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## ► To cite this version:

Laurent Bordez, Yvon Cavaloc, Philippe Jourand, Marc Ducouso, Farid Juillot, et al.. Effects of ultramafic topsoil stockpiling during mine activities on its microbial diversity and other microbiological and physicochemical characteristics. *Ecological Engineering*, 2022, 177, pp.106563. 10.1016/j.ecoleng.2022.106563 . hal-03581999

**HAL Id: hal-03581999**

**<https://hal.science/hal-03581999>**

Submitted on 1 Mar 2022

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# Ecological Engineering

## Effects of ultramafic topsoil stockpiling during mine activities on its microbiota diversity and other characteristics

--Manuscript Draft--

<b>Manuscript Number:</b>	
<b>Article Type:</b>	Research Paper
<b>Keywords:</b>	ultramafic soils; topsoil; microbiota diversity; bacteria; Fungi; AMF
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<b>Abstract:</b>	<p>Nowadays, the ecological restoration is considered as the best solution for the rehabilitation of mining-degraded areas, particularly when it concerns valuable ecosystems. This is the case in New Caledonia considered as a hot spot of biodiversity, with 82% of endemic plant species in ultramafic soils. The use of topsoil to restore mined areas is an important practice. However, topsoil stockpiling can reduce soil fertility. We studied the evolution of different properties of two topsoils stored in 10 m height piles on mined areas, focusing particularly on the microbiota diversity. The effects of storage duration, depth and topsoil origin were analysed. The studied ultramafic soils were characterized by a relatively high microbiota diversity with, for all tested samples, a total of 53 315 bacterial OTUs corresponding to 45 phyla, and 21 486 fungal OTUs corresponding to 7 phyla. Bacterial and fungal general diversity were not affected after 12 months of storage, but community structures of these groups were changed. Arbuscular mycorrhizal fungi (AMF) diversity was significantly reduced by the storage. Microbial activity, AMF spore numbers, soil organic carbon, total nitrogen, cation exchange capacity and C/N decreased significantly starting from 3 to 9 months of topsoil storage, whereas soil bulk density and pH increased, and extractable concentrations of potentially toxic metals varied only slightly. These changes may affect negatively microbial functions of the soils, in relation to ecosystem diversity. It is then recommended to reduce to a minimum possible the topsoil storage before its use in ecosystem restoration.</p>
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## **COVER LETTER**

This research article is about changes that occur in ultramafic topsoils during stockpiling operations before ecological restoration of nickel mining-degraded areas.

It concerns biosphere (ecosystem restoration), anthroposphere (mining activity) and lithosphere (ultramafic rocks and nickel ore).

It is a global ecological approach using a multifactorial microbiological and physicochemical characterization of soils evolution during their storage in mines. It is then a contribution to environmental remediation of anthropogenic degradations, including the reduction of erosion and lagoon pollution.

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**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:

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41

42 **ABSTRACT**

43 Nowadays, the ecological restoration is considered as the best solution for the  
44 rehabilitation of mining-degraded areas, particularly when it concerns valuable  
45 ecosystems. This is the case in New Caledonia considered as a hot spot of biodiversity,  
46 with 82% of endemic plant species in ultramafic soils. The use of topsoil to restore  
47 mined areas is an important practice. However, topsoil stockpiling can reduce soil  
48 fertility. We studied the evolution of different properties of two topsoils stored in 10

49 m height piles on mined areas, focusing particularly on the microbiota diversity. The  
50 effects of storage duration, depth and topsoil origin were analysed. The studied  
51 ultramafic soils were characterized by a relatively high microbiota diversity with, for all  
52 tested samples, a total of 53 315 bacterial OTUs corresponding to 45 phyla, and 21 486  
53 fungal OTUs corresponding to 7 phyla. Bacterial and fungal general diversity were not  
54 affected after 12 months of storage, but community structures of these groups were  
55 changed. Arbuscular mycorrhizal fungi (AMF) diversity was significantly reduced by the  
56 storage. Microbial activity, AMF spore numbers, soil organic carbon, total nitrogen,  
57 cation exchange capacity and C/N decreased significantly starting from 3 to 9 months  
58 of topsoil storage, whereas soil bulk density and pH increased, and extractable  
59 concentrations of potentially toxic metals varied only slightly. These changes may  
60 affect negatively microbial functions of the soils, in relation to ecosystem diversity. It is  
61 then recommended to reduce to a minimum possible the topsoil storage before its use  
62 in ecosystem restoration.

63

64 **Keywords :** Ultramafic soils, topsoil, microbiota diversity, bacteria, fungi, AMF

65



## 66 **1. Introduction**

67 The soil surfaces stripped by mine activities over the world are constantly rising in  
68 relation to the increase of industrial needs, particularly for metals (Deshaies, 2011;  
69 Harrison and Rajakaruna, 2011). These practices create important environmental and  
70 societal problems (Bradshaw, 1997, Tordoff et al., 2000). Nowadays, ecological  
71 restoration is considered as the best option for rehabilitation of these degraded areas  
72 (L'Huillier et al., 2010 ; McDonald et al., 2016). Indeed, only this practice can ensure  
73 the long-term ecosystem resilience by allowing the reinstallation of at least a part of  
74 the original biodiversity. Such a long-term view is vital to face the global crisis affecting  
75 the biosphere (Aronson and Alexander, 2013), as stressed by United Nations (UN)  
76 general assembly with the proclamation of UN Decade on Ecosystem Restoration for  
77 2021-2030 (Fischer et al., 2020; UN Environment Agency, 2019). However, ecological  
78 restoration needs a high level of scientific and technical knowledge, particularly when  
79 facing extreme environmental conditions.

80 In New Caledonia, metal rich ultramafic areas represent one third of the main land  
81 containing 20 to 30% of the world nickel resources (L'Huillier and Jaffré, 2010). These  
82 ultramafic substrates are covered by very valuable ecosystems, with a high level of  
83 endemism (Isnard et al., 2016), up to 82% for plants (Morat et al., 2012). These  
84 ecosystems are threatened by mine activities, fire and other anthropogenic pressures,  
85 and have been listed as one of the world biodiversity hot spots (Myers et al., 2000;  
86 Marchese, 2015). The soils of these areas are generally deficient in macronutrients,  
87 particularly P, K and N, and enriched in potentially toxic metals such as Ni, Co, Cr and  
88 Mn (Jaffré and L'Huillier, 2010; Proctor, 2003). In addition, Mg is in excess and thus  
89 competes with Ca for root absorption, accentuating the selection pressure on plant  
90 species (Jaffré and L'Huillier, 2010; Proctor and Nagy, 1992). Finally, most of these

91 soils that are colonized by ultramafic maquis (shrub heathlands) are subject to large  
92 fluctuations of water availability ranging from waterlogging to drought (Jaffré and  
93 L’Huillier, 2010). Despite these extreme conditions, these soils contain an important  
94 seed bank (Bordez, 2015; L’Huillier et al., 2010) and a high microbial diversity (Bordez  
95 et al., 2016; Gourmelon et al., 2016), including original and efficient populations of  
96 mycorrhizal fungi (Perrier et al., 2006; Amir et al., 2014a; 2019; Crossay et al., 2019;  
97 Waseem et al., 2017).

98 In relation with these biological properties, the soil stripped during nickel mine  
99 activities (generally called topsoil) must be replaced back after mining operations to  
100 restore the degraded areas. This practice is considered as fundamental (Ghose, 2001;  
101 Sheoran et al., 2010); however the topsoil is often stockpiled before being spread for  
102 mined areas restoration (Ghose, 2001). It is frequently the case in New Caledonia,  
103 because mines recently operated do not have yet enough surfaces to restore. In other  
104 cases, mining activities are not sufficiently coordinated with rehabilitation activities to  
105 provide an area for restoration after each stripping operation. Topsoil storage during  
106 more than few months can induce a loss of seed bank and a degradation of its fertility  
107 (Amir et al. 2014b; Ghose, 2001; Koch, 2007; Stahl et al., 2002). However, only few  
108 studies have reported detailed effects of topsoil storage on soil characteristics,  
109 including microbiota diversity and structure (Block, 2018; Ezeokoli et al., 2019;  
110 Mashigo, 2018). Moreover, to our knowledge, no data on this subject are available for  
111 ultramafic topsoils. Yet, the resilience of soil microbiota functions directly depends on  
112 microbial community structure (Griffith et al., 2008), in relation to ecological niches of  
113 the different taxa, and ecosystem functions also depend on microbiota diversity  
114 (Deng, 2012). Different studies reported the covariation of soil microbial and vegetal

115 communities (Djukic et al. 2010; Yergeau et al. 2010), suggesting that changes in  
116 microbiota can induce changes in plant species structure.

117 We studied the evolution of two topsoils stored in 10 m height piles on mine areas,  
118 in New Caledonia. The first topsoil was taken from an open ligno-herbaceous maquis,  
119 whereas the second one came from a bushy maquis. We chose a multifactorial  
120 approach to analyse the changes that appeared during the storage for microbiota  
121 diversity and structure, as well as different other microbial and physico-chemical  
122 characteristics. We tested the effects of time storage, depth and topsoil origin.

123

## 124 **2. Materials and methods**

### 125 *2.1. Site description, topsoil characteristics and sampling*

126 New Caledonia is located approximately 1,500 km east of Australia and 1,800 km  
127 north of New Zealand in the South Pacific. The climate is subtropical to tropical, with a  
128 rainy season from December to May and a dry season from September to November.

129 The two sites where the topsoils were stripped are located on the Koniambo massif  
130 (Fig. S1). The monthly temperature in Koniambo fluctuates between 14.5 °C and 21.1  
131 °C, with daily variations of about 5°C. The average monthly rainfall is about 250 mm  
132 during the wet season and 100 mm during the dry season.

