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Altered fungal communities in contaminated soils from French industrial brownfields

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Highlights:

- Fungal diversity of polycyclic aromatic hydrocarbon (PAH) and metal contaminated soils
- Differences at phylum and OTU level among soils of slag heap, settling pond and control groups
- Saccharomycetes, Leotiomyces and Agaricomycetes favoured in uncontaminated soils
- Higher relative abundance of Cryptomycota in metal-contaminated soils
- Higher relative abundance of Dothideomycetes in PAH-contaminated soils

| | | Controls | Slag heaps | Settling ponds |
|----------------------------------|-----------------------|---|---|---|
| Contamination levels | PAH |  |  |  |
| | Metal (Zn, Pb) |  |  |  |
| Alpha-diversity Indicator levels | Abundance & Richness |  |  |  |
| | Diversity & Evenness |  |  |  |
| Fungal community | Repressed fungal taxa | | Agaricomycetes Saccharomycetes Leotiomycetes Chytridiomycota | |
| | Favored fungal taxa | | Cryptomycota Pucciniomycetes Dothideomycetes Eurotiomycetes | |

Abstract

Polycyclic aromatic hydrocarbons (PAHs) and metals are contaminants of industrial brownfield soils. Pollutants can have harmful effects on fungi, which are major actors of soil functioning. Our objective was to highlight fungal selection following long-term contamination of soils. Fungal diversity was assessed on 30 top-soil samples from ten sites gathered in three groups with different contamination levels and physico-chemical characteristics: 1) uncontaminated controls, 2) slag heaps displaying high PAH and moderate metal contaminations, and 3) settling ponds displaying high metal and intermediate PAH contaminations.

Although fungal abundance and richness were similar among the soil groups, the diversity and evenness indices were lower for the slag heap group. Fungal diversity differed among soil groups at the phylum and OTU levels, and indicator species were identified. The relative abundance of Agaricomycetes, Saccharomycetes, Leotiomycetes and Chytridiomycota was higher in the control soils than in the two groups of contaminated soils. Cryptomycota LKM11 representatives were favoured in the slag heap and settling pond groups, and their relative abundance was correlated to the zinc and lead contamination levels. Dothideomycetes – positively linked to PAH contamination – and Eurotiomycetes were specific to the slag heap group. Pucciniomycetes and especially *Gymnosporangium* members were favoured in the settling pond soils.

1 **Altered fungal communities in contaminated soils from French industrial** 2 **brownfields**

3
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10 11 **Abstract**

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17 contaminations, and 3) settling ponds displaying high metal and intermediate PAH contaminations.

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21 Leotiomycetes and Chytridiomycota was higher in the control soils than in the two groups of contaminated soils.
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24 PAH contamination – and Eurotiomycetes were specific to the slag heap group. Pucciniomycetes and especially
25 *Gymnosporangium* members were favoured in the settling pond soils.

26
27
28 **Keywords:** Fungal communities; 18S rDNA sequencing; Soil pollution; Polycyclic aromatic hydrocarbon;
29 **Metals.**

31 **1. Introduction**

32 Fungi are known to play a fundamental role in terrestrial ecosystems: they contribute to carbon cycling through
33 organic matter mineralisation, or mediate plant mineral nutrition (Tedersoo *et al.*, 2014). Soil fungal communities
34 and the relative abundance of the different fungal taxa are mostly shaped by climatic and edaphic factors
35 (Beauregard *et al.*, 2010; Hannula *et al.*, 2012; Tedersoo *et al.*, 2014). In addition to these natural environmental
36 and biogeographic filters, anthropogenic constraints linked to human activities also contribute to shape fungal
37 communities. Due to their toxicity, soil contaminants act as selection factors that shape microbial communities.
38 An increase in soil metal (Pb and Zn) concentrations has been shown to decrease arbuscular mycorrhizal fungal
39 richness of plant species in pit mine, whereas one taxon was positively selected and developed in the highly
40 contaminated area (Zarei *et al.*, 2010). As a consequence, the presence of contaminants and their toxicity over
41 the long term could: 1) decrease diversity owing to the extinction of sensitive taxa, 2) disrupt the recycling of
42 organic matter, biogeochemical cycles and soil functioning, and 3) select suitable taxa tolerant to pollutants,
43 possibly involved in contaminant biodegradation or bioaccumulation (Harms *et al.*, 2011). There is still a need
44 nowadays to increase our knowledge on the ecology and adaptation capacities of fungal communities and taxa to
45 soil contaminants, and to evidence the selection of certain taxa displaying potentially interesting properties in
46 bioremediation.

47 Various anthropogenic activities have been negatively affecting the air, water and soil matrices by releasing
48 solid, liquid and gaseous wastes containing diverse pollutants. To date, large brownfield lands, where coking and
49 steel industry took place during the 20th century, are still highly contaminated with metals and organic
50 compounds such as polycyclic aromatic hydrocarbons (PAHs) (Rachwał *et al.*, 2015) that pose serious threats to
51 public and environmental health. Only few studies have assessed fungal taxonomic diversity in contaminated
52 soils (Bourceret *et al.*, 2016), especially in soils contaminated for several decades and with various
53 contamination levels and contamination types. PAHs and metals are known for their toxicity, carcinogenicity, or
54 mutagenicity (US EPA, 2014) that can also target soil fungal communities. For example, PAH contamination
55 can reduce fungal diversity (Bell *et al.*, 2014), or species richness (Zhou *et al.*, 2017). Some fungal genera such
56 as *Fusarium*, *Cladosporium*, *Mucor* (Salvo *et al.*, 2005), and the *Sordariales* and *Pleosporales* orders (Zhou *et al.*,
57 2017) have been found more represented in PAH-contaminated soil or sediment than in uncontaminated
58 environments. Ascomycota appear to be more prevalent in metal-contaminated soil, while Basidiomycota
59 dominate in uncontaminated areas (Narendrula and Nkongolo, 2017). More specifically, zinc and cadmium
60 contamination in a forest soil modified fungal community composition, with a dominance of *Sistotrema sp.*,
61 *Suillus luteus* and *Wilcoxina mikolae*, but did not affect fungal diversity indices (Op De Beeck *et al.*, 2015). This
62 dominance of various species or genera in contaminated soils suggests fungal selection and adaptation to
63 contamination. For bacterial taxonomic diversity in soils with various PAH and metal contamination levels, the
64 occurrence of certain OTUs was directly correlated to the presence of contaminants (Lemmel *et al.*, 2019). We
65 therefore wondered whether the fungal community response was similar or not in these soils.

66 In this context of soils contaminated by PAHs and heavy metals (Zn, Cd, Pb...) for decades following industrial
67 activities, we hypothesized that the type and level of contamination led to the selection of microorganisms, and
68 to similar fungal community compositions of soils sharing similar contamination types (i.e. with a similar
69 industrial history). Fungal species specific to these extremely contaminated soils could be identified as indicators

70 and considered for bioremediation. Additionally, a better understanding of fungal community assemblages living
71 in historically multi-contaminated soils could improve our knowledge of fungal ecology and soil functioning.
72 The aim of this study was to determine the taxonomic diversity and composition of fungal communities from
73 long-term contaminated soils presenting both PAH and metal contaminations. Slag heap and settling pond soils
74 that evolved from former French steel industries were compared with control uncontaminated soils from the
75 same region. The slag heap sites displayed high PAH and medium metal contamination, while the settling pond
76 sites displayed high metal and low PAH contamination. Taxonomic diversity was assessed through 18S rRNA
77 gene tag-amplicon sequencing (Illumina MiSeq). The links between soil physico-chemical parameters such as
78 contaminants and fungal alpha diversity indices or relative abundance of fungal phyla or OTUs were
79 investigated, and indicator species were identified.
80

81 **2. Materials and methods**

82 The ten sites and their physico-chemical soil characteristics were previously described in Lemmel *et al.* (2019),
83 but are briefly summarized in **Table 1**. These ten sites are located in the same geographical area of northeastern
84 part of France (Grand-Est region; **Figure S1**) and are located less than 100 km from one another. Briefly, three
85 were considered as the control soils (ctrl), i.e. three forest soils collected at Hémilly (He), Montiers-sur-Saulx
86 (Mo), and Dieulouard (Di) with low anthropogenic influence and without PAH or metal contamination from
87 steel activity. Seven brownfield soils from former steel industries known to be contaminated by metals and/or
88 PAHs were collected from: i) former slag heaps (sh) at Homécourt (Ho), Terville (Te), Uckange (Uc), and
89 Neuves-Maisons (NM), and ii) former settling ponds (sp), at Pompey (Po), Mont-St-Martin (MsM), and
90 Russange-Micheville (RM). All sites presented a similar dense tree cover (dominated by birch and/or beech),
91 except for Uc (sparse birch trees) and NM (colonized neither by trees nor by other plant species, i.e. bare soil).
92 Three independent bulk soil samples (triplicates collected 1 m apart) were collected in November 2015 (early
93 autumn season with a daily mean temperature of 11°C) on each site, as follows: after removing the litter layer, if
94 present, three blocks of 20 cm side and 30 cm depth were sampled, brought back to the laboratory, air-dried,
95 sieved at 2 mm and homogenised before analysis. Aliquots of soil for DNA extraction were immediately frozen
96 and stored at -80°C. The 30 top-soil samples from the ten sites were gathered in three groups (control, slag heap,
97 settling pond).

