.06	3.61 $\pm$ 0.29	0.87 $\pm$ 0.10	0.25 $\pm$ 0.03	0.08 $\pm$ 0.005
F-value	1.03		2.64		31.8		2267		0.002		38.72		83.37		27.36	
p-value	0.31		0.11		<0.001 ***		<0.001 ***		0.96		<0.001 ***		<0.001 ***		<0.001 ***	
DF	1		1		1		1		1		1		1		1	

1 the effect of density on the microbial parameters (ANOVA,  $\alpha = 5\%$ ,  $n_{bio} = 3$ ,  $n_{tech} = 24$  and 36

Figure 1

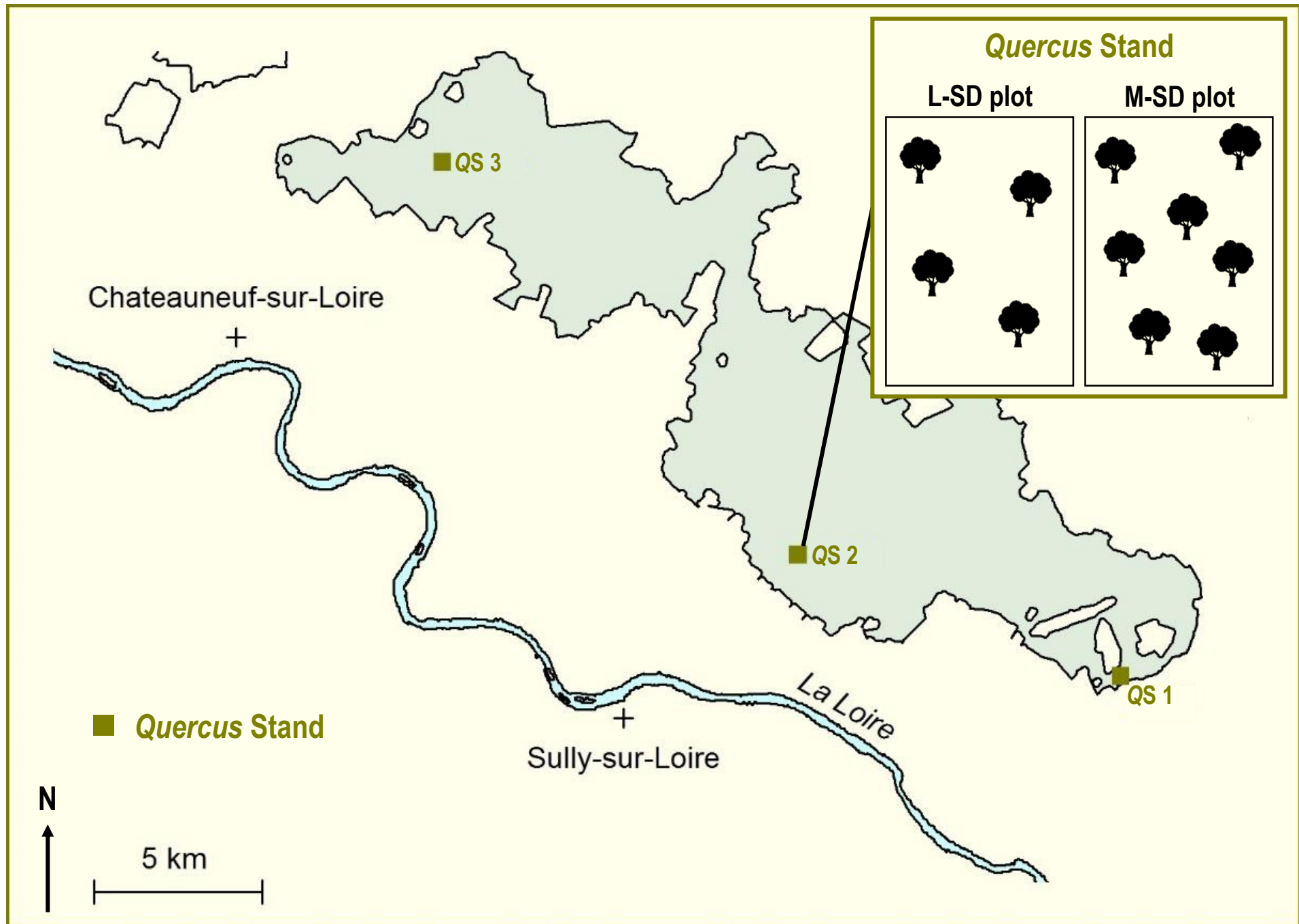
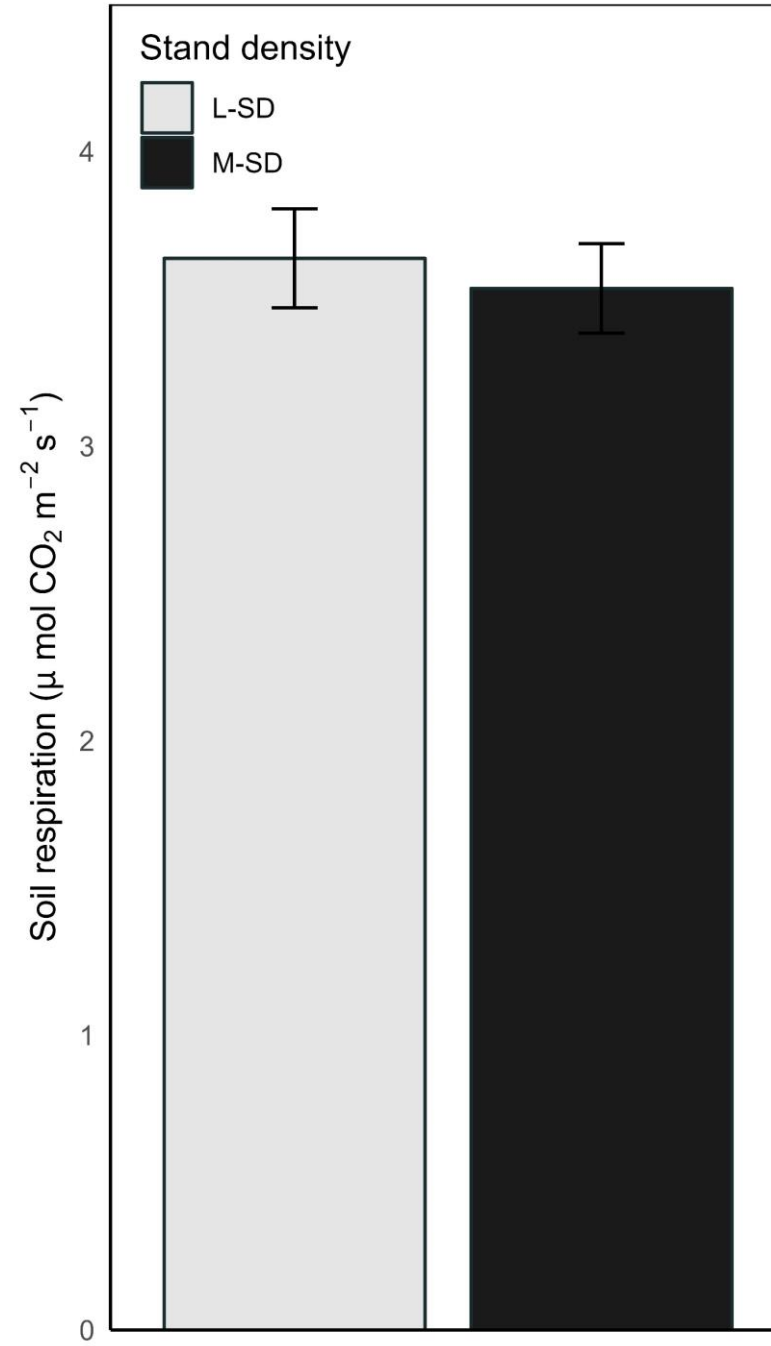
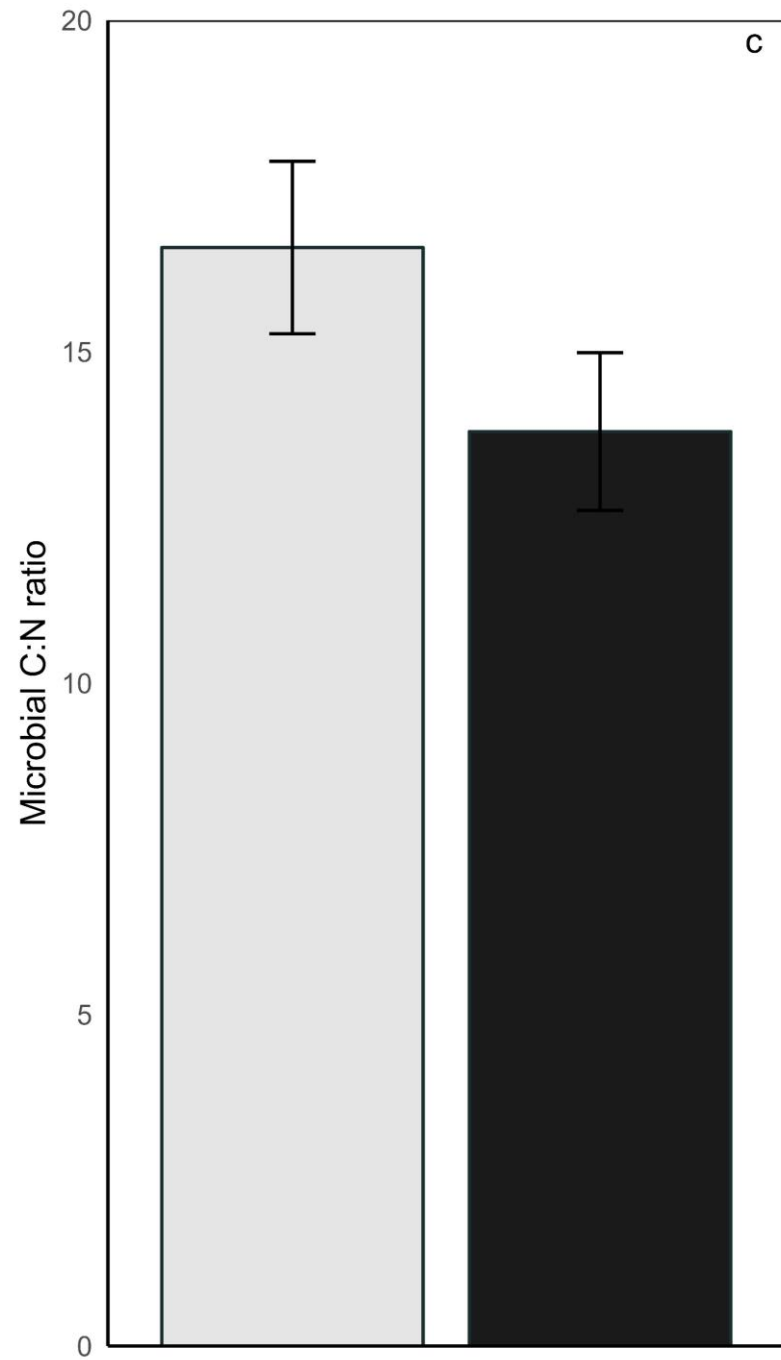
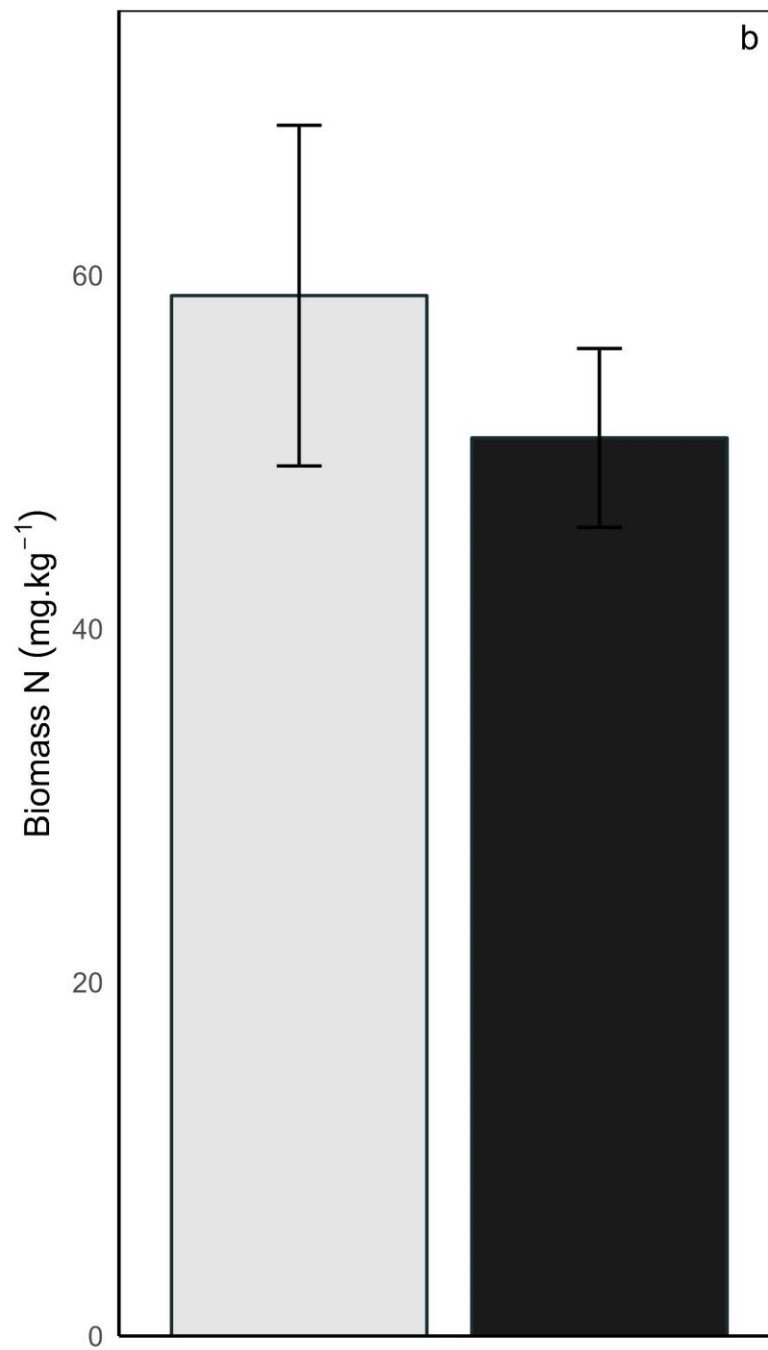
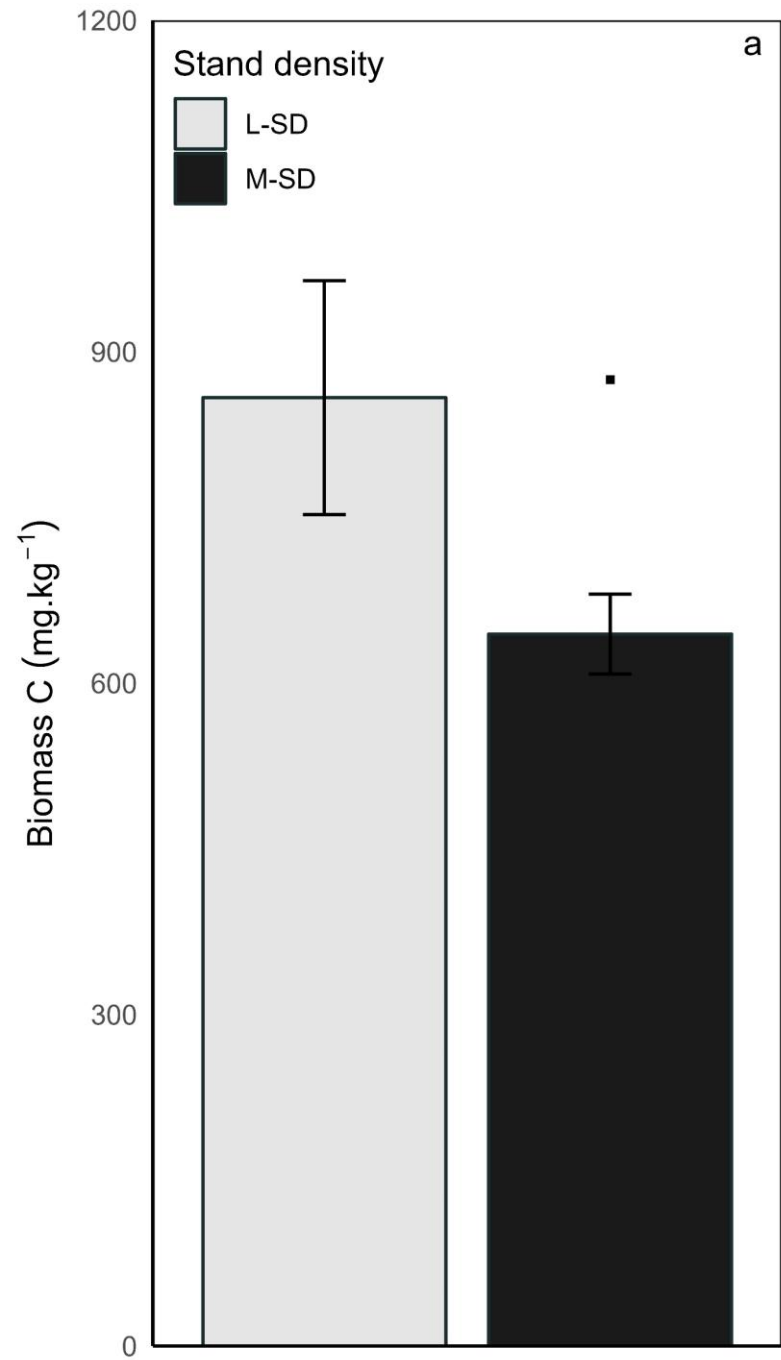