133 The first topsoil pile (called topsoil 1) was obtained by stripping a ligno-  
134 herbaceous open maquis at an altitude of 450 to 900 m (20° 59.311' S, 164° 49.037'  
135 E). The maquis was dominated by the cyperaceous species *Costularia nervosa*, *C.*  
136 *araundinacea*, *Schoenus neocaledonicus* and *Lepidosperma perteres*. The most  
137 frequent shrubby species were *Nomandia neocaledonica*, *Hibertia altigena*, *H.*  
138 *pancheri*, *Eucarpha deplanchei*, *Montrouziera gabriellae*, *Myodocarpus floribundus*,  
139 *Codia montana* and *Ixora francii*.

140 The soil was a highly weathered oxisol (ferralsol) with following physico-chemical  
 141 characteristics (analysis done before the stripping): pF 4.2: 14.85; clay (%): 11.5; silt  
 142 (%): 27.8; sand (%): 55.2; pH (H<sub>2</sub>O): 5.44; organic carbon (%): 2.47 %; total N (%): 0.09;  
 143 total P (mg kg<sup>-1</sup>): 120.48; available P (Olsen mg kg<sup>-1</sup>): 0.00; Ca (g Kg<sup>-1</sup>): 0.65; CEC (meq  
 144 100g<sup>-1</sup>): 7.50; K CE (meq 100g<sup>-1</sup>): 0.08; Mg (%): 0.78; Fe (%): 41.60; Mn (%): 0.41; Ni  
 145 (%): 0.55; Cr (%): 1.94; Co (%): 0.08; Fe DTPA (extractable by  
 146 dimethylenetriaminopentaacetic acid, mg kg<sup>-1</sup>): 77.05; Mn DTPA (mg kg<sup>-1</sup>): 667.61; Ni  
 147 DTPA (mg kg<sup>-1</sup>): 76.69; Cr DTPA (mg kg<sup>-1</sup>): 0.54; Co DTPA (mg kg<sup>-1</sup>): 121.86.

148 The second topsoil pile (called topsoil 2) was obtained by stripping a dense bushy  
 149 maquis at the same altitude (20° 58.331' S, 164° 47.618' E). The main plant species of  
 150 the ecosystem were: *Tristaniopsis Callobuxus*, *Alstonia spp.*, *Codia montana*, *Hibbertia*  
 151 *lucens*, *Metrosideros nitida*, *Costularia arundinacea*, *Lepidosmerma perteres*. The soil  
 152 was also a weathered oxisol (ferralsol) with following physico-chemical characteristics  
 153 (analysis done before the stripping): pF 4.2: 16.07; clay (%): 11.5; silt (%): 26.0; sand  
 154 (%): 55.0; pH (H<sub>2</sub>O): 5.36; organic carbon (%): 4.07 %; total N (%): 0.13; total P (mg kg<sup>-1</sup>):  
 155 126.91; available P (Olsen mg kg<sup>-1</sup>): 0.33; Ca (g Kg<sup>-1</sup>): 0.60; CEC (meq 100g<sup>-1</sup>): 9.81; K  
 156 CE (meq 100g<sup>-1</sup>): 0.11; Mg (%): 0.82; Fe (%): 44.6; Mn (%): 0.41; Ni (%): 0.48; Cr (%):  
 157 1.93; Co (%): 0.09; Fe DTPA (extractable by dimethylenetriaminopentaacetic acid, mg  
 158 kg<sup>-1</sup>): 129.62; Mn DTPA (mg kg<sup>-1</sup>): 629.21; Ni DTPA (mg kg<sup>-1</sup>): 108.43; Cr DTPA (mg kg<sup>-1</sup>):  
 159 0.27; Co DTPA (mg kg<sup>-1</sup>): 112.88.

160 The two soils and associated vegetation were stripped with a 25-ton articulated  
 161 hydraulic excavator. The stripped vegetation was integrated to the topsoils. The  
 162 topsoil piles were placed on relatively flat places and had a height of 10 m (Fig. S2).

163 Topsoil samples were collected at the constitution of the topsoil piles (t0); and  
 164 every three months until 18 months (t3; t6; t9; t12; t18). For sampling, an opening was

165 done on the pile with an excavator and samples were collected at three different  
166 depths (0.1 m; 2 m; 10 m) with a disinfected planter, after scraping of the first cm.  
167 Except for the determination of bulk density, soil samples were sieved at 2 mm mesh.

168

## 169 *2.2. General physico-chemical analyses*

170 Sieved and dried soil samples were sent to the Laboratoire des Moyens  
171 Analytiques (LAMA US IMAGO) of the IRD Noumea Center for physico-chemical  
172 characterization (see physico-chemical properties listed above) using standard  
173 methods (<https://imago.ird.fr/moyens-analytiques/noumea/les-prestations>).

174

## 175 *2.3. Soil DNA extraction and bar-coded pyrosequencing of bacteria and fungi*

176 DNA pyrosequencing analyses were realized at the beginning of the storage and  
177 after 12 months for the three depth levels. For each soil sample, eight DNA extractions  
178 were performed as replicates from 0.25 g subsamples using the Mobio PowerSoil  
179 extraction kit (MO BIO Laboratories, Inc., Carlsbad, CA, USA). We performed the  
180 alternative lysis method proposed by the manufacturer. Soil subsamples were  
181 vortexed for 3–4 s and heated to 70 °C for 5 min three times. The DNA samples were  
182 then stored at -20 °C until needed. The bacterial community diversity was assessed  
183 using the DNA ~545-bp region covering V1-V3 within the 16S gene region and  
184 considered by Woese (1987) as the broadly conserved DNA barcode. The primers 27F  
185 and 553R were used to amplify this region. The fungal community was analyzed by  
186 targeting the DNA region of the ITS2 rRNA gene, which is widely used as a DNA  
187 metabarcoding marker (Bates et al., 2013). The primers ITS3 and ITS4 were used to  
188 amplify this region. As shown by White et al. (1990), the primer set provides 95% of  
189 fungal and 99% of Glomeromycota DNA. The full set of primers used is reported in

190 Table S1. The PCRs were conducted in 25  $\mu\text{L}$  reactions with 0.625  $\mu\text{L}$  (0.25  $\mu\text{M}$ ) of each  
191 primer, 2.5 ng of the template DNA for each soil subsample, 1 X PCR buffer and 0.08 U  
192 of GoTaq<sup>R</sup> Flexi DNA polymerase (Promega Corporation, Madison, WI, USA). The  
193 cycling conditions were: initial denaturation at 94 °C for 3 min; 30 cycles of 94 °C for  
194 30 s, 57 °C for 1 min and 72 °C for 1.5 min, and finally a 10 min extension at 72 °C. The  
195 amplified DNA was purified by filtration (Sephadex G50; Sigma-Aldrich, St. Louis, MO,  
196 USA) and the product purity was visualized on agarose 1.5% gel in a TAE buffer. Gels  
197 were stained with ethidium bromide (0.5  $\mu\text{g}/\text{mL}$ ) and visualized under UV light. After  
198 DNA quality control, the subsample replicates of PCR products from a same soil  
199 sample were pooled to obtain one final amplicon per soil sample for each microbial  
200 domain. The DNA concentration of each amplicons was determined using a NanoDrop  
201 spectrophotometer (Nano- Drop 2000; Thermo Scientific, Wilmington, DE, USA).  
202 Finally, for both bacteria and fungi, 4 libraries of 9 amplicons pooled in equimolar  
203 ratios, were generated, as recommended by 454 Life Sciences (Margulies et al., 2005).  
204 The pyrosequencing was performed by Beckman Coulter Genomics (Beckman Coulter  
205 Genomics, Danvers, MA, USA).

206

#### 207 *2.4. Processing of pyrosequencing data*

208 The quality of the data was analyzed as described by Nilsson et al. (2012) and  
209 processed using the Quantitative Insights Into Microbial Ecology (QIIME) toolkit  
210 (Caporaso et al., 2010). Bacterial and fungal sequences were quality-trimmed to a  
211 score >25 and assigned to soil samples based on their barcodes. Ambiguous bases and  
212 mismatches in primer sequences were not admitted. Chimeras were checked and  
213 removed with the Chimera Slayer algorithm in QIIME. Both bacterial and fungal  
214 sequences were binned into operational taxonomic units (OTUs) using a 97% identity

215 threshold with uclust (Kunin et al., 2010), and the most abundant sequence from each  
216 OTU was selected as a representative sequence for that OTU. Singletons were  
217 removed. We used ITSX SOFTWARE (version 1.0.10) (Bengtsson-Palme et al., 2013) to  
218 remove non-fungal ITS2 sequences. Taxonomy was assigned to bacterial OTUs by using  
219 the Basic Local Alignment Search Tool (BLAST) for each representative sequence  
220 against a subset of the SILVA database (the full database filtered at 97% sequence  
221 identity using BLAST) (SILVA SSU 111) (Quast et al., 2013). The fungal pyrosequencing  
222 reads were BLASTed against a database of the ITS2 rRNA sequences from UNITE-INSD  
223 V6 (Abarenkov et al., 2010) using the species hypothesis (Kõljalg et al., 2013). The  
224 percentage similarity to the assigned sequence ranged from 97% to 98.5%, with an  
225 average value of 98.3%. After this step, the unidentified fungi sequences were  
226 extracted and BLASTed against the MAARJAM database using a 97% identity threshold  
227 (Opik et al., 2010) to identify the Glomeromycota. According to McMurdie and Holmes  
228 (2014), who showed that the rarefaction of microbiome count data is inefficient in the  
229 statistical sense, the EDGER package within the R statistical environment  
230 ('EDGERPACKAGE', Robinson et al., 2010), was used to normalize the differential  
231 sequencing depth analysis and the OTU composition for the two microbial domains.  
232 The 'calcNormFactors' function was used to find a set of scaling factors for the library  
233 sizes that minimize the log-fold changes between the samples for most genes. We  
234 then generated an OTU normalized matrix for both fungi and bacteria. These new  
235 matrixes were used to the downstream statistical analysis.