98 The methods used for soil characterisation are described in Lemmel *et al.* (2019). The soil collection presented a
99 gradient of PAH contamination ranging from 0.03 to 1,095.90 mg kg⁻¹ dw soil (based on the 16 priority PAHs of
100 the US Environmental Protection Agency, US-EPA) and a gradient of metal contamination mainly represented
101 by Zn (60 to 119,000 mg kg⁻¹), Pb (23 to 39,500 mg kg⁻¹) and Cd (0.09 to 152.00 mg kg⁻¹) (**Table 1**). The slag
102 heap and settling pond soils displayed significantly higher PAH concentrations than the control soils. The
103 settling pond soils displayed significantly higher concentrations of metals (Zn, Cd, and Pb; fluorhydric acid
104 extraction) than the control and slag heap soils. The pH (measured from a soil suspension prepared in distilled
105 water, 1:5 w/v) was rather similar among the soils from all 10 sites (7.2 to 8.0) except for soil He (5.4). Other
106 soil characteristics, namely cation exchange capacity (CEC), carbonates, total organic carbon (24 to 159 g kg⁻¹),
107 total nitrogen (0.56 to 7.19 g kg⁻¹; Dumas method, ISO 10694), and texture (from silty to sandy; ISO 11277
108 method) varied among soils, but no significant statistical difference (Kruskal Wallis test, p>0.05) was found
109 among the three groups of sites (**Table 1**). To sum up, the slag heap soils were mainly characterised by high
110 PAH contamination and an intermediate level of metal contamination, while the settling pond soils were mainly
111 characterised by high metal contamination and intermediate PAH contamination. The control soils showed the
112 lowest PAH and metal contamination levels, but significantly higher concentrations of major elements (Fe, K,
113 Al) than all contaminated soils.

114

115 **2.2. DNA extraction, real-time quantitative PCR, sequencing**

116 Genomic DNA was extracted from *ca.* 0.5 g of the soil samples (30 top-soil samples from the ten sites) using a
117 Fast DNA spin kit for Soil (MP Biomedicals), then diluted to 5 ng µl⁻¹ to be used as templates for further PCR
118 analysis.

119 Fungal abundance was estimated by real-time PCR quantification (Thion *et al.*, 2012), using the primer sets
120 Fung5F/FF390R (Lueders *et al.*, 2004), as explained in Lemmel *et al.* (2019).

121 The V7-V8 region of fungal 18S rRNA genes was amplified (*ca.* 390 bp) using primers FR1 and FF390 (Vainio
122 and Hantula, 2000) and following a previously described dual-index strategy (Kozich *et al.*, 2013) using PCR
123 primers with Illumina adaptor, pad and index sequences (Thomas and Cébron, 2016). PCR reactions were
124 performed on 2 μ l of diluted gDNA using Phusion high-fidelity polymerase (Thermo Scientific). PCR reactions
125 consisted of 34 cycles with a touchdown annealing temperature for 20 cycles (63 °C to 53 °C with a decrease of
126 0.5 °C/cycle) and 14 cycles at 53 °C. Amplification products were checked by 1 % agarose gel electrophoresis,
127 and purified using the UltraClean-htp 96 Well PCR Clean-Up kit (Qiagen) following the manufacturer's
128 instructions. After Quant-iT Picogreen ds-DNA assay Kit (Invitrogen) quantification, an amplicon library was
129 prepared (10 nM equimolar pool), purified on a QIAquick PCR purification kit column (Qiagen), and sent for
130 sequencing to Genewiz platform (South Plainfield, NJ, USA) using an Illumina MiSeq V2 Kit for 2 x 250 bp
131 paired-end sequencing. Illumina MiSeq paired-end reads were deposited in the SRA database under BioProject
132 accession number PRJNA601915. Sequence data were analysed following the MiSeq SOP procedure available
133 in March 2017 and described in Kozich *et al.* (2013), using Mothur v.1.36.0 (Schloss *et al.*, 2009). Paired-end
134 reads were trimmed using the following criteria: QS > 20, 312 bp < length < 320 bp, and no more than 3
135 ambiguous bases and 8 homopolymers. Chimeras detected using Uchime (Edgar *et al.*, 2011) and singletons
136 (sequences occurring only once among all samples) were removed. Unique sequences were aligned and taxa
137 were assigned using the Silva V132 database (cutoff = 80) and following Wang's method (Wang *et al.* 2007).
138 Singletons and sequences not affiliated to fungi were removed. Sequences were clustered in Operational
139 Taxonomic Units (OTUs) at 97 % similarity. Finally, datasets were rarefied to the lowest number of sequences
140 *per* sample (6,181 reads/sample) to compare the soil diversity levels. The number of reads for each OTU was
141 expressed as a percentage, to express the relative proportion or relative abundance of each OTU within the total
142 community. Alpha diversity was expressed by calculating Chao1 richness, Pielou's evenness J' , and Shannon
143 diversity H' indices (Hill *et al.*, 2003) using Mothur.

144

145 **2.3. Statistical analyses**

146 Statistical analyses were performed using RStudio v1.1.463. Significant differences (for fungal abundance, alpha
147 diversity indices, and relative abundance of the different phyla and classes) among the three soil groups (ctrl, sh
148 and sp) were assessed using multiple comparison Kruskal-Wallis test using '*kruskal*' function of the agricolae R
149 package (Mendiburu, 2013), with $p < 0.05$ and Fisher's least significant difference *post hoc* test.

150 Variation in fungal community composition among sites was visualized by nonmetric multidimensional scaling
151 (NMDS) using the function '*metaMDS*' on a Bray-Curtis dissimilarity matrix ('*vegdist*' function), and square-
152 root transformed ('*sqrt*' function) to limit the influence of abundant OTUs. Differences in fungal community
153 OTU composition among *a priori* defined groups of sites were evaluated using permutation-based multivariate
154 analysis of variance (PERMANOVA; Anderson 2001) in the '*adonis2*' function of the vegan R package
155 (Oksanen *et al.* 2017), yielding tests of significance using a 999-permutation-based F-test. To validate
156 PERMANOVA, we examined homogeneity of variance using a permutational test of multivariate group
157 dispersions ('*betadisper*' function).

158 Relationships between soil characteristics and soil fungal community diversity (alpha diversity indices, relative
159 abundance of the different phyla and of OTUs) were further analysed through three redundancy analyses (RDA;
160 '*rda*' function of the vegan R package; Oksanen *et al.* 2017) that classify the effects of soil characteristics on the

161 mean community composition *per* site. The carbon to nitrogen concentration ratio (C/N), organic matter (OM),
162 dissolved organic carbon (DOC), phosphorus (P₂O₅), carbonates, PAH concentrations (PAH), total metal
163 concentrations (Cu, Zn, Pb, Cd), and major element concentrations (Al, Ca, Fe, Na, K), pH, CEC and texture
164 (silt and sand percentages) were included to calculate the explained variance in RDA. The relative abundances of
165 fungal phyla and fungal OTUs were normalized (log(x+1)), and the most appropriate statistical model for RDA
166 was determined using forward selection as implemented in *vegan*'s '*ordistep*' and '*anova*' functions. Only
167 significantly explanatory variables (1,000 Monte Carlo permutations, $\alpha < 0.05$) were included in the final
168 selected RDA models.

169 To investigate the linear relation between soil parameters and fungal diversity (alpha diversity indices and
170 relative abundance of phyla or classes), we calculated Pearson's *r* between all pairs of measures using the
171 function '*rcorr*' in the *Hmisc* R package (Harrell, 2012).