Figure 2



**Figure 3**





**Figure 4**

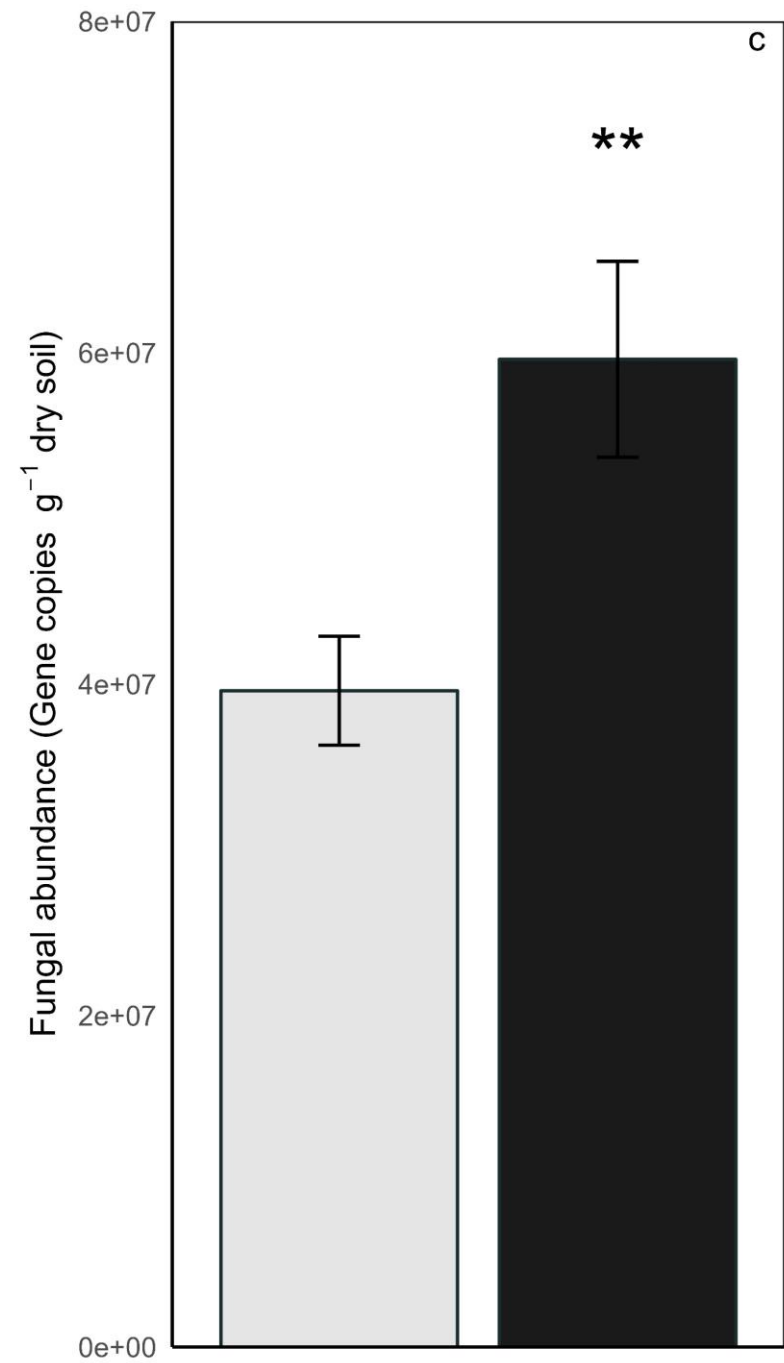