236

### 237 *2.5. Global microbial activity (carbon mineralisation)*

238 Soil microbial activity is a good index of fertility as it releases mineral elements for  
239 plant nutrition. We chose to measure the CO<sub>2</sub> produced by microbial respiration,

240 corresponding to carbon mineralisation. The CO<sub>2</sub> can be easily captured and dosed.  
241 Each soil sample (40g of equivalent dry soil adjusted to 80% of retention capacity) was  
242 incubated 28 days in an hermetic bottle (500 mL) containing a small flask with 10 mL  
243 of NaOH 0.25 N. To avoid the saturation of the NaOH solution, the flask was replaced  
244 after 14 days and the sample was then incubated 14 days more. The CO<sub>2</sub> produced  
245 was quantified with 0.1 M and 0.5 M HCl solutions (Joergensen, 1996) for each of the  
246 two periods of 14 days and the values were then summed to obtain the final value.  
247 Three soil samples, each representing a mix of two, were reacted for each depth and  
248 each time of storage.

249

## 250 *2.6. Arbuscular mycorrhizal fungi spore extraction and quantification*

251 Arbuscular mycorrhizal symbiosis is very important in New Caledonian ultramafic  
252 maquis (Amir and Ducouso, 2010; Perrier et al., 2006). It was then of interest to  
253 follow up the abundance of arbuscular mycorrhizal fungi (AMF) during topsoil storage.  
254 Regarding the high number of samples, we chose to count the viable AMF spores after  
255 humid sieving followed by sucrose gradient centrifugation (method modified from  
256 Daniels and Skipper, 1982). Four soil samples were used for each time of storage and  
257 depth. For each sample, 50 g of sieved and dried soil were placed in a 255 µm mesh  
258 sieve adjusted above a 36 µm mesh sieve. The soil was then wet sieved through the  
259 two sieves and the harvested material in the 36 µm mesh sieve was suspended in 20  
260 mL of water in a 50-mL Falcon tube. A 25-mL sucrose solution (70% v/w) was injected  
261 at the bottom of the tube, forming a stepped density gradient that was centrifuged at  
262 800 rpm for 5 min. Spores of AMF were collected from the interface with the sucrose  
263 solution, washed with tap water on a 36 µm sieve, and transferred to a 50-mL Falcon  
264 tube with 10 mL of water. Finally, the Falcon tube was vortexed and 5 successive



265 aliquots of 100  $\mu$ L were immediately transferred to Petri dishes for spore counting  
266 under a stereomicroscope. Only viable spores containing protoplasm and not broken  
267 were counted.

268

### 269 *2.7. Bulk density*

270 As the topsoil situated in depth inside the piles can be compressed, it was  
271 Regarding the possible compression of soil within the piles, it was important to  
272 measure the changes of the bulk density during topsoil storage. This analysis was done  
273 by weighting 300 cm<sup>3</sup> of non-sieved soil, before and after drying at 105°C during 72h.  
274 Then we calculated two parameters: i) the bulk density value  $\rho$  (g.cm<sup>-3</sup>) as the  
275 mass/volume ratio of the dry soil; ii) the Water-Filled Pore Space (Robertson and  
276 Groffman, 2015) as  $WFPS = (TE \times 100 \times \rho) / (1 - (\rho / \rho_S))$ , where TE is the soil water  
277 content in % (soil dried 72h at 105°C). Four soil samples were tested for each level and  
278 topsoil pile.

279

### 280 *2.8. Statistical analyses*

281 The statistical analyses were performed using R statistical environment ('VEGAN  
282 PACKAGE', R.3.1.1, R foundation). The differences between the variable means were  
283 tested with a permutational multivariate analysis of variance (PERMANOVA). In case of  
284 significant differences, PERMANOVA pairwise comparisons were used to identify the  
285 differences. The results were illustrated by nonmetric multidimensional scaling  
286 (NMDS) analysis. The relationship between fungal or bacterial communities and  
287 edaphic variables was tested using spearman correlation (PRIMER V7). When a  
288 significant relationship was found, a distance-based linear model (DISTLM) was built to  
289 identify the significant predictors edaphic variables. This model does a partitioning of

290 variation using a multiple regression model. Permutation methods are used to assess  
291 statistical significance of each predictor variable. In addition, preliminary diagnostics  
292 are made to assess and to avoid multicollinearity among predictor variables that could  
293 bias the results (McArdle and Anderson, 2001).

294

### 295 **3. Results**

#### 296 *3.1. Changes in the composition of bacterial communities*

297 From the 454-pyrosequencing data, we obtained global numbers of 138 932 and  
298 269 046 exploitable sequences of bacteria respectively for topsoil 1 and topsoil 2.  
299 These sequences could be classified in 24 382 OTUs for topsoil 1 and 33 449 OTUs for  
300 topsoil 2. At the beginning of the storage (t0) the mean number of OTUs was 2917 for  
301 topsoil 1 and 4730 for topsoil 2 which has a significantly higher bacterial diversity (Fig.  
302 1). This diversity did not change significantly after 12 months of storage whatever the  
303 depth considered.

304 A total of 45 bacterial phyla were identified. Overall, for the two topsoils and the  
305 three depths, the dominant phyla were: Proteobacteria ( $43.0 \pm 2.9\%$ ), Acidobacteria  
306 ( $29.1 \pm 1.6\%$ ), Actinobacteria ( $13.0 \pm 0.2\%$ ), Planctomyces ( $8.4 \pm 1.1\%$ ), Chloroflexi ( $5.8$   
307  $\pm 0.7\%$ ), Verucomicrobia ( $5.1 \pm 0.8\%$ ), Bacteroidetes ( $4.1 \pm 1.4\%$ ), Firmicutes ( $2.7 \pm$   
308  $0.4\%$ ). The other phyla accounted for less than 2% each (Fig. 2A). The same bacterial  
309 orders were present in the two topsoils (Fig. 2B). After 12 months of storage, these  
310 taxa differed on their relative abundance. Respectively 45% and 50% of the observed  
311 differences between t0 and t12 were due to the variations in 12 taxa. It corresponded  
312 mainly to a reduction of the abundance of Planctomycetales, Rhodospirillales,  
313 Acidobacteriaceae and Chtoniobacterales, and an increase of the abundance of  
314 Burkholderiales, Sphingomonadales, Sphingobacteriales and Nocardiodaceae. Details

315 of the abundance values and corresponding standard errors for the different taxa are  
316 given in table S2.

317 A non-metric multidimensional scaling (NMDS) ordination (Fig. 3) showed  
318 significant differences ( $p < 0.001$ ) of the bacterial OTU assemblages, between the two  
319 storage periods tested (t0 and t12), and this for the two topsoils. These latter were  
320 also clearly different for their bacterial community structure ( $p < 0.001$ ). No  
321 differences were detected between the three topsoil depths. Furthermore, the  
322 distance based linear model (DISTLM) approach (Fig. S3) showed that 16% of the  
323 variability between t0 and t12 storage periods was explained significantly by  
324 variations, during the storage, of pH (for 10% of the variability) and organic carbon (6%  
325 of the variability).

326

### 327 *3.2. Changes in the composition of fungal communities*

328 The global numbers of exploitable fungal sequences obtained were 181 096 and  
329 307 254 respectively for topsoil 1 and topsoil 2. These sequences were classified in  
330 9 357 fungal OTUs for topsoil 1 and 11 779 for topsoil 2. The number of fungal OTUs at  
331 t0 was 533 for topsoil 1 and 965 for topsoil 2, which has a significantly higher fungal  
332 diversity (Fig. 4). Except for a significant increase of the fungal diversity after 12  
333 months at the surface layer of topsoil 1, the other samples did not show any  
334 significant differences in relation with the time of storage or the depth.

335 Overall, for the two topsoils and the three depths, the dominant phyla, were:  
336 Ascomycota ( $46.2 \pm 5.7\%$ ) and Basidiomycota ( $25.6 \pm 7.2\%$ ) (Fig. 5A). The non-  
337 identified phyla represented  $28.4 \pm 0.2\%$ . The other phyla were less abundant  
338 Zygomycota ( $3.3 \pm 0.5\%$ ), Chytridiomycota ( $> 1\%$ ), Glomeromycota ( $> 1\%$ ),  
339 Blastocladiomycota ( $> 1\%$ ) were. The composition of the phyla changed significantly

340 after 12 months of storage for the two topsoils, with a reduction of the Basidiomycota  
341 abundance and an increase of the Ascomycota and the unidentified phyla abundance.  
342 The two topsoils significantly differed for fungal community structure ( $p < 0.001$ ). A  
343 total of 41 fungal classes were identified. Among them 10 were dominant (Fig. 5B).  
344 Moreover, 68.3% (topsoil 1) and 69.8% (topsoil 2) of the observed changes after 12  
345 months of storage were related to a reduction of the Agaricomycetes, and an increase  
346 of the Sordariomycetes and an unidentified group. Details of the abundance values  
347 and corresponding standard errors for the different taxa are given in table S3.

348 NMDS ordination (Fig. 6) showed that significant changes in the fungal OTU  
349 assemblages appeared after 12 months of storage for the two topsoils ( $p < 0.001$ ), but  
350 without significant effect of the depth. The DISTLM approach (Fig. S4) indicated that  
351 29.3% of these changes in fungal communities during topsoil storage were explained  
352 by variation in pH H<sub>2</sub>O (13.4%), carbon mineralization (7.3%), extractable Mn content  
353 (4.4%) and exchangeable K (4.3%).

354 Arbuscular mycorrhizal fungi (AMF) sequences (Glomeromycota) were also  
355 analysed; respectively 77 and 177 sequences were obtained for Topsoil 1 and Topsoil  
356 2. At t<sub>0</sub>, these sequences corresponded respectively to 22 and 47 OTUs (Fig. 7),  
357 Topsoil 2 being significantly richer. After 12 months of storage, the number of OTUs  
358 decreased significantly (*i.e.* 3 to 4 times), for the two topsoils. Overall, the depth did  
359 not affect the OTUs number, except for 10 m depth in Topsoil 1 which showed  
360 significantly less OTUs than those of the two other depths. The structure of AMF  
361 communities was affected by the storage for the two topsoils. However, the OTU  
362 numbers for each taxa were too small to obtain reliable statistical analysis (data not  
363 shown).