172 We employed indicator species analysis using '*multipatt*' function and '*IndVal.g*' attribute of the *indicspecies* R
173 package (Dufrene and Legendre, 1997; De Cáceres *et al.*, 2010) to identify significant ($p < 0.05$) indicator fungal
174 OTUs of the control, slag heap and settling pond sites by combining information of species abundances and
175 occurrences in each group. Indicator values were tested using Monte Carlo permutation tests with 9,999
176 permutations.

177

178 **3. Results**

179 **3.1. Fungal community diversity descriptors**

180 Fungal abundance (estimated using 18S rRNA gene quantification), the number of OTUs determined at 97%
181 similarity and the taxonomic alpha diversity estimators based on OTU data are shown in **Table 2**. Fungal
182 abundance, the number of observed OTUs, and Chao1 richness indices were not significantly different among
183 the three groups of soils (**Table 2**). Slag heap sites harboured less diversified fungal communities, with lower
184 Shannon's diversity and Pielou's evenness indices than the control and settling pond sites. Redundancy analysis
185 was performed to identify the soil parameters that most influenced the alpha diversity indices (Chao1 richness,
186 Shannon diversity H' , and Pielou's evenness J'). However, no significant soil characteristic among those
187 measured was identified as contributing significantly to the variation of alpha diversity descriptors.

188

189 **3.2. Patterns of fungal phyla and classes**

190 We investigated fungal diversity at the phylum level and at the class level for the two major phyla, *i.e.*
191 Basidiomycota and Ascomycota, and explored their relative proportions in each site and in the three groups of
192 soils (**Figure 1**). The relative proportions of each phylum varied among soils, with a majority of OTUs
193 belonging to Ascomycota, Basidiomycota and Cryptomycota (**Figure 1A**). Fungal communities from soils
194 classified in the same group had different compositions. For example, the relative abundance of Eurotiomycetes
195 was at least fifteen times higher in sh-Te and sh-Uc than in the other two slag heap soils. However,
196 PERMANOVA analysis indicated that the fungal assemblage at the phylum level was significantly different
197 among the three groups of soils (sum of squares = 0.47, $R^2 = 0.41$, $F_{\text{model}} = 9.27$, $p < 0.001$). In general, all
198 soils were dominated by Ascomycota (40.5 % to 90.5 % on average), except those from sites ctrl-He and sp-RM
199 that were dominated by Basidiomycota (61.8 %) and Cryptomycota (73.2 %), respectively (**Figure 1A**). The
200 relative abundance of Ascomycota was significantly higher in the slag heap sites than in the other 2 sites (**Figure**

201 **1B**). The relative abundance of Cryptomycota (98-100 % of the OTUs belonging to this phylum were affiliated
202 to the environmental clade LKM11) was significantly higher in the contaminated sites than in the control sites
203 (**Figure 1B**). The relative abundance of Basidiomycota representatives was significantly lower in the slag heap
204 sites than in the settling pond sites (**Figure 1B**). Within the phylum Ascomycota, the relative abundance of fungi
205 affiliated to the Leotiomycetes, Pezizomycetes and Saccharomycetes classes was greater in the control sites than
206 in the contaminated sites (**Figure 1C**). Although Dothideomycetes were not dominant, OTUs affiliated to this
207 class represented 21.8 % and 23.3 % on average of fungal community in sites sh-Ho and sh-NM, respectively,
208 and were significantly enriched in the slag heap sites compared with the control sites, where this class
209 represented only 2.0 to 4.2 % on average (**Figure 1C**). Within the Basidiomycota phylum, the relative
210 abundance of the Agaricomycetes, Geminibasidiomycetes and Tremellomycetes classes was significantly higher
211 in the control sites than in the contaminated sites (**Figure 1D**).

212 Redundancy analysis (**Figure 2A**) was performed to identify the soil parameters that influenced the fungal
213 diversity structure at the phylum level. The first two axes of the RDA accounted for 89.4% of variability in the
214 relative abundance of fungal phyla. This variability was best explained by four of the measured soil parameters
215 (silt, $p=0.001$; Zn, $p=0.001$; K, $p=0.003$; PAH, $p=0.035$; ANOVA). The three main phyla – Ascomycota,
216 Basidiomycota and Cryptomycota – were largely discriminated in the RDA plot. Ascomycota and Cryptomycota
217 were separated on the first axis, mostly explained by soil texture (silt percentage) and metal contamination (total
218 zinc concentration). The plot also showed that the relative abundance of Cryptomycota was positively influenced
219 by zinc concentration, and this observation was confirmed by a significant Pearson correlation between the
220 relative abundance of Cryptomycota and the total zinc and lead concentrations in the soils ($p < 0.001$, $r = 0.93$
221 and $r = 0.70$, respectively; **Table S1**). The relative abundance of Ascomycota was influenced by soil texture
222 (negatively by the presence of silt) and by the PAH concentration (**Figure 2A**). We indeed confirmed a
223 significant Pearson correlation between the relative abundance of Ascomycota and the silt percentage ($p < 0.001$,
224 $r = -0.77$, **Table S1**). In addition, the relative proportion of fungi affiliated to Dothideomycetes – a class of the
225 Ascomycota phylum – was positively correlated to the PAH concentration (Pearson, $p < 0.001$, $r = 0.96$, **Table**
226 **S1**). The relative abundance of Basidiomycota appeared positively influenced by the potassium (K)
227 concentration (**Figure 2A**). The Pearson correlation between the relative abundance of Basidiomycota and the
228 soil characteristics evidenced a negative correlation with the soil pH ($p < 0.001$, $r = -0.84$, **Table S1**), and a
229 positive correlation with the dissolved organic carbon content ($p < 0.001$, $r = 0.84$, **Table S1**), two variables not
230 considered significant in the RDA model.

231

232 **3.3. Patterns of fungal OTUs and indicator species**

233 The analysis at the OTU level generated 1,614 OTUs, which ranged from 101 (sp-RM) to 191 (sp-Po) mean
234 OTUs *per* site, without any significant difference among the three groups of soils (**Table 2**). PERMANOVA
235 analysis indicated that the fungal OTU assemblage structure was significantly different among the three groups
236 of soils (sum of squares = 1.5224, $R^2 = 0.24$, $F_{\text{model}} = 4.273$, $p < 0.001$). Redundancy analysis (**Figure 2B**) was
237 performed to identify the soil parameters that mostly influenced fungal diversity at the OTU level. The first two
238 axes of the RDA accounted only for 37.0% of variability in the relative abundance of fungal OTUs, which was
239 significantly explained by two of the measured soil parameters (Al, $p=0.002$; PAH, $p=0.031$). This low
240 percentage of variability explained means that there were other non-measured parameters influencing most the

241 fungal diversity at OTU level. However, the control soils were separated from the 2 groups of contaminated soils
242 on the first axis by aluminium concentrations. The two groups of slag heap and settling pond soils were not
243 separated, but two soils of the slag heap group were separated from all the other contaminated soils on the
244 second axis due to their higher PAH concentrations. The RDA plot highlighted a few OTUs explaining most of
245 the variation among soil samples, and some were also identified as indicator species.

246 We searched for indicator species for the three groups of soils. **Figure 3** shows the indicator species with the
247 lowest p -values (< 0.01 ; see **Table S2** for the complete list of indicator species). In the control group, 86
248 indicator species were identified, with more than half (45 OTUs) belonging to Basidiomycota and one third (27
249 OTUs) belonging to Ascomycota. Among the 29 highly significant indicator species of the control soils (**Figure**
250 **3**), 11 were abundant OTUs representing 1 to 12% relative abundance of the whole fungal community in some of
251 the control soils. Seventeen were affiliated to Basidiomycota, five to Ascomycota, four to Chytridiomycota, two
252 to Cryptomycota, and one to Mucoromycota. The Basidiomycota indicator species belonged to nine different
253 orders (Agaricales, Pucciniales, Cantharellales, Russulales, Tremellales, Geminibasidiales, Trichosporonales,
254 Kriegeriales, and Corticiales). In the slag heap group, 22 indicator species were identified, 15 of which belonged
255 to Ascomycota. Among the 7 highly significant ones, four belonged to Ascomycota, one to Basidiomycota, one
256 to Cryptomycota LKM11 division and one to Mucoromycota (**Figure 3**). The Ascomycota indicator species
257 belonged to two main orders, namely Pleosporales and Chaetothyriales. In the settling pond group, 36 indicator
258 species were identified, 10 of which belonged to Cryptomycota LKM11 division. Among the four highly
259 significant indicator species, two belonged to Ascomycota, one to Basidiomycota and one to Mucoromycota
260 affiliated to the Glomeromycetes class.