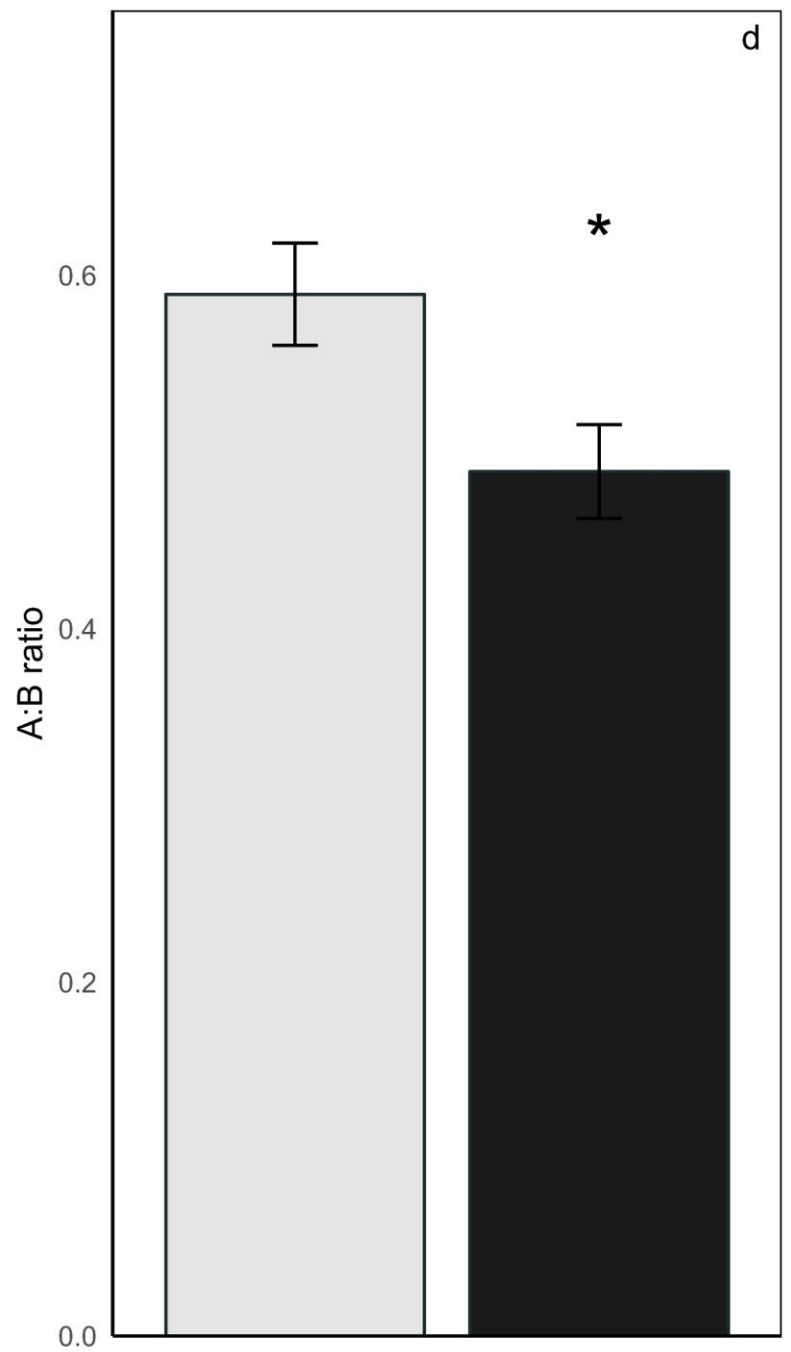
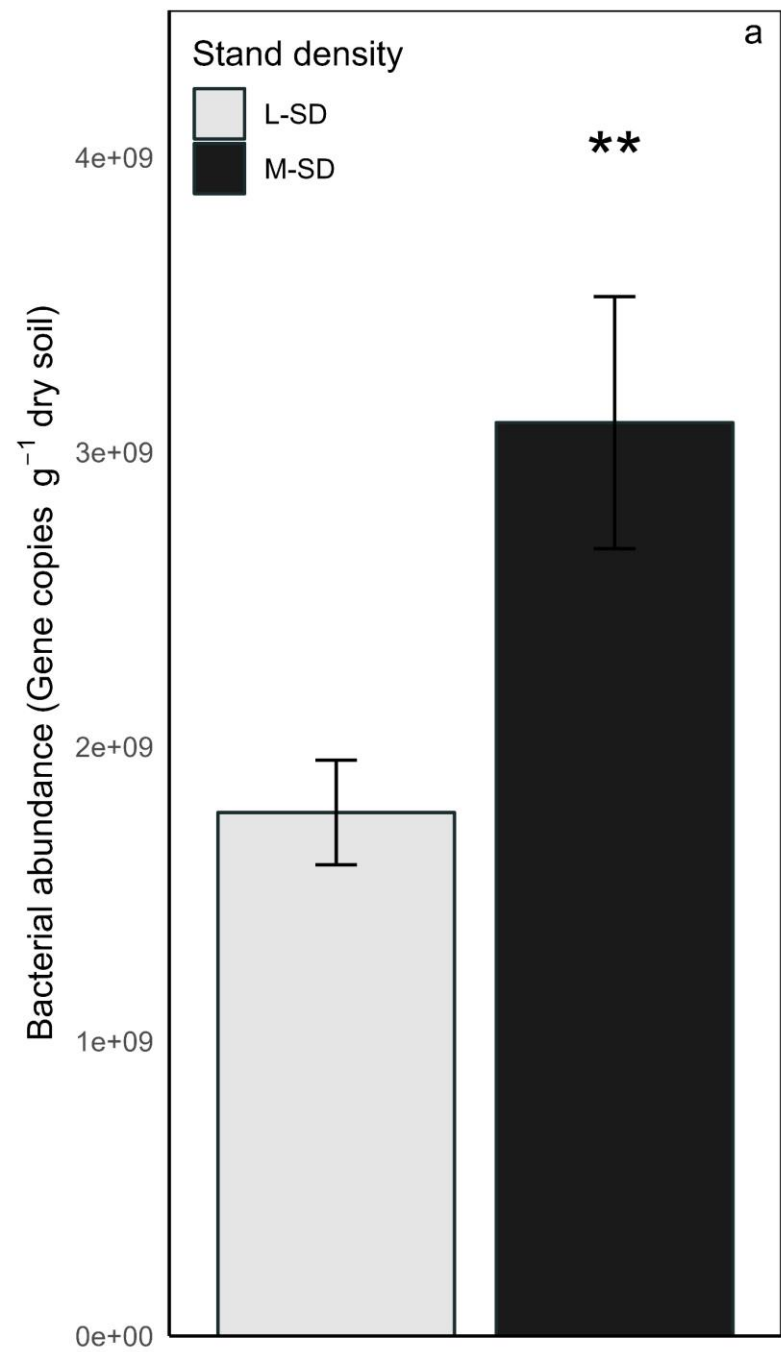