364

### 365 3.3. Evolution of the global microbial activity

366 Global microbial activity expressed by microbial respiration in the incubated soil  
367 samples was negatively affected by the topsoil storage (Fig. 8). In topsoil 1, the activity  
368 dropped significantly (*i.e.* 5 to 7 times) after 6 months and then tended to stabilize.  
369 The same dynamic was observed for topsoil 2, but with a lower reduction of the  
370 activity. Some values (between 3 and 9 months) were significantly lower at the  
371 deepest layer in comparison with the surface of the piles. Negative correlations  
372 between microbial activity on the one hand and depth and bulk density on the other  
373 hand were detected (respectively  $r = -0.338$ ,  $p < 0.01$  and  $r = -0.330$ ,  $p = 0.01$ ).

374

### 375 3.4. Evolution of AMF viable spore numbers

376 The number of viable AMF spores in topsoil 1 dropped significantly 3 times, after  
377 3 months of storage in the surface of the topsoil pile and more than 4 times after 6  
378 months, before stabilizing until 18 months (Fig. 9). In topsoil 2, the decrease was less  
379 rapid until 6 months, but the decline was finally stronger (*i.e.* 10 times) after 12  
380 months. A significant difference between the two topsoils for this parameter was  
381 detected ( $p < 0.001$ ). The reduction of viable spore numbers was significantly stronger  
382 in the deepest layer for the two topsoil piles during the first period (*i.e.* 6 to 9  
383 months), before dropping equally for all depths. A negative correlation between the  
384 number of viable spores and the depth was noted ( $r = -0.527$ ,  $p < 0.0001$ ). AMF viable  
385 spore richness was also negatively correlated to bulk density ( $r = -0.322$ ,  $p < 0.01$ ), and  
386 positively correlated to organic carbon ( $r = 0.412$ ,  $p < 0.0001$ ), and Cation Exchange  
387 Capacity ( $r = 0.42$ ,  $p < 0.0001$ ).

388

### 389 3.5. Evolution of the physico-chemical characteristics

390 Topsoil bulk density varied similarly (*i.e.* from 1.2-1.3 g.cm<sup>-3</sup> to 1.8-1.9 g.cm<sup>-3</sup>) for  
391 the two topsoil piles during storage (Fig 10). Soil density increased mostly during the  
392 first three months. However, differences between the three tested depths were  
393 significant only after 9 months, with higher densities in the deepest layer (10 m). The  
394 Water-Filled Pore Space (Fig. 10) did not change significantly (*i.e.* from 50 to 53 %) in  
395 the surface layer during storage. However, this parameter showed a significant  
396 decrease in the deepest layer after 9 and 18 months of storage for topsoil 1 and after  
397 6 months of storage for topsoil 2.

398 The organic carbon stock in topsoil 1 decreased from 31 g.kg<sup>-1</sup> to less than 10 g.kg<sup>-1</sup>  
399 after 9 months storage, with significant differences compared to the initial soil (t<sub>0</sub>)  
400 that after 6 months storage (Fig. 11). In topsoil 2, the loss in organic carbon after 9  
401 months storage was lower (*i.e.* from 42 g.kg<sup>-1</sup> to 20 g.kg<sup>-1</sup>). For the two topsoils, no  
402 significant differences were detected between the three depths. The dynamic of total  
403 nitrogen was very close to that of organic carbon (Fig. 11). Organic carbon and total  
404 nitrogen were negatively correlated with the duration of storage (respectively  $r = -$   
405 0.417,  $p = 0.0001$ ; and  $r = - 0.324$ ,  $p = 0.01$ ).

406 The pH values (Fig. S5) increased from 5.4 to 6.2 during storage in topsoil 1 and  
407 from 5.2 to 6.6 in topsoil 2. Significant differences appearing after 3 to 6 months of  
408 storage. Overall, pH values were higher in the deep layers of the piles. The C/N values  
409 (Fig. S6) dropped significantly from 23-25 to 3-5 after three months of storage for the  
410 two topsoils. The values did not vary significantly for longer duration of storage.

411 Among the other physico-chemical parameters that have been followed (Table S4),  
412 it can be noticed that topsoil storage induced a reduction of CEC, exchangeable K and  
413 Ca as well as the Ca/Mg ratio after 6 to 9 months for the two topsoils. In topsoil 1,  
414 extractable Fe, Ni, Mn and Co decreased significantly after 3 to 9 months of storage,

415 whereas extractable Cr increased 6 to 10 times after three months of storage and then  
416 stabilized. In topsoil 2, only extractable Fe was reduced and extractable Co increased  
417 after three months of storage, before stabilizing.

418

#### 419 **4. Discussion**

420 The storage of 10 m height piles of ultramafic topsoils during 18 months induced  
421 modifications of their microbiota structure and affected different other characteristics.

422

##### 423 *4.1. Effects of topsoil stockpiling on microbiota diversity*

424 Despite their extreme edaphic characteristics related to the ultramafic nature of  
425 their parent rocks, the studied soils from ultramafic ecosystems, showed unexpectedly  
426 high microbial diversity richness (4730 bacterial OTUs and 965 fungal OTUs for topsoil  
427 2), comparable to those reported for temperate forests or Amazonian forests (Bordez  
428 et al., 2016). In a previous study, Gourmelon et al. (2016) found similar results for  
429 different other ultramafic soils from New Caledonia. This high richness is consistent  
430 with Mouillot et al. (2013) assertion that ultramafic ecosystems host highly complex  
431 microbial communities. Topsoil 2, from a bushy maquis with a denser vegetation, was  
432 characterized by a higher bacterial and fungal diversity. This relation between  
433 ecosystem productivity and microbial diversity richness agrees with different reported  
434 studies for other types of ecosystems (Cong et al., 2015; Kennedy et al., 2005; Li et al.  
435 2015). Bordez et al. (2016) showed that the gradient in vegetation density and  
436 productivity of 6 ultramafic ecosystems from New Caledonia is the main factor driving  
437 microbial diversity richness, even if the edaphic properties (especially total N, pH and  
438 CEC) also influence the richness of microbial communities.

439 In the present study, the microbiota global diversity of the two topsoils was not  
440 affected by 12 months storage. Bacterial and fungal OTU numbers were not  
441 significantly different compared to the non-stored control topsoil, whatever the depth  
442 considered. At the order level, all taxa survived during topsoil storage, at least at a low  
443 abundance. In a study comparing a 5-years stockpiled topsoil with the non-mined  
444 corresponding topsoil, Ezeokoli et al. (2019) found a higher microbiota diversity in the  
445 latter. The duration of storage can probably explain the differences with our study.  
446 Indeed, in our experiment organic carbon and mineralization activity started to decline  
447 only after 6 to 9 months of storage. The number of cultivable aerobic bacteria also  
448 dropped significantly starting from 6 to 9 months and was only 7 times lower after 12  
449 months (data given in Bordez, 2015). Some species becoming rare after 12 months  
450 could thus disappear from the stored topsoil after few years. In fact, contrary to the  
451 diversity, the structure of microbial communities changed after 12 months of storage.  
452 For bacteria, these changes were mainly driven by variations on the relative  
453 abundance of 12 taxa (at the order level), some of them showing a decreasing  
454 abundance and others showing an increasing abundance. Fungal taxa showed a similar  
455 type of changes, with variations in the relative abundance of three classes.

456 Studies discussing variations in microbial taxa composition in relation with soil  
457 properties are relatively recent and quite limited in number. Among these studies,  
458 Gourmelon et al. (2016) found the same 4 dominant bacterial phyla than those found  
459 in the present study (*i.e.* Proteobacteria, Planctomyces, Acidobacteria and  
460 Actinobacteria) in different ultramafic soils from New Caledonia. The domination of  
461 Proteobacteria, Acidobacteria and Actinobacteria was also reported by other studies  
462 in different soil types and conditions (Herrera et al., 2007; Ko et al., 2017; Liang et al.,  
463 2019). Ko et al. (2017) showed changes of bacterial phyla structure in deep layers of



464 the soil, with an increase of Acidobacteria in relation with carbon reduction caused by  
465 microbial activity. In our study, Acidobacteria did not vary with depth and reduction of  
466 organic carbon after 12 months of storage. However, the DISTLM analysis indicated  
467 that the reduction of organic carbon caused by microbial activity affected the bacterial  
468 community structure of the two stockpiled topsoils. As observed by Waldropt et al.  
469 (2006) and Lauber et al. (2008), organic carbon reduction can have different  
470 consequences, particularly in terms of competition and starvation of a part of the  
471 microbiota. In our study, the phyla of Planctomycetes was less represented after 12  
472 months of topsoil storage in comparison with the non-stored ones. This phylum has  
473 indeed been reported as sensitive to soil history (Buckley et al., 2006). Our DISTLM  
474 analysis also detected an effect of soil pH on the bacterial community structure. Soil  
475 pH is commonly reported as regulating the structure of microbiota communities  
476 (Lauber et al., 2008; Rousk et al., 2010; Sheng et al., 2019). In our case, it is interesting  
477 to note that Acidobacteria, the second most important bacterial phylum in the studied  
478 topsoils, declined after storage in topsoil 2 where the pH value raised from 5.2 to 6.5.  
479 This result is in agreement with Sait et al. (2006) who indicated that Acidobacteria  
480 grow optimally from pH 4 to 5.5 and poorly or not at all for pH above 6.5.

481 Concerning fungi, Ascomycota and Basidiomycota were generally dominant in our  
482 study. This result is in agreement with previous studies on ultramafic soils from New  
483 Caledonia (Bordez et al., 2016; Gourmelon et al., 2016) and on other soil types (Karst  
484 et al., 2013; Ko et al., 2017; Weber et al., 2013; Wang et al., 2020). The main detected  
485 variables that were found to structure these communities were pH, carbon  
486 mineralization, exchangeable K and DTPA extractable Mn. The statistical analysis  
487 showed that the relative importance of the Basidiomycota was positively correlated  
488 with organic carbon ( $p = 0.02$ ) and carbon mineralisation ( $p < 0.01$ ) (data not shown).