261

262

263 **4. Discussion**

264 We hypothesized that in a context of long-term soil contamination, the type and level of contamination selected
265 adapted microorganisms and conducted to similar fungal community composition among soils sharing similar
266 industrial history and contamination type and level. To test this hypothesis, we analysed fungal communities in a
267 collection of 30 soils from 10 sampling sites classified in three groups according to their physico-chemical and
268 contamination characteristics. As previously shown in Lemmel *et al.* (2019), slag heap soils presented a high
269 PAH concentration and an intermediate level of metal contamination, and settling pond soils presented a high
270 level of metal contamination (especially high Zn, Pb and total Cd concentrations) and intermediate PAH
271 concentrations. The control soils were characterised by the lowest level of both PAH and metal contaminations.
272 Although the soils affiliated to one group showed variable fungal community structures, our data confirmed our
273 hypothesis: significant differences were highlighted among the control, slag heap and settling pond soil groups,
274 at various taxonomic levels (from the phylum to the OTU), and indicator species specific to the three soil groups
275 were identified. Fungal abundance and specific richness did not differ among soil groups, indicating that
276 differences were mostly explained by changes in fungal taxonomic composition and/or relative taxon
277 abundances.

278

279 **4.1. Fungal taxa repressed by contamination**

280 Our study showed a negative impact of contamination on many fungal taxa. In Basidiomycota phylum, the
281 Agaricomycetes, Geminibasidiomycetes and Tremellomycetes classes were represented in higher relative
282 proportions in the uncontaminated soils of the control group. Among the indicator species identified in the group
283 of control soils, more than half (45 out of 86) belonged to Basidiomycota. Members of the Agaricomycetes class,
284 which dominate numerous terrestrial ecosystems (Lauber *et al.*, 2008; Tedersoo *et al.*, 2014), represented the
285 majority (31 out of 45) of the Basidiomycota indicator species, and belonged to the Agaricales, Boletales,
286 Cantharellales, Corticiales, Trechisporales, and Russulales orders. Agaricomycetes fill a wide range of ecological
287 niches, but a very large number of species are wood-decay fungi that play a vital role in carbon cycling (de
288 Mattos-Shiple *et al.*, 2016). Furthermore, the relative abundance of Basidiomycota, and among them
289 Agaricomycetes, was positively correlated to different soil fertility indicators, i.e. increased Na, K and DOC
290 concentrations. Canini *et al.* (2020) also found a positive correlation between the potassium soil concentration
291 and the relative abundance of Basidiomycota and Agaricomycetes, as highlighted by our RDA analysis. A higher
292 DOC concentration could enhance the development of Agaricomycetes or be the result of a higher organic
293 matter degradation rate in these soils. For example, lignin degradation activity can lead to the release of
294 dissolved organic carbon (Kalbitz *et al.*, 2006). Industrial PAH- and metal-contaminated soils are disturbed
295 ecosystems that usually have lower fertility than unanthropised soils (Vetterlein & Hüttle 1999). A decrease of
296 the relative proportion of Agaricomycetes in our contaminated soils could indicate a disruption of the carbon
297 cycle and slower organic matter mineralisation due to metal toxicity (Clemente *et al.*, 2006). More globally,
298 Basidiomycota have been shown to be affected by metal contamination, with higher proportions in reference
299 soils than in metal-contaminated ones (Narendrula and Nkongolo, 2017).

300 The relative abundance of representatives of the Saccharomycetes and Leotiomycetes (both belonging to
301 Ascomycota) was higher in the control soils than in the two groups of contaminated soils. At a global scale, the
302 Saccharomycetes and Leotiomycetes classes represent 0.4 % and 7.1 % of fungal communities on average
303 (Tedersoo *et al.*, 2014), respectively, and were present at 1.4 to 5.5 % and 7.7 to 22.0 % in our control soils,
304 respectively. OTUs belonging to Saccharomycetes and Leotiomycetes were identified as indicator species of the
305 group of control soils. Among Saccharomycetes, *Blastobotrys adenivorans*, *Debaryomyces hansenii*,
306 *Sugiyamaella lignhabitans*, and *Ogataea methanolica*, identified as control group indicator species, have been
307 described as versatile yeasts able to use a large variety of lignocellulosic and carbohydrate carbon sources mostly
308 on rotting wood or decaying organic matter and could have xerotolerance properties such as halotolerance or
309 thermotolerance (Kunze *et al.* 2014; Michán *et al.* 2013). Despite these adaptive properties, we could
310 hypothesize that Saccharomycetes- and Leotiomycetes-affiliated taxa are negatively impacted by soil
311 contamination. Ferrari *et al.* (2011) indeed found that Saccharomycetes and Leotiomycetes were dominant
312 among pristine-soil isolates but were replaced by other taxa after diesel addition.

313 Members of the Sordariomycetes class (Hypocreales, Sordariales, Ophiostomatales and Atractospora) were
314 identified among indicator species of the control group, even if no difference appeared in their relative
315 abundance between the groups of soils. Sordariomycetes are the second largest class of Ascomycota that
316 represent 8.0 % of the fungal community in soils at a global scale (Tedersoo *et al.*, 2014) and are known as
317 common soil fungal taxa belonging to many different functional guilds (Maharachchikumbura *et al.*, 2016). In
318 the same line as in the present study, the relative abundance of Sordariomycetes was affected by high soil oil
319 contamination (Bell *et al.*, 2014).

320 The relative proportion of fungi affiliated to the Chytridiomycota phylum was lower in the settling pond soils
321 than in the control soils, where Chytridiomycota OTUs were identified as indicator species. A similar
322 observation was made by Ventorino *et al.* (2018) who found that even if this phyla did not dominate the fungal
323 community, its proportion decreased in heavily hydrocarbon- and copper-contaminated soils compared with
324 uncontaminated ones. Chytridiomycota can also be inhibited by long-term heavy metal pollution (Chen *et al.*,
325 2014). In the present work, the relative abundance of Chytridiomycota was negatively correlated to the zinc, lead
326 and iron concentrations, but positively correlated to soil nutrient availability (K and Na). The combination of
327 specific physico-chemical characteristics encountered in settling pond soils could explain why members of this
328 ancestral fungal phylum were found less adapted and less competitive than other fungi. For example,
329 Chytridiomycota are the only fungi producing unwallied, asexual spores that swim by means of a single
330 flagellum (Longcore and Simmons, 2020) giving them a better ability to colonize humid or aquatic
331 environments, in quite different conditions from those found in metal-contaminated soils.

332

333 **4.2. Fungal taxa favoured in slag heap soils**

334 We assessed the impact of high PAH and medium metal contamination on the soil fungal community diversity
335 of the slag heap group. Fungal Shannon diversity and Pielou's evenness indices were lower in the slag heap
336 soils, indicating a disturbance of the relationships among fungal species, while no difference in the bacterial
337 alpha diversity indices was previously found for the same soils (Lemmel *et al.*, 2019). Such reduced Shannon
338 diversity was previously observed in oil-contaminated soil (Bell *et al.* 2014). Lower diversity and evenness but
339 similar richness indicate that a few species dominate the fungal community, increasing the discrepancy between
340 the abundances of the different taxa.

341 Members of the Dothideomycetes class (Ascomycota) were in higher relative proportions in the slag heap soils
342 than in the control soils. The relative abundance of Dothideomycetes was positively correlated to soil PAH
343 contamination. At the world scale, this class represents 4.5 % of the fungal soil communities on average
344 (Tedersoo *et al.*, 2014), but represented 21.8 and 23.3 % of the fungal communities of soils Ho and NM,
345 respectively – the two soils displaying the highest PAH contamination. An increase in culturable
346 Dothideomycetes strains at high hydrocarbon concentrations was found by Ferrari *et al.* (2011). This class could
347 have been selected in PAH-contaminated soils due to its PAH tolerance or its PAH degradation capacity. In our
348 soils, the Dothideomycetes class was mostly represented by OTUs affiliated to Pleosporales, which was also one
349 dominant order found in hydrocarbon-contaminated soils (Bell *et al.*, 2014). Moreover, a quarter of the indicator
350 species identified in the slag heap group belonged to the Pleosporales order, with representatives of the *Preussia*,
351 *Corynespora*, and *Alternaria* genera. *Preussia* is a soft rot fungi with important role in lignin degradation and
352 having ability to cleave aromatic rings compounds (Horwath 2007). Interestingly, several Dothideomycetes
353 genera, including *Preussia*, have been shown to harbour endohyphal bacteria able to biodegrade hydrocarbons
354 (Hoffman and Arnold, 2010). Moreover, members of the *Corynespora* genus have been described as steroid and
355 limonene-hydrocarbon degraders (Demyttenaere *et al.*, 2001; Nassiri and Faramarzi, 2015), and found to
356 preferentially develop in a soil spiked with high amounts of phenanthrene (Li *et al.*, 2019). Concerning members
357 of the *Alternaria* genus, some have been isolated for their PAH degradation capacities (Chávez-Gómez *et al.*,
358 2003).