Figure 4

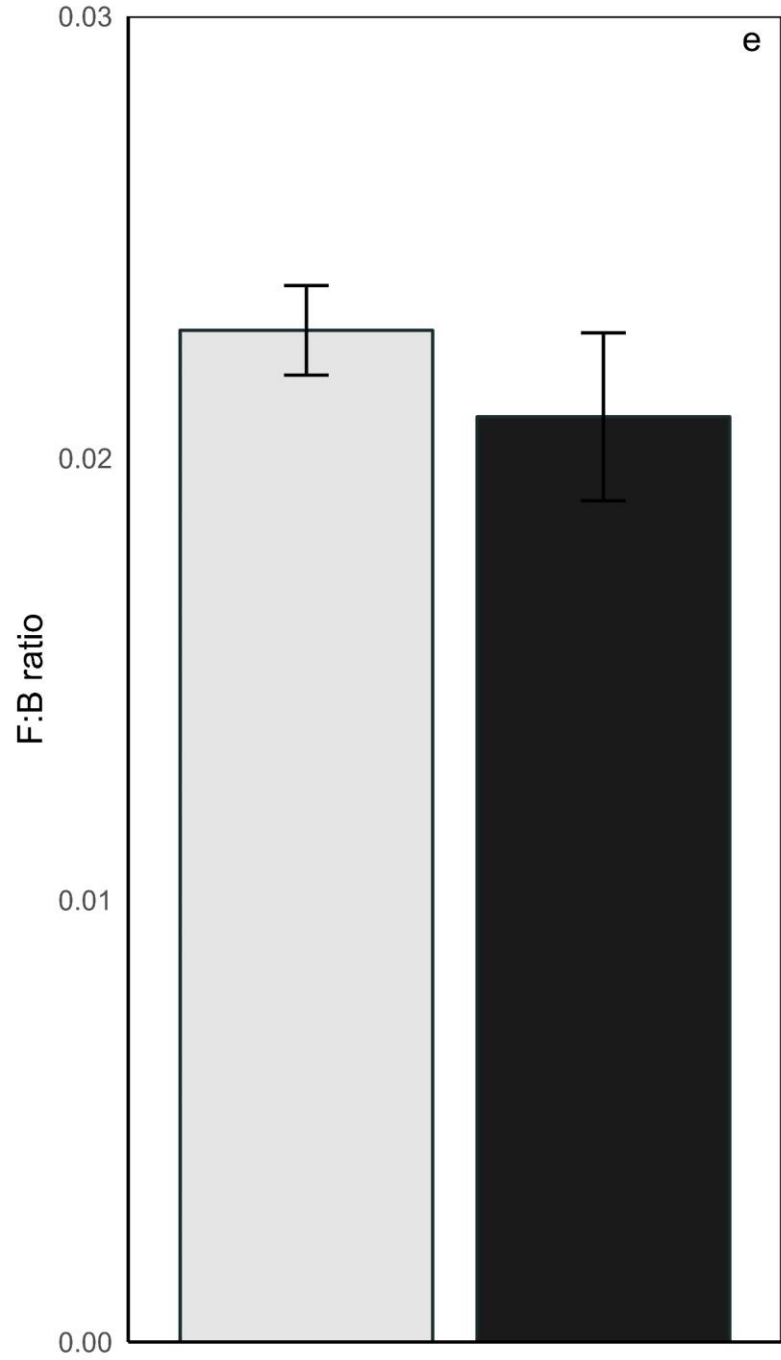
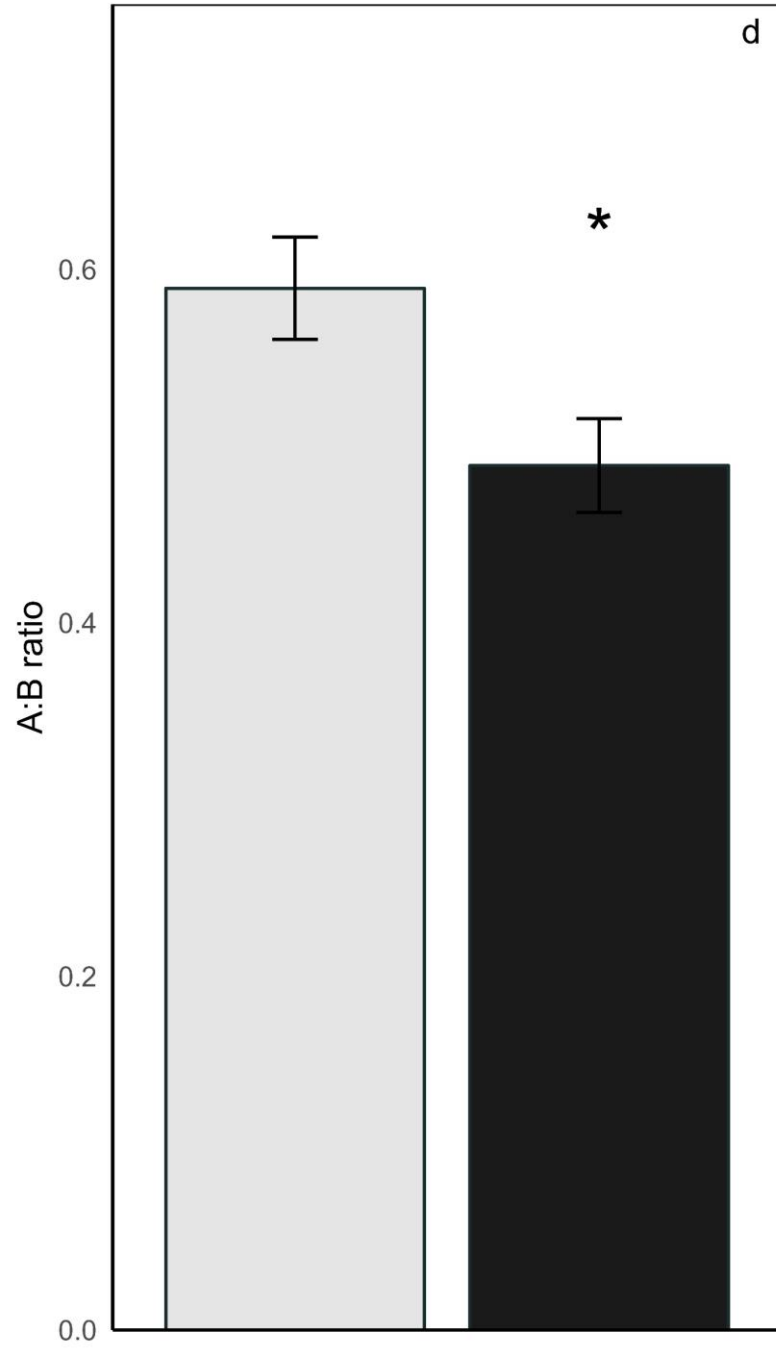
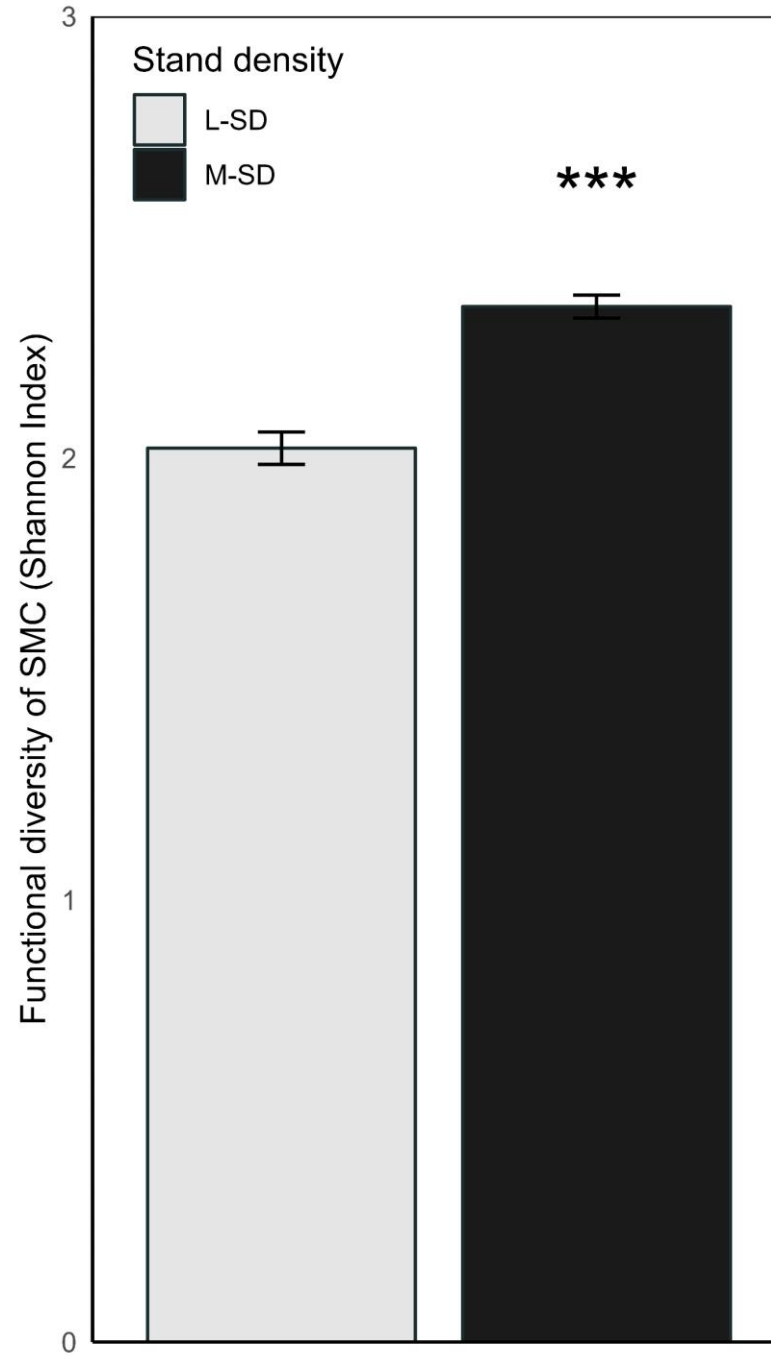