489 Indeed, we observed a clear reduction of the Basidiomycota group after 12 months of  
490 topsoil storage, as carbon concentration and C/N dropped drastically. This phylum is  
491 known for its ability to decompose organic matter, particularly litter and wood  
492 (Purahong et al., 2016; 2018; Wang et al., 2020). In addition, many Basidiomycota  
493 species are ectomycorrhizal and may then be disturbed by the topsoil storage. Among  
494 the Basidiomycota, the class of Agaricomycetes was dominant, which is frequently  
495 reported in similar studies (Karst et al., 2013; Ko et al., 2017; Sheng et al., 2019).  
496 Inversely, Ascomycota were more represented after 12 months of storage, particularly  
497 in deep layers. This result can probably be linked to the high resistance of this group to  
498 many soil stresses (Egidi et al., 2019), such as pH variations and organic carbon  
499 rarefaction. Indeed, Ascomycota are mostly saprotroph with a large variety of  
500 enzymes that enable them to use recalcitrant substrates (Ko et al., 2017).

501 AMF communities were also relatively diverse, with 22 and 47 OTUs found for the  
502 two non-stored topsoils. On average, these values correspond to those reported for  
503 different other soil types (Schechter and Burns 2008; Yu et al. 2012; Hassan et al.  
504 2014; Kruger et al. 2017). The 12 months storage clearly reduced this diversity for the  
505 two topsoils. As obligatory symbionts, AMF can survive mainly as spores in absence of  
506 plants, but the spores were also affected as indicated by the reduction of their  
507 numbers during the storage. Regarding the ecological importance of AMF in ultramafic  
508 soils from New Caledonia (Amir et al., 2010; Amir et al., 2019; Crossay et al. 2019), this  
509 reduction of AMF diversity and density in stored topsoils may slow down the relative  
510 growth of plant species in restored areas. According to Birnbaum et al. (2017), AMF  
511 communities of stockpiled sandy topsoils naturally recolonized by plants take 5 to 10  
512 years, before returning to their initial level.

513 Other changes can also influence the structure of the microbiota in relation with  
514 the specific needs of each taxa. A compaction of the soil after 12 months of storage  
515 has been noted for the three topsoil layers, particularly in the 10 m depth layer. Such  
516 an increase of the bulk density with the depth has also been observed by Ezeokoli et  
517 al. (2019) in stockpiled soils. This increase can create an anoxia and inhibit taxa that  
518 require high concentrations of oxygen.

519

#### 520 *4.2. Effects of topsoil stockpiling on some physico-chemical and microbiological* 521 *properties*

522 The evolution of bulk density and water-filled pore space values showed that the  
523 two studied topsoils have been compacted during the first months of storage,  
524 especially in deep layers. The sandy nature of the soils (*i.e.* 55% sand fraction) and  
525 their relatively low organic C contents suggest that compaction could have been  
526 driven by aggregates destruction upon driving rain and high pressure in deep layers.  
527 Such a compaction effect with reduction of soil aggregates has been reported by other  
528 studies for stockpiled topsoils (Abdul-kareem and McRae, 1984; Strohmayer, 1999;  
529 Wick et al., 2007). Organic C and total N contents decreased 2 to 3 times after 9  
530 months of storage, without significant differences between the three tested depth  
531 levels. Several studies showed a reduction of organic matter in stored soils (Visser et  
532 al., 1984; Harris and Birch, 1989; Gobat et al., 2010; Birnbaum et al., 2017) as a  
533 consequence of microbial mineralization in absence of any carbon flux. This  
534 assumption is supported by our results, which showed a positive correlation between  
535 organic C and microbial activity. We also observed a strong decrease, before  
536 stabilization, of the microbial activity after 6 months of storage in the two topsoils, in

537 agreement with those from other studies (Visser et al., 1984; Harris et al., 1989;  
538 Strohmayer, 1999).

539 Other chemical properties showed some variations in relation to organic matter  
540 reduction, and/or leaching after driving rains during topsoils storage. Among those,  
541 CEC, as well as exchangeable K and Ca decreased after 3 to 9 months of storage.  
542 However, no significant differences could be found between the three tested depths.  
543 Organic carbon reduction and leaching can also explain the reduction of extractable  
544 Fe, Mn and Co, since the fraction of these metals that is associated with organic  
545 matter through complexation is usually the most extractable (McGrath, 1996; Menzies  
546 et al., 2007). This explanation is supported by the lack of variation of extractable Fe,  
547 Mn and Co in topsoil 2 where organic carbon reduction is much lower. The increase of  
548 extractable Cr during storage can also be attributed to organic carbon reduction since  
549 organic matter is known to counteract Cr oxidation by Mn-oxides and thus to reduce  
550 Cr extractability in soils (Fendorf et al., 1992; 1995).

551

## 552 **5. Conclusions**

553 The two topsoils stockpiled in mine conditions during 18 months showed different  
554 modifications of their microbiota diversity and other microbiological and physico-  
555 chemical properties. Overall, these variations differed significantly for the two topsoils  
556 and some were influenced by the depth of storage. Our results thus suggest that New  
557 Caledonian ultramafic soils are sensitive to management during mine activities. If the  
558 changes in physico-chemical characteristics in these conditions are relatively well  
559 known, the perturbations induced on microbiota community structure of the stored  
560 topsoils must be regarded with attention, considering the possible degradations of soil  
561 microbial functions (Griffith et al., 2008; Ezeokoli et al., 2019). Indeed, some studies

562 reported variations of the structure of plant communities in relation to differences of  
563 microbial communities (Djukic et al., 2010; Yergeau et al., 2010). Yu et al. (2012) found  
564 a clear relationship between root-associated fungal communities and plant health  
565 status. Changes on microbial community structure upon topsoil stockpiling could thus  
566 induce degradations of soil functions and then perturbations of the dynamic of the  
567 reconstructed ecosystems. Considering all the above-mentioned results, the duration  
568 of the stockpiling of ultramafic topsoils should be reduced as far as possible and in all  
569 cases should not exceed 6 months. It is also recommended to reduce the height of the  
570 stockpiles when possible to 1 m or less to minimize the compaction and the  
571 anaerobiosis (Amir et al., 2014b).

572

### 573 **Acknowledgments**

574 The authors gratefully acknowledge CNRT Koniambo Nickel Society for providing the  
575 financial support.

576

### 577 **Supplementary data**

578 Supplementary data associated with this article can be found online at: doi...

579

### 580 **Author Contribution**

581 H. A. supervised and designed the research, provided the funding and wrote the  
582 manuscript; L. B. realized the research studies, contributed to their design and  
583 checked the manuscript text; Y. C. contributed to the design and supervision of  
584 molecular studies ; P. J. contributed to the design and supervision of molecular studies  
585 and checked the manuscript text; M. D. contributed to the supervision and checked

586 the manuscript text; F. J. contributed to the supervision of physico-chemical  
587 characterizations and their interpretation and checked the manuscript text.