359 Although representatives of the Eurotiomycetes class were detected in variable proportions in our soil collection,
360 they largely dominated fungal communities in two slag heap soils (77.7 % in sh-Uc and 62.7 % sh-Te). This
361 class of Ascomycota represented a high proportion of fungal strains isolated from heavy-metal-contaminated
362 sediments (Abdel-Azeem *et al.*, 2015). Among indicator species of the slag heap group, we found 10 OTUs (half
363 of the indicator species) belonging to the Eurotiomycetes class, among which members of the Chaetothyriales
364 Sclerococcales and Eurotiales orders. Chaetothyriales have been detected in a wide range of habitats (Gueidan *et*
365 *al.*, 2014), so that one cannot be conclusive about their ecological niche. However, six OTUs belonging to
366 Herpotrichiellaceae family (Chaetothyriales order), with three belonging to *Exophiala calicioides* and one to
367 *Cladophialophora chaetospora* species, were among the 22 indicator species of slag heap soils. Members of
368 *Cladophialophora* and *Exophiala* genera, described as black yeast-like fungi, were largely studied for their
369 bioremediation potential (Prenafeta-Boldú *et al.* 2004). The genus *Exophiala*, is comprised of opportunistic
370 pathogens as well as species recovered from hydrocarbon contaminated environments, such as *E. xenobiotica*
371 (De Hoog *et al.* 2006) and *E. sideris* (also having the ability to tolerate arsenic; Seyedmousavi *et al.* 2011).
372 Strains with the ability to degrade hydrocarbon (benzene, toluene, ethylbenzene or xylene) were often isolated,
373 such as *E. maquariensis* (Zhang *et al.* 2019), *E. xenobiotica* and *E. bergeri* (Zhao *et al.* 2010).

374

375 **4.3. Fungal taxa favoured in settling pond soils**

376 The alpha diversity (richness, evenness and diversity) of the settling pond soils was not different from that of the
377 control soils. However, fungal community structure differences were observed between the settling pond and
378 control soils. Members of the Pucciniomycetes class (Basidiomycota) were in a higher relative proportion in the
379 settling pond soils. The relative abundance of Pucciniomycetes was positively correlated to the Ni, Co and Mo
380 metal concentrations. A quarter (8 out of 36) of the settling ponds indicator species belonged to Pucciniomycetes
381 and were affiliated to the *Gymnosporangium* genus, whose members are well known phytopathogens causing
382 rust (Yun *et al.*, 2009). Similarly, one of the slag heap indicator species belonged to *Rhizoctonia* genus known as
383 plant pathogen with a wide host range and worldwide distribution and causing collar, crown or root rots (Lenart-
384 Boroń & Boroń, 2014). We can therefore hypothesize that chronic soil pollution induced the selection of these
385 opportunistic plant pathogens, and maybe others, which is worrying for the ecosystem balance. Besides, the
386 settling pond group was the only one that harboured one OTU belonging to the Glomeromycetes class
387 (Mucoromycota phylum) and affiliated to *Entrophospora infrequens* as an indicator species. This fungus is
388 known as an arbuscular mycorrhizal fungus (AMF) beneficial for plants (Koziol and Bever, 2016). Seven OTUs
389 identified as settling pond soil indicator species belonged to Pezizomycotina sub-division of the Ascomycota,
390 but they belong to various classes and genera having various ecological preference, being geophilic fungi such as
391 *Dothidea*, *Parascedosporium*, and *Helvella* genera, being lichenicolous fungi such as *Lichina* and
392 *Rhymbocarpus*, or entomopathogen such as *Metarhizium*.

393

394 **4.4. Fungal taxa favoured in the two contaminated soils**

395 The relative abundance of fungi belonging to the Cryptomycota phylum was higher in the slag heap and settling
396 pond soils than in the control soils. Nearly half of the indicator species of the settling pond group and two
397 indicator species of slag heap group were affiliated to Cryptomycota. This newly proposed phylum is composed
398 of atypical fungi that have been mostly detected in freshwater ecosystems to date. They are single-cell fungi able

399 to form epibiont associations (Jones *et al.*, 2011), they lack a rigid chitin-/cellulose-rich wall, and they are
400 possibly capable of phagotrophy, as described for the *Rozella* genus (Pöggeler and Wöstemeyer, 2011).
401 Although, Cryptomycota represented 0.3 to 72.7% of the fungal communities in our contaminated soils, they
402 represent only a slight part (0.9 % on average) of fungal soil communities at the world scale (Tedersoo *et al.*,
403 2014), but Lazarus and James (2015) found that they were ubiquitous in terrestrial and aquatic ecosystems.
404 Cryptomycota members could be tolerant to metal contamination because their relative abundance was positively
405 correlated to the total zinc and lead concentrations. They were recently detected as dominant fungi colonizing
406 microplastics in aquatic ecosystems (Kettner *et al.*, 2017), which are well-known metal adsorption matrices
407 (Cole *et al.*, 2011; Brennecke *et al.*, 2016). In our study, Cryptomycota were mostly represented by sequences
408 belonging to OTUs related to the LKM11 environmental clade. This clade forms a deep branch of true fungi, but
409 its ecology and physiology is still unclear because no culturable representative currently exists (Lara *et al.*,
410 2010). However, based on our data, we can suppose that this LKM11 clade is well adapted to metal
411 contamination in soil ecosystems, even if it was recently detected as dominant in pristine limnetic ecosystems
412 (Rojas-Jimenez *et al.*, 2017). Therefore, its presence in contaminated soils could depend more on other soil
413 parameters such as carbon resources because positive correlation between relative Cryptomycota abundance and
414 organic matter has also been detected.

415

416 **5. Conclusions**

417 Our study brings the first report about the diversity and structure of fungal communities in aged multi-
418 contaminated soils, and identifies fungal taxa affected or favoured in PAH- and/or metal-contaminated soils vs.
419 control soils. Fungal abundance and richness were not significantly different among soil groups, while Shannon
420 diversity and evenness indices were lower in the slag heap soils than in the control soils. The differences among
421 the three groups of soils were confirmed based on fungal diversity at the phylum, class and OTU levels.
422 concerning the occurrence of certain fungal phyla, classes and indicator species depending on the soil physico-
423 chemical and contaminant characteristics were highlighted but need to be confirmed on a wider range of sites.
424 Basidiomycota, among which Agaricomycetes, as well as Saccharomycetes and Leotiomycetes (Ascomycota),
425 well known as wood-decaying fungi, were repressed in the two contaminated soil groups compared with the
426 control soils, potentially having an impact on carbon cycle. Cryptomycota representatives, such as the LKM11
427 clade, were favoured in the slag heap and settling pond soils, probably due to the presence of metals such as zinc
428 and lead. Members of the Dothideomycetes (*Preussia*, *Corynespora*, and *Alternaria*) and Eurotiomycetes
429 (*Exophiala calicioides* and *Cladophialophora chaetospora*) classes were identified as indicator species of the
430 slag heap soils and could be interesting PAH-degraders as their relative abundance was positively linked to the
431 soil PAH contamination level. Indicator species of the settling pond group mainly belonged to the
432 *Gymnosporangium* genus (Pucciniomycetes) known as phytopathogen. The contaminated soils could be
433 reservoirs of plant or animal pathogens thus having concern for environmental health but the selection of
434 protective species such as mycorrhizal fungi (Glomeromycetes class) could indicate resistance of the ecosystem.
435 Briefly, the fungal communities of contaminated soils could be altered for organic matter recycling functions,
436 and harbour pathogens, but it also potentially contain useful fungi for improving bioremediation strategies.

437

438

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444

445

446 **Table 1. Physico-chemical characteristics of the soils from the 10 studied sites and mean values for the**
 447 **three groups:** controls ctrl (n = 3), slag heaps sh (n = 4), and settling ponds sp (n = 3). *: soil classification is
 448 based on WRB 2016. Significant results of Kruskal-Wallis tests ($p < 0.05$), testing differences among the 3 soil
 449 groups, are indicated by different letters and no letter appears when non-significant differences were found.
 450 CEC: Cationic Exchange Capacity.