Figure 5



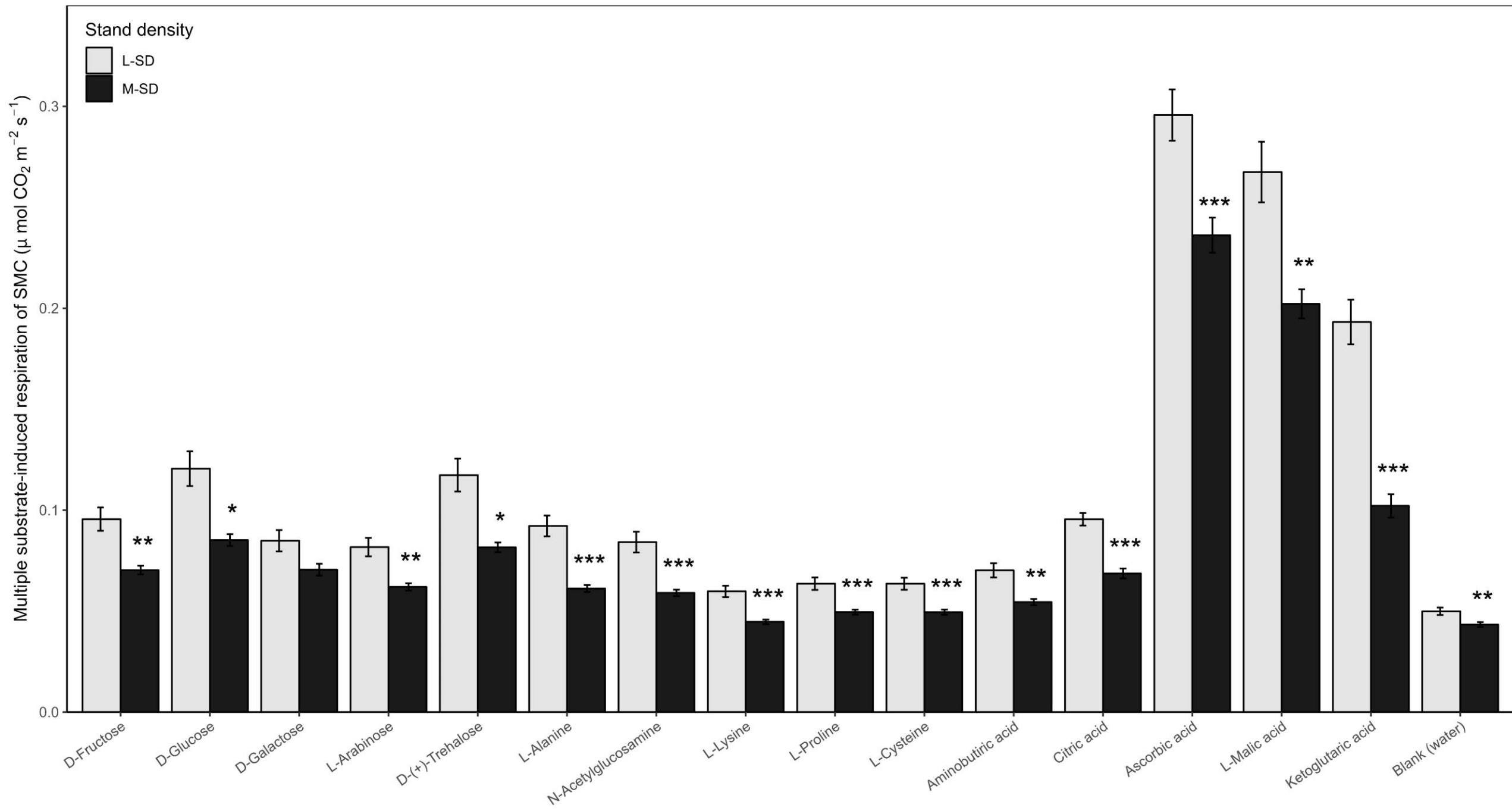
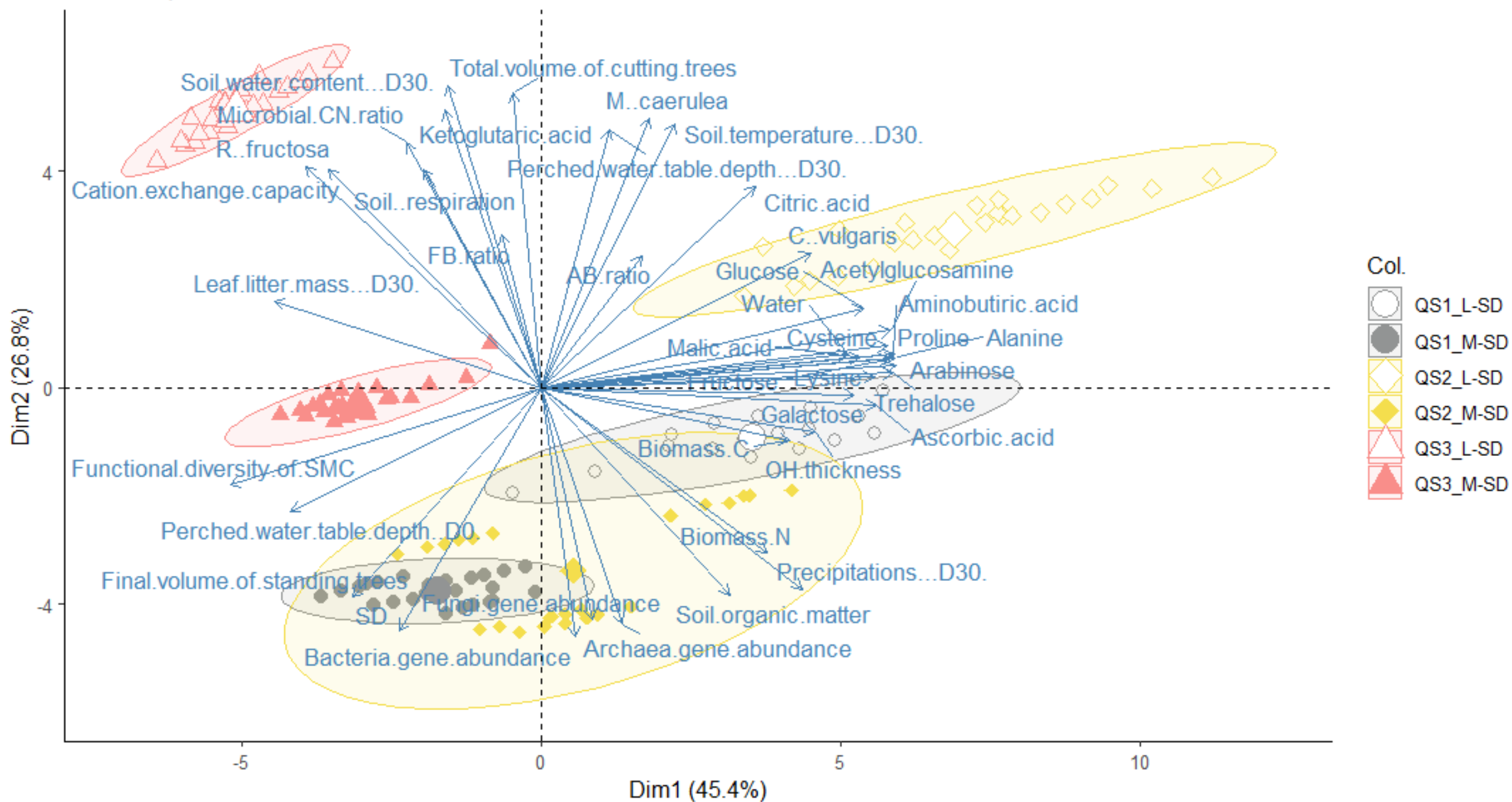
**Figure 6**