588

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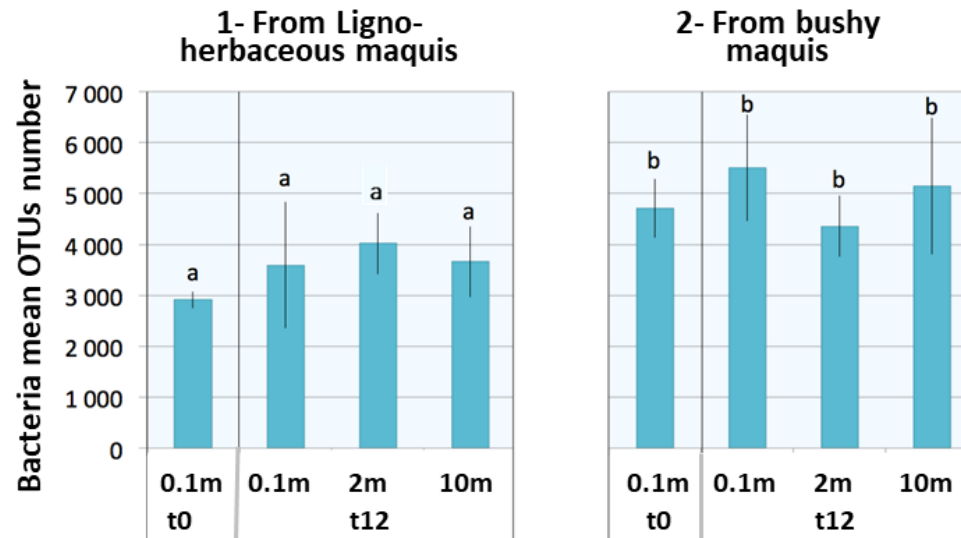
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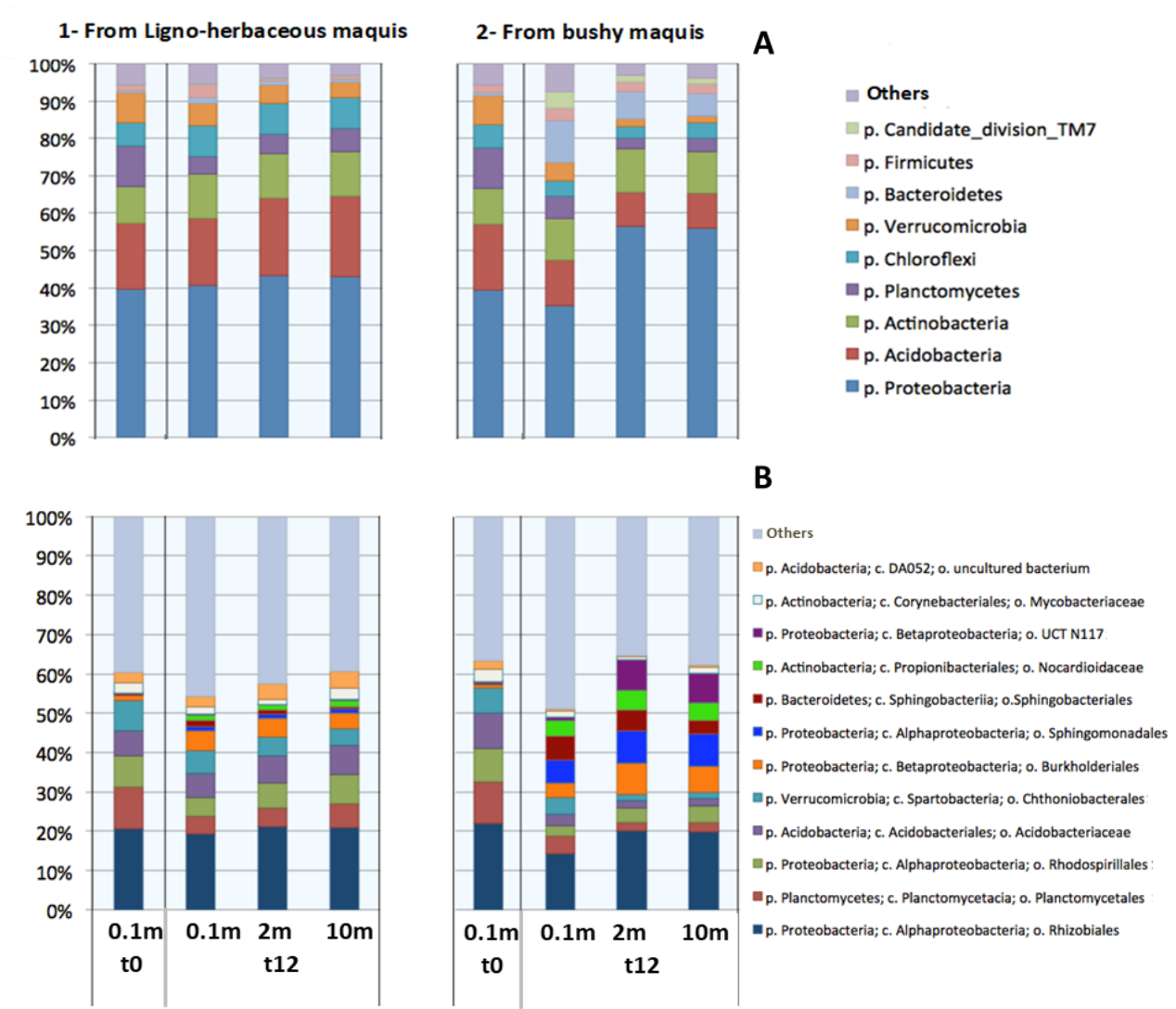
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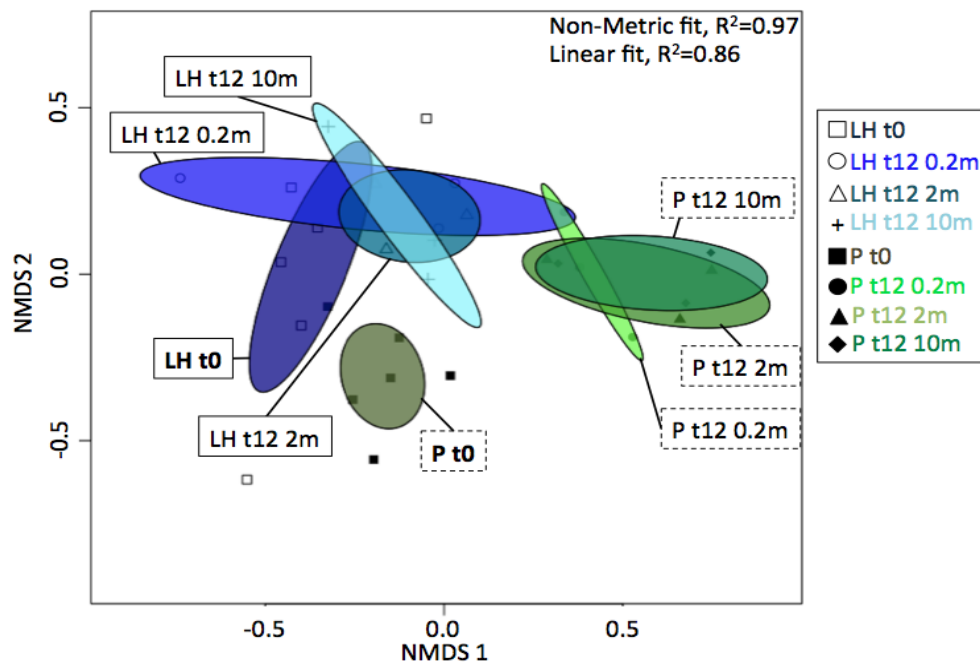
**Color should be used for all figures in print****Fig. 1**

**Fig. 1.** Bacterial OTU numbers (mean  $\pm$  se) in 12 months stockpiled topsoils (t12) compared to the non-stored topsoils (t0) at different depth (0.1m, 2 m and 10 m). For each graph, bars with the same letter are not significantly different according to a one-way PERMANOVA followed by Pair-wise permutation test (n = 10).

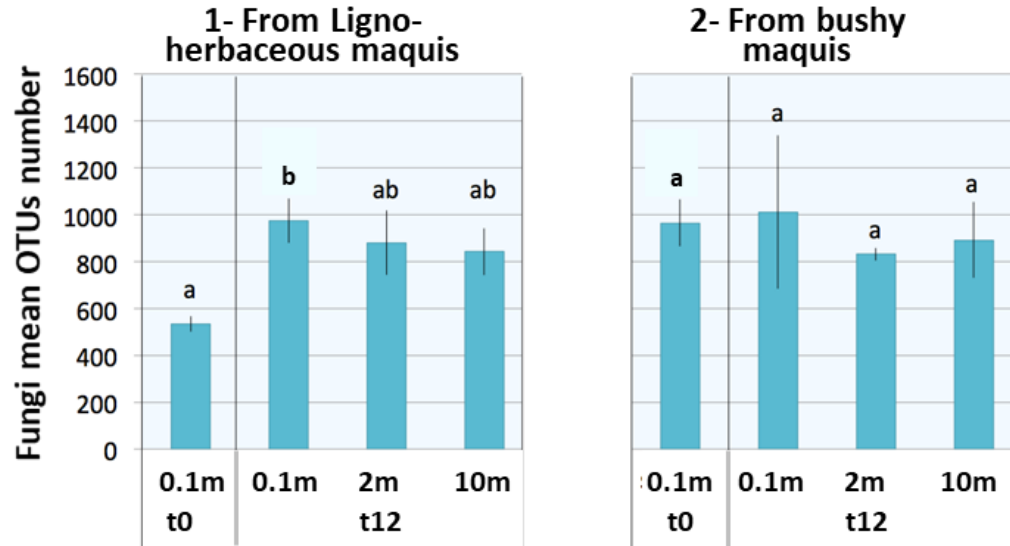


**Fig. 2.** Relative abundance of bacterial phyla (A) and orders (B) in 12 months stockpiled topsoils (t12) compared to the non-stored topsoils (t0).