451

| Soil site group | Soil classification* | pH | CEC (cmol kg ⁻¹) | carbonates (g kg ⁻¹) | organic C (g kg ⁻¹) | total N | Texture (%) clay:silt:sand | Fe | Al | K | Zn | Pb | Cd | Cu | PAH | |
|-----------------|----------------------|-----------|---------------------------------|-------------------------------------|------------------------------------|---------|-------------------------------|--------------|------------------|-----------------|-------------------|--------------------|--------------------|-------------------|-----|--------------------|
| | | | | | | | | | | | | | | | | |
| Mo | ctrl | Cambisol | 7.0 | 35 | 5 | 81 | 4.6 | 49 : 46 : 5 | 44 | 67 | 14.6 | 144 | 44 | 0.7 | 16 | 3.5 |
| He | ctrl | Luvisol | 5.4 | 15 | 1 | 72 | 2.8 | 26 : 69 : 5 | 27 | 52 | 18.6 | 248 | 93 | 0.3 | 13 | 0.1 |
| Di | ctrl | Fluvisol | 7.3 | 10 | 14 | 24 | 1.7 | 12 : 14 : 74 | 18 | 45 | 28.6 | 60 | 31 | 0.2 | 10 | 0.9 |
| Uc | sh | Technosol | 8.0 | 4 | 8 | 30 | 0.6 | 5 : 18 : 77 | 452 | 20 | 0.6 | 74 | 23 | 0.1 | 9 | 0.9 |
| Te | sh | Technosol | 7.7 | 20 | 181 | 88 | 4.3 | 10 : 21 : 69 | 108 | 25 | 5.4 | 1650 | 475 | 4.5 | 146 | 114.7 |
| NM | sh | Technosol | 7.4 | 17 | 14 | 75 | 2.3 | 13 : 28 : 59 | 193 | 40 | 9.2 | 2540 | 653 | 3.3 | 144 | 1095.9 |
| Ho | sh | Technosol | 7.5 | 10 | 114 | 159 | 2.9 | 7 : 12 : 81 | 66 | 40 | 5.8 | 314 | 303 | 1.1 | 94 | 937.7 |
| Po | sp | Technosol | 7.8 | 51 | 89 | 109 | 7.2 | 18 : 53 : 29 | 93 | 40 | 5.4 | 29600 | 34700 | 152.0 | 148 | 21.2 |
| MsM | sp | Technosol | 7.2 | 16 | 152 | 119 | 4.2 | 11 : 73 : 16 | 304 | 21 | 1.5 | 55400 | 14400 | 17.6 | 186 | 49.6 |
| RM | sp | Technosol | 7.6 | 11 | 219 | 149 | 3.8 | 11 : 75 : 14 | 54 | 22 | 3.4 | 119000 | 39500 | 22.0 | 42 | 6.6 |
| Mean per group | ctrl | | 6.6 | 20 | 7 | 59 | 3.1 | 29 : 43 : 28 | 30 ^b | 55 ^a | 20.6 ^a | 151 ^b | 56 ^b | 0.4 ^b | 13 | 1.5 ^b |
| | sh | | 7.6 | 13 | 80 | 88 | 2.5 | 9 : 20 : 71 | 205 ^a | 31 ^b | 5.2 ^b | 1144 ^b | 363 ^b | 2.2 ^b | 98 | 537.3 ^a |
| | sp | | 7.6 | 26 | 154 | 126 | 5.1 | 13 : 67 : 20 | 150 ^a | 27 ^b | 3.4 ^b | 68000 ^a | 29533 ^a | 63.9 ^a | 125 | 25.8 ^a |

452

453

454 **Table 2. Fungal abundance and taxonomic alpha diversity indices of the soils from the 10 studied sites.**

455 Values are means (n = 3) ± standard error of the mean. For soil groups, values are means (n=3 for ctrl and sp
 456 soils, and n = 4 for sh soils) ± standard error of the mean. Significant results of Kruskal-Wallis tests ($p < 0.05$),
 457 testing differences among the 3 soil groups, are indicated by different letters, and no letter appears when non-
 458 significant differences were found.

| Soil site group | 18S rRNA gene copy number (x 10 ³ g ⁻¹) | Observed number of OTUs | Chao1 index | Shannon's diversity index H' | Pielou's evenness index J' | |
|-----------------|--|-------------------------|-------------|------------------------------|----------------------------|----------------------------|
| Mo | ctrl | 4.8 ± 0.6 | 162 ± 15 | 215 ± 31 | 3.40 ± 0.02 | 0.669 ± 0.009 |
| He | ctrl | 45.0 ± 1.8 | 176 ± 10 | 281 ± 48 | 3.08 ± 0.04 | 0.596 ± 0.018 |
| Di | ctrl | 21.8 ± 1.2 | 155 ± 7 | 203 ± 15 | 2.77 ± 0.09 | 0.549 ± 0.014 |
| Uc | sh | 5.1 ± 0.3 | 103 ± 4 | 175 ± 7 | 1.25 ± 0.05 | 0.271 ± 0.011 |
| Te | sh | 34.2 ± 3.0 | 113 ± 2 | 147 ± 15 | 2.08 ± 0.06 | 0.440 ± 0.011 |
| NM | sh | 3.1 ± 0.3 | 150 ± 7 | 179 ± 20 | 2.92 ± 0.03 | 0.581 ± 0.002 |
| Ho | sh | 11.8 ± 0.2 | 190 ± 9 | 257 ± 6 | 2.75 ± 0.01 | 0.529 ± 0.005 |
| Po | sp | 18.1 ± 1.6 | 196 ± 8 | 317 ± 22 | 3.14 ± 0.02 | 0.593 ± 0.006 |
| MsM | sp | 10.3 ± 0.9 | 148 ± 7 | 186 ± 9 | 3.11 ± 0.02 | 0.623 ± 0.002 |
| RM | sp | 8.5 ± 0.7 | 101 ± 5 | 158 ± 15 | 1.46 ± 0.05 | 0.313 ± 0.007 |
| Mean per group | ctrl | 23.8 ± 5.9 | 164 ± 6 | 233 ± 21 | 3.08 ± 0.10 ^a | 0.605 ± 0.019 ^a |
| | sh | 13.5 ± 3.8 | 139 ± 11 | 190 ± 14 | 2.25 ± 0.20 ^b | 0.455 ± 0.036 ^b |
| | sp | 12.3 ± 1.6 | 148 ± 14 | 220 ± 26 | 2.57 ± 0.28 ^a | 0.510 ± 0.049 ^a |

459

460 **Figure captions**

461

462 **Figure 1 | Taxonomic composition of the fungal communities of soil samples from the 10 studied sites and**
463 **differences among groups of sites.** A) Relative abundance of major fungal phyla and major classes for
464 Ascomycota and Basidiomycota in the 10 studied sites. Values are means (n = 3), and error-bars represent
465 standard errors of the mean for each site. The group named 'Others' corresponded to Blastocladiomycota,
466 LKM15, Neocallimastigomycota, Zoopagomycota, Entorrhizomycota, unclassified fungi, and taxa affiliated to
467 Basidiomycota and Ascomycota but not shown on the graph due to their minor relative abundances. B) Relative
468 abundance of the five major phyla in the three groups of sites, C) Relative abundance of Ascomycota major
469 classes in the three groups of sites, and D) Relative abundance of Basidiomycota **major classes in the three**
470 **groups of sites.** Values are means (n=9 for *ctrl* and *sp* group and n=12 for *sh* group). Statistical differences
471 among phylum and class proportions in the soil groups are indicated by different letters (Kruskal-Wallis test, * p
472 < 0.05, ** p < 0.001, ***p < 0.0001, n.s: not significant).