Table 5		Stand characteristics			Hydric properties			Soil physicochemical properties				Litter mass		Vegetation cover of understory species		
		Stand density	Final volume of standing trees.ha <sup>-1</sup>	Total volume of cutting trees .ha <sup>-1</sup>	Precipitations (-D30)	Soil water content (-D30)	Perched water table depth (-D30)	Perched water table depth (D0)	Organic matter	OH thickness	Cation Exchange Capacity	Soil temperature (-D30)	Leaf litter mass (-D30)	<i>Calluna vulgaris</i>	<i>Molinia caerulea</i>	<i>Rubus fruticosus</i>
Respiration <i>in situ</i>	Soil basal respiration (June 2019)	<i>ns</i>	<i>ns</i>	0.49***				-0.70***	<i>ns</i>	0.59***			<i>ns</i>	0.50***	0.60***	
	Biomass C	<i>ns</i>	-0.37**	<i>ns</i>	0.71***	<i>ns</i>	<i>ns</i>	-0.53***	0.57***	<i>ns</i>	-0.47***	<i>ns</i>	-0.66***	<i>ns</i>	-0.49***	
Microbial biomass	Biomass N	<i>ns</i>		<i>ns</i>	0.84***	-0.50***	0.46***	<i>ns</i>	0.85***	0.39**	-0.55***	<i>ns</i>	-0.54***	<i>ns</i>	-0.59***	
	Microbial C:N ratio	<i>ns</i>	<i>ns</i>	0.59***	-0.74***	0.70***	-0.71***	<i>ns</i>	-0.83***	-0.41**	0.46***	0.35*	0.41**	<i>ns</i>	0.62***	0.62***
Microbial gene abundance	Bacterial	0.42**	<i>ns</i>	-0.62***	0.44***	-0.63***	0.63***	<i>ns</i>	0.58***	0.38**	-0.41***	-0.45***	<i>ns</i>	<i>ns</i>	-0.52***	-0.47***
	Archaeal	<i>ns</i>	<i>ns</i>	-0.58***	0.54***	-0.61***	0.64***	<i>ns</i>	0.71***	0.49***	-0.42***	-0.34*	<i>ns</i>	<i>ns</i>	-0.48***	-0.51***
	Fungal	0.41**	<i>ns</i>	-0.62***	0.39**	-0.65***	0.67***	<i>ns</i>	0.56***	0.47***	-0.41***	-0.46***	<i>ns</i>	<i>ns</i>	-0.54***	-0.48***
	A:B ratio	-0.49***	-0.50***	0.38**	<i>ns</i>	<i>ns</i>	<i>ns</i>	-0.39**	<i>ns</i>	<i>ns</i>	<i>ns</i>	0.50***	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>
	F:B ratio	<i>ns</i>	<i>ns</i>	0.34*	-0.46***	0.34*	<i>ns</i>	<i>ns</i>	-0.40**	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	0.35*
Functional diversity and activity (multiple substrate induced-respiration)	Functional diversity	0.57***	0.64***	<i>ns</i>	-0.38**	<i>ns</i>	<i>ns</i>	0.62***	<i>ns</i>	-0.58***	<i>ns</i>	-0.55***	0.40**	-0.63***	-0.39**	<i>ns</i>
	Fructose	-0.37**	-0.48***	<i>ns</i>	0.58***	<i>ns</i>	<i>ns</i>	-0.61***	0.37**	0.67***	-0.52***	0.35*	-0.67***	0.70***	0.33*	-0.56***
	Glucose	-0.36**	-0.46***	<i>ns</i>	0.44***	<i>ns</i>	-0.47***	-0.65***	<i>ns</i>	0.63***	-0.44***	0.39**	-0.63***	0.83***	0.55***	-0.48***
	Galactose	<i>ns</i>	<i>ns</i>	<i>ns</i>	0.62***	<i>ns</i>	<i>ns</i>	-0.59***	0.43**	0.52***	-0.49***	<i>ns</i>	-0.63***	0.60***	<i>ns</i>	-0.54***
	Arabinose	-0.38**	-0.50***	<i>ns</i>	0.60***	<i>ns</i>	<i>ns</i>	-0.62***	0.41**	0.73***	-0.53***	0.36**	-0.65***	0.72***	0.33*	-0.58***
	Trehalose	-0.38**	-0.48***	<i>ns</i>	0.64***	<i>ns</i>	<i>ns</i>	-0.64***	0.45***	0.63***	-0.52***	0.35*	-0.68***	0.67***	<i>ns</i>	-0.56***
	Alanine	-0.49***	-0.59***	<i>ns</i>	0.62***	<i>ns</i>	<i>ns</i>	-0.64***	0.44***	0.69***	-0.51***	0.44***	-0.67***	0.67***	<i>ns</i>	-0.55***
	Acetylglucosamine	-0.42**	-0.53***	<i>ns</i>	0.55***	<i>ns</i>	-0.37**	-0.70***	<i>ns</i>	0.72***	-0.48***	0.43**	-0.66***	0.83***	0.49***	-0.54***
	Lysine	-0.44***	-0.55***	<i>ns</i>	0.57***	<i>ns</i>	<i>ns</i>	-0.47***	0.42**	0.66***	-0.53***	0.35*	-0.62***	0.52***	<i>ns</i>	-0.55***
	Proline	-0.40**	-0.52***	<i>ns</i>	0.56***	<i>ns</i>	<i>ns</i>	-0.60***	0.37**	0.74***	-0.51***	0.37**	-0.63***	0.72***	0.35*	-0.56***
	Cysteine	-0.39**	-0.51***	<i>ns</i>	0.52***	<i>ns</i>	<i>ns</i>	-0.58***	<i>ns</i>	0.71***	-0.51***	0.37**	-0.64***	0.72***	0.37**	-0.55***
	Aminobutiric	-0.39**	-0.51***	<i>ns</i>	0.57***	<i>ns</i>	<i>ns</i>	-0.64***	0.37**	0.74***	-0.51***	0.38**	-0.64***	0.77***	0.39**	-0.57***
	Citric	-0.51***	-0.52***	0.39**	<i>ns</i>	0.32	-0.59***	-0.43***	<i>ns</i>	0.34*	<i>ns</i>	0.53***	<i>ns</i>	0.61***	0.64***	<i>ns</i>
	Ascorbic	-0.40**	-0.53***	<i>ns</i>	0.65***	<i>ns</i>	<i>ns</i>	-0.58***	0.61***	0.74***	-0.45***	0.35*	-0.51***	0.56***	<i>ns</i>	-0.53***
	Malique	-0.35*	-0.43***	<i>ns</i>	0.55***	<i>ns</i>	<i>ns</i>	-0.63***	0.36**	0.54***	-0.42**	0.35*	-0.60***	0.65***	0.33*	-0.47***
Ketoglutarique	-0.5***	-0.39**	0.79***	-0.77***	0.82***	-0.49***	<i>ns</i>	-0.67***	<i>ns</i>	0.75***	0.54***	0.54***	<i>ns</i>	0.49***	0.79***	
Water	<i>ns</i>	-0.42**	<i>ns</i>	0.39**	<i>ns</i>	<i>ns</i>	-0.38**	<i>ns</i>	0.70***	-0.47***	<i>ns</i>	-0.48***	0.60***	<i>ns</i>	-0.49***	

Figure A.1

PCA - Biplot



1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

**Declaration of interests**

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

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