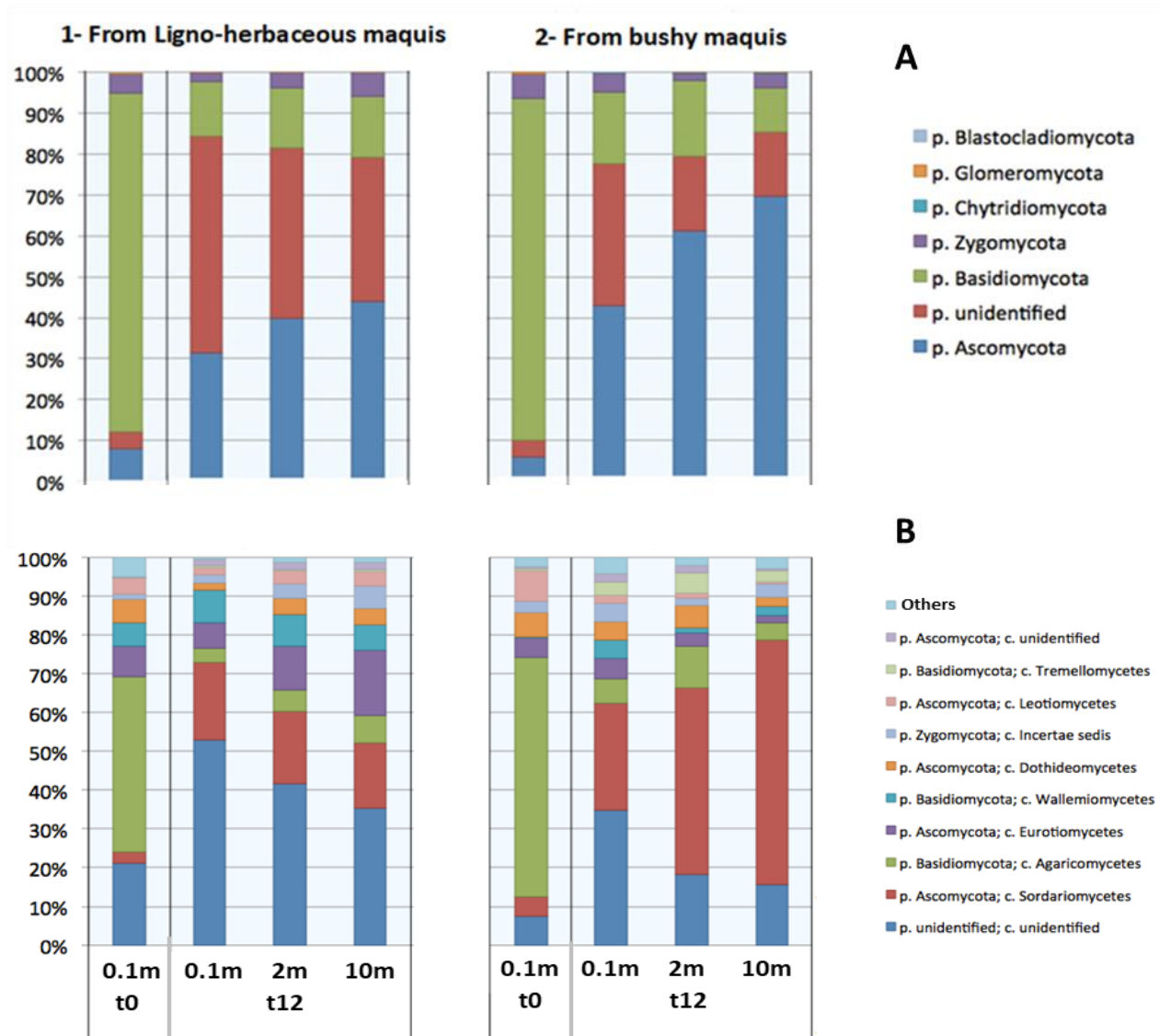
Fig. 3



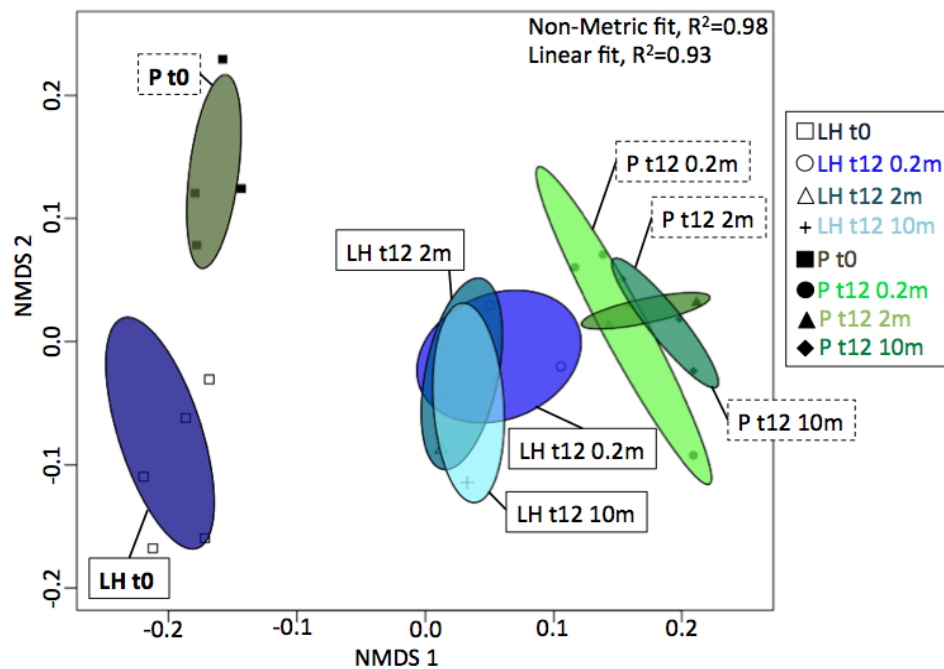
**Fig. 3.** Non-metric multidimensional scaling analysis (NMDS) of bacterial community structure of 12 months stockpiled topsoils (t12) compared with the non-stored topsoils (t0) at different depth (0.1m, 2 m and 10 m). The distance between the ellipses indicates the degree of similarity between the communities. T0 bacterial communities differed significantly between the two topsoils and with the corresponding stored topsoils. LH = ligno-herbaceous maquis topsoil; P = bushy maquis topsoil.



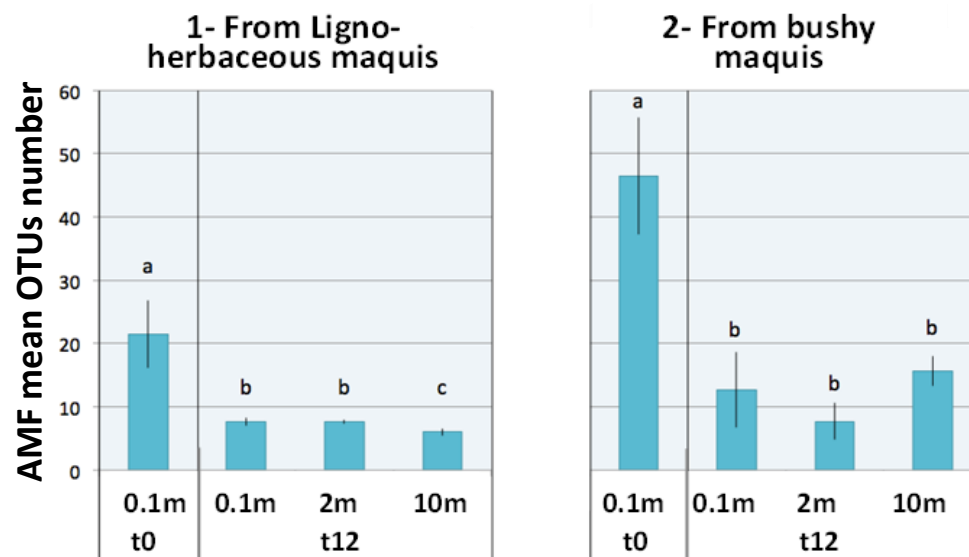
**Fig. 4.** Fungal OTU numbers (mean  $\pm$  se) in 12 months stockpiled topsoils (t12) in comparison with the non-stored corresponding topsoil (t0) at different depth (0.1m, 2 m and 10 m). For each graph, bars with the same letter are not significantly different according to a one-way PERMANOVA followed by Pair-wise permutation test ( $n = 10$ ). The topsoil 2 was found significantly richer than topsoil 1 at t0.



**Fig. 5.** Relative abundance of fungal phyla (A) and classes (B) in 12 months stockpiled topsoils (t12) in comparison with the non-stored corresponding topsoil (t0).

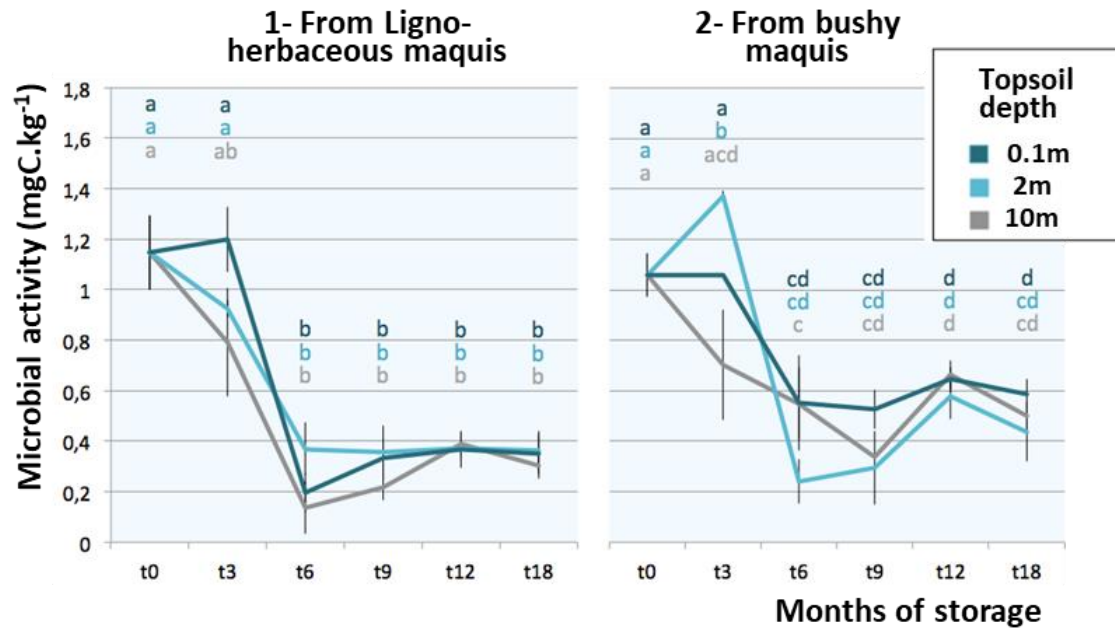


**Fig. 6.** Non-metric multidimensional scaling analysis (NMDS) of fungal community structure of 12 months stockpiled topsoils (t12) in comparison with the non-stored corresponding topsoil (t0) at different depth (0.1m, 2 m and 10 m). The distance between the ellipses indicates the degree of similarity between the communities. TO fungal communities differed significantly between the two topsoils and with the corresponding stored topsoils. LH = ligno-herbaceous maquis topsoil; P = bushy maquis topsoil.



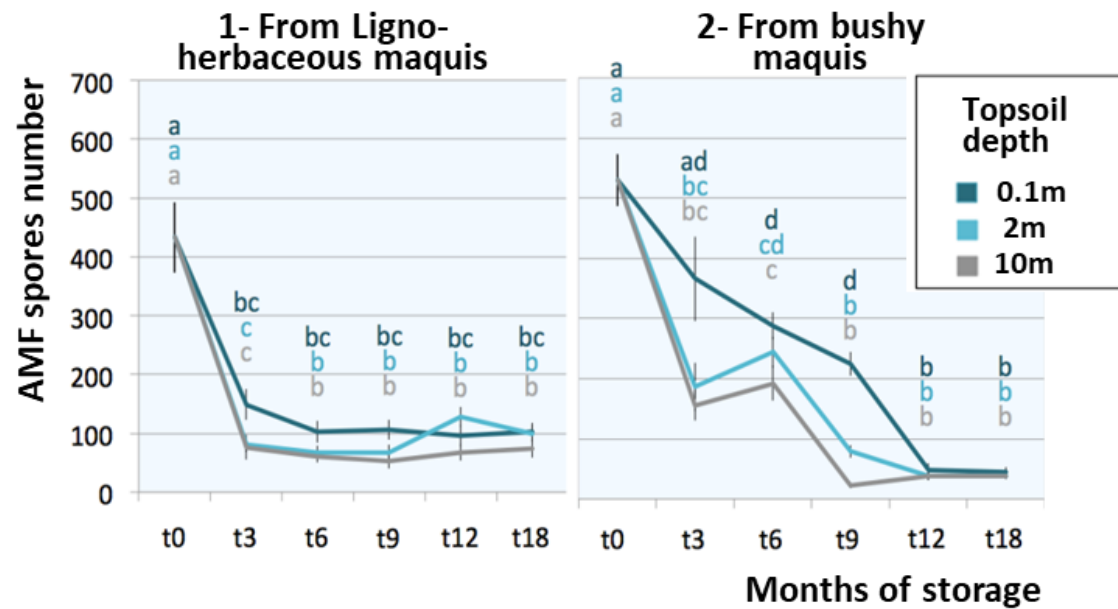
**Fig. 7.** AMF OTU numbers (mean  $\pm$  se) in 12 months stockpiled topsoils (t12) compared with the non-stored topsoils (t0) at different depth (0.1m, 2 m and 10 m). For each graph, bars with the same letter are not significantly different according to a one-way PERMANOVA followed by Pair-wise permutation test ( $n = 10$ ).