473

474

475 **Figure 2 | Redundancy analysis (RDA) plots showing influence of significant soil characteristics (blue**
476 **vectors) on the fungal communities of the ten studied sites.** A) Fungal phyla relative abundance (in red) and
477 B) Fungal OTUs relative abundance (in red; only the names of some OTUs highly discriminating samples on
478 first plan are shown, those circled in blue and green are those from indicator species, cf. Figure 3). *Soils (Di:*
479 *Dieulouard, Ho: Homécourt, He: Hémilly, MsM: Mont St Martin, Mo: Montiers, RM: Russange-Micheville,*
480 *NM: Neuves-Maisons, Po: Pompey, Te: Terville, Uc: Uckange) were coloured according to the group they*
481 *belong to, ie. settling ponds in red, slag heaps in green and controls in blue. Soil characteristics: PAH,*
482 *polycyclic aromatic hydrocarbons; K, Zn and Al: total concentration of potassium, zinc and aluminium,*
483 *respectively; silt: the percentage of silt of the soil texture.*

484

485

486 **Figure 3 | Indicator species identified for control, slag heap and settling pond groups of soil (in blue, green**
487 **and red, respectively), their mean relative abundance in the group and indicator value (Indval).** Only
488 indicator species with the lower p-values (p< 0.01) are presented (see Table S2 for complete list of indicator
489 species). OTU lowest possible taxonomic classifications are given next to the OTU number with the prefix
490 corresponding to taxonomic rank (s-d = sub-division, c = class, o = order, f = family, g = genus, no prefix =
491 species). Indicator species also appearing as discriminant OTUs on RDA plot (Figure 2B) are indicated with *.

492

493

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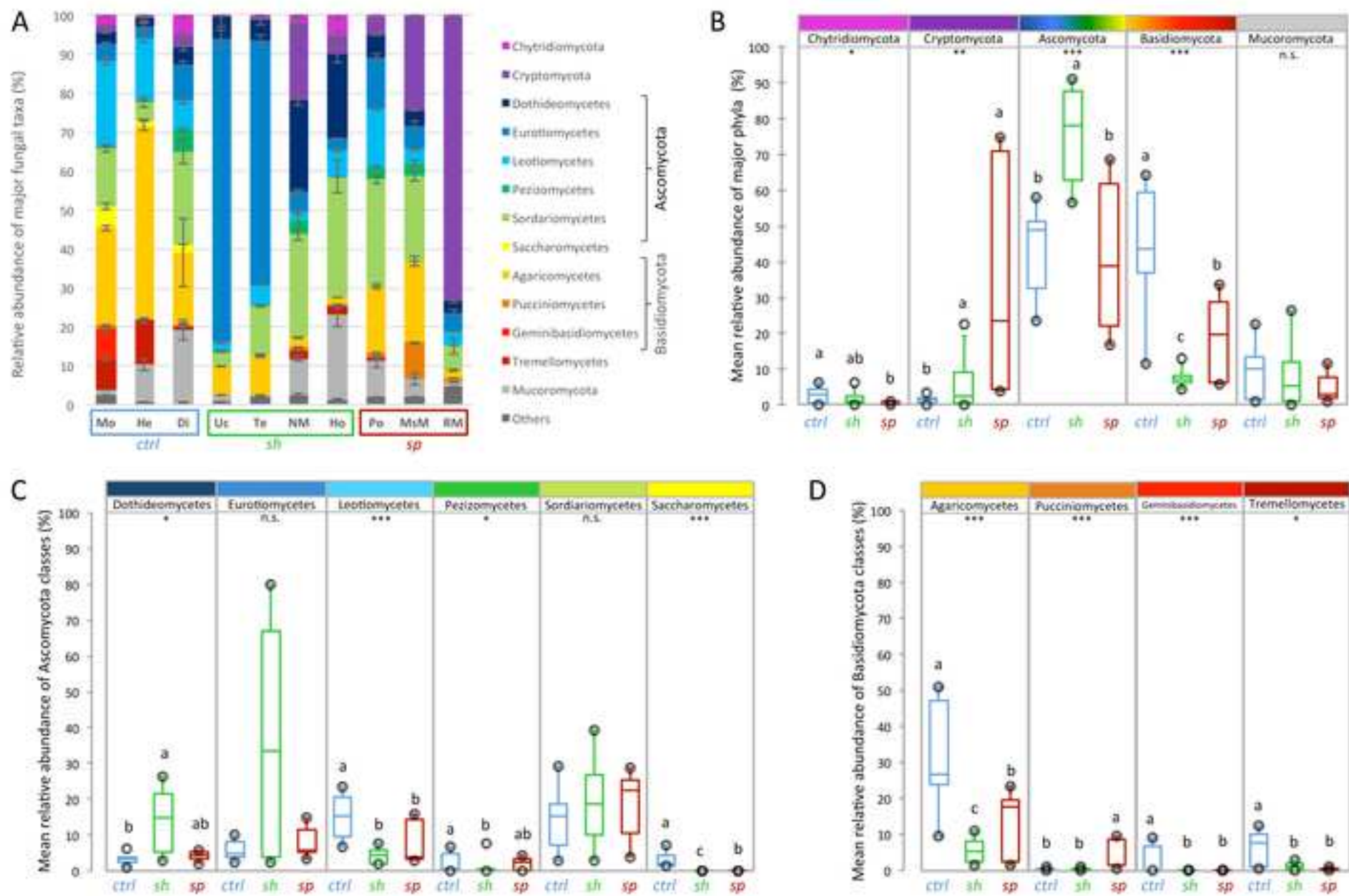
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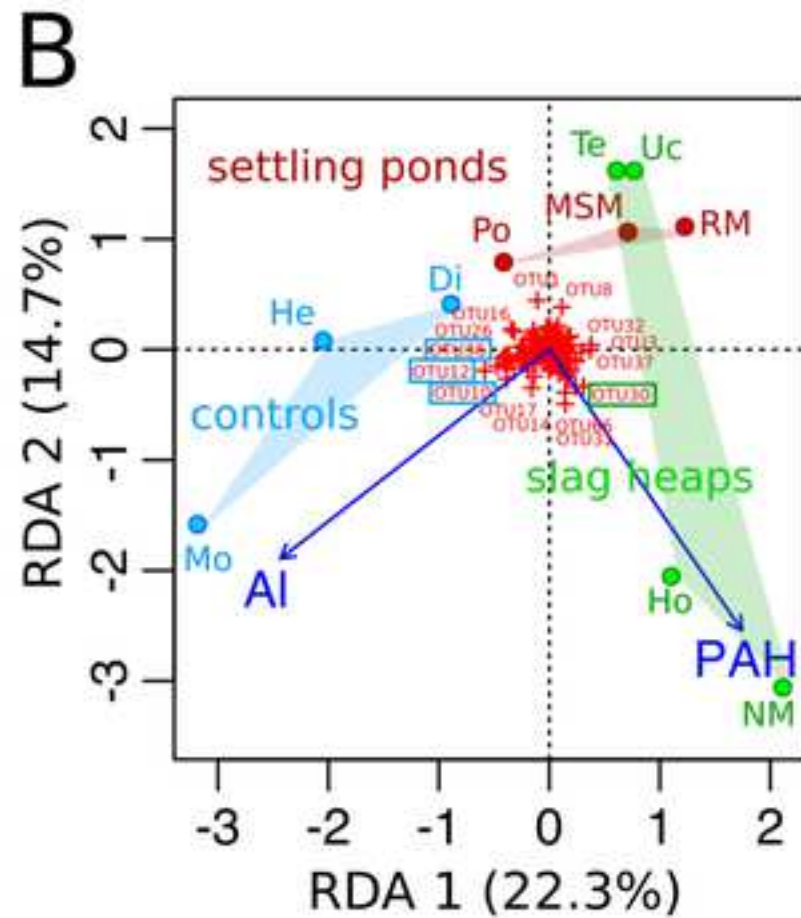
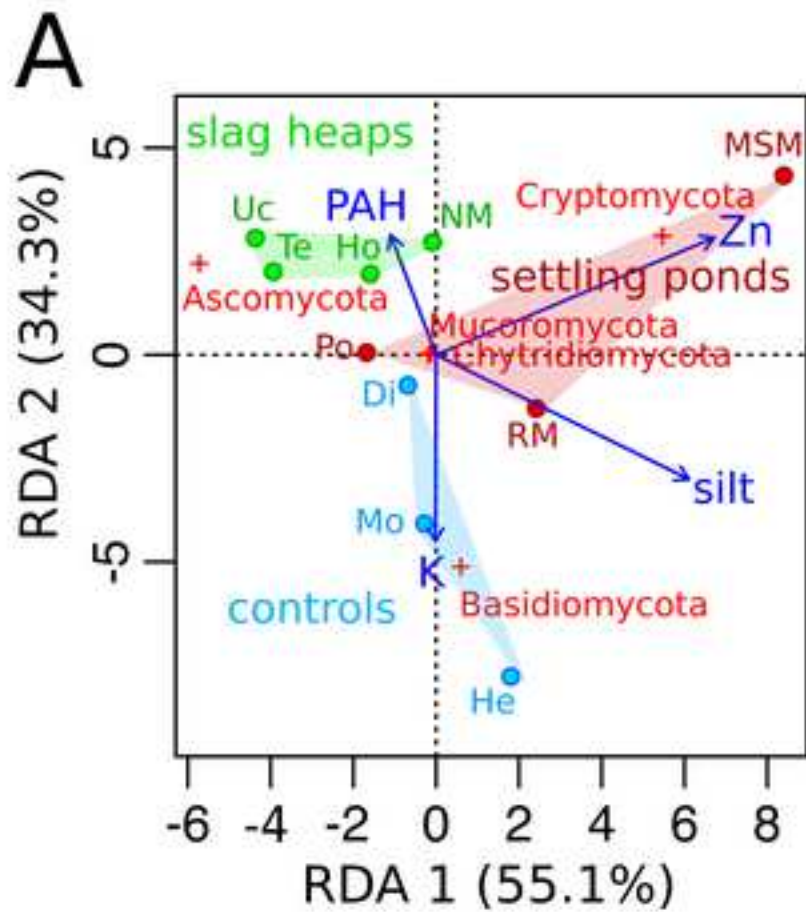
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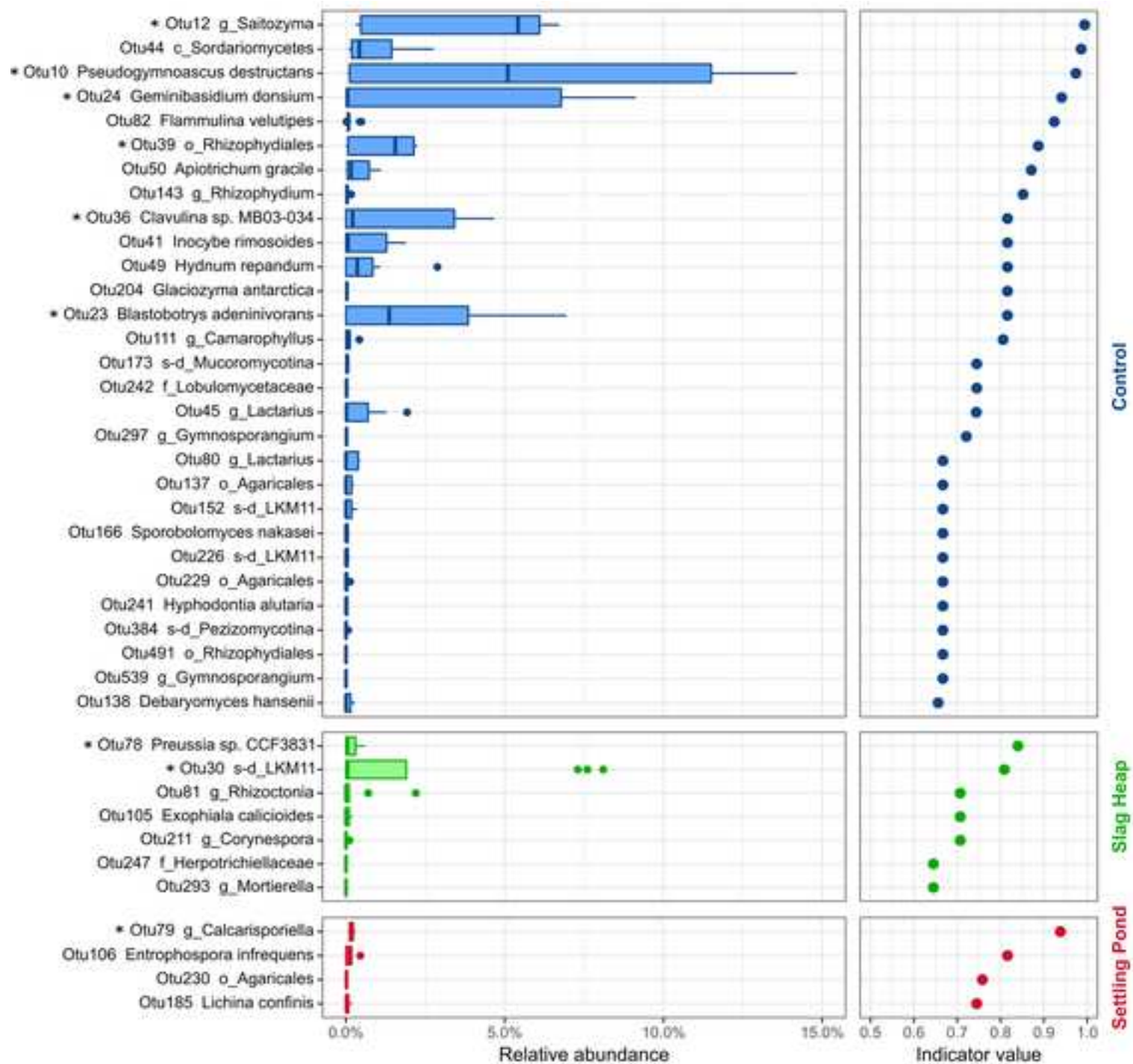


Table1&2

| | Soil site group | Soil classification | pH | CEC (cmol kg^{-1}) | carbonate s | organic C (g kg^{-1}) | total N | |
|----------------|-----------------|---------------------|-----------|----------------------------------|----------------|-------------------------------------|---------|-----|
| | Mo | ctrl | Cambisol | 7.0 | 35 | 5 | 81 | 4.6 |
| | He | ctrl | Luvisol | 5.4 | 15 | 1 | 72 | 2.8 |
| | Di | ctrl | Fluvisol | 7.3 | 10 | 14 | 24 | 1.7 |
| | Uc | sh | Technosol | 8.0 | 4 | 8 | 30 | 0.6 |
| | Te | sh | Technosol | 7.7 | 20 | 181 | 88 | 4.3 |
| | NM | sh | Technosol | 7.4 | 17 | 14 | 75 | 2.3 |
| | Ho | sh | Technosol | 7.5 | 10 | 114 | 159 | 2.9 |
| | Po | sp | Technosol | 7.8 | 51 | 89 | 109 | 7.2 |
| | MsM | sp | Technosol | 7.2 | 16 | 152 | 119 | 4.2 |
| | RM | sp | Technosol | 7.6 | 11 | 219 | 149 | 3.8 |
| Mean per group | ctrl | | | 6.6 | 20 | 7 | 59 | 3.1 |
| | sh | | | 7.6 | 13 | 80 | 88 | 2.5 |
| | sp | | | 7.6 | 26 | 154 | 126 | 5.1 |

| Texture (%) | Fe | Al | K | Zn | Pb | Cd | Cu | PAH |
|----------------|-----------------------|-----------------|-------------------|------------------------|--------------------|-------------------|-----|--------------------|
| clay:silt:sand | (g kg ⁻¹) | | | (mg kg ⁻¹) | | | | |
| 49 : 46 : 5 | 44 | 67 | 14.6 | 144 | 44 | 0.7 | 16 | 3.5 |
| 26 : 69 : 5 | 27 | 52 | 18.6 | 248 | 93 | 0.3 | 13 | 0.1 |
| 12 : 14 : 74 | 18 | 45 | 28.6 | 60 | 31 | 0.2 | 10 | 0.9 |
| 5 : 18 : 77 | 452 | 20 | 0.6 | 74 | 23 | 0.1 | 9 | 0.9 |
| 10 : 21 : 69 | 108 | 25 | 5.4 | 1650 | 475 | 4.5 | 146 | 114.7 |
| 13 : 28 : 59 | 193 | 40 | 9.2 | 2540 | 653 | 3.3 | 144 | 1095.9 |
| 7 : 12 : 81 | 66 | 40 | 5.8 | 314 | 303 | 1.1 | 94 | 937.7 |
| 18 : 53 : 29 | 93 | 40 | 5.4 | 29600 | 34700 | 152.0 | 148 | 21.2 |
| 11 : 73 : 16 | 304 | 21 | 1.5 | 55400 | 14400 | 17.6 | 186 | 49.6 |
| 11 : 75 : 14 | 54 | 22 | 3.4 | 119000 | 39500 | 22.0 | 42 | 6.6 |
| 29 : 43 : 28 | 30 ^b | 55 ^a | 20.6 ^a | 151 ^b | 56 ^b | 0.4 ^b | 13 | 1.5 ^b |
| 9 : 20 : 71 | 205 ^a | 31 ^b | 5.2 ^b | 1144 ^b | 363 ^b | 2.2 ^b | 98 | 537.3 ^a |
| 13 : 67