Fig. 8



**Fig. 8.** Evolution of the microbial activity (carbon mineralisation) of stockpiled topsoils in relation to time storage and depth. For each graph, means with the same letter are not significantly different according to a two-way PERMANOVA followed by Pair-wise permutation test ( $n = 6$  mixed 2 by 2).





**Fig. 9.** Evolution of viable AMF spore numbers in stockpiled topsoils in relation to time storage and depth. For each graph, means with the same letter are not significantly different according to a two-way PERMANOVA followed by Pair-wise permutation test ( $n = 6$  mixed 2 by 2).

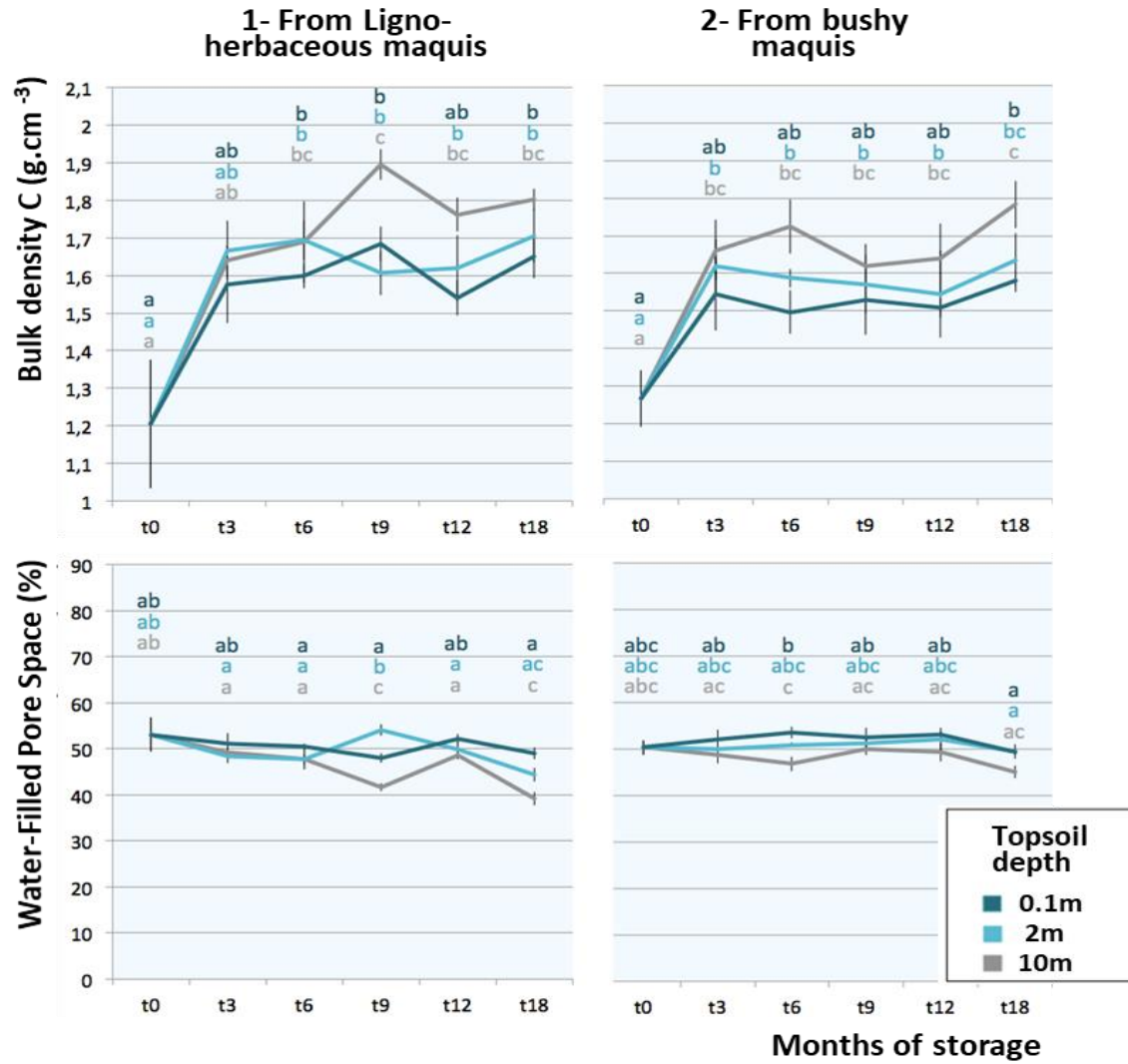
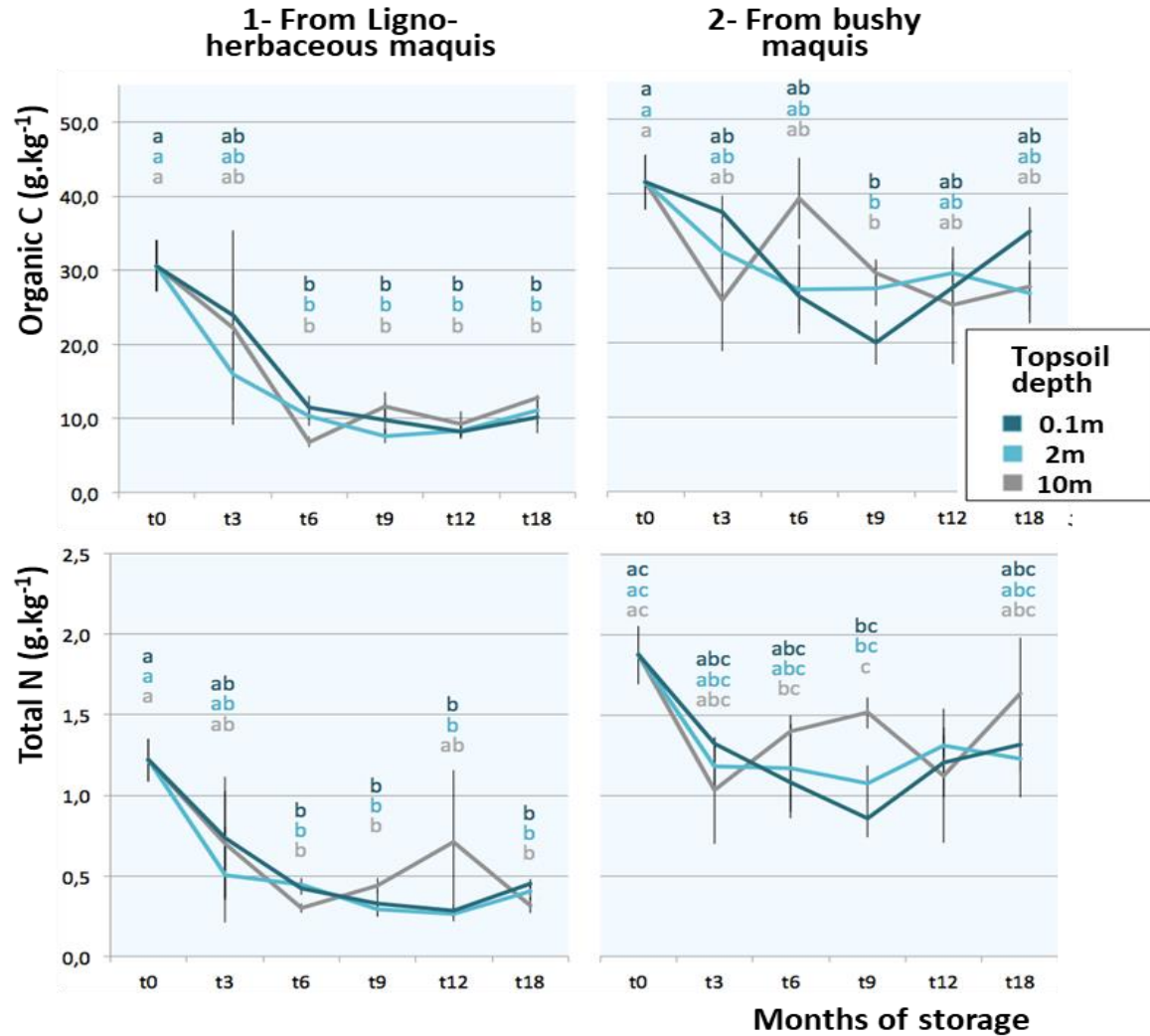


Fig. 10. Evolution of bulk density and water-filled pore space of stockpiled topsoils in relation to time storage and depth. For each graph, means with the same letter are not significantly different according to a two-way PERMANOVA followed by Pair-wise permutation test (n = 4).



**Fig. 11.** Evolution of organic carbon and total nitrogen of stockpiled topsoils in relation to time storage and depth. For each graph, means with the same letter are not significantly different according to a two-way PERMANOVA followed by Pair-wise permutation test (n = 4).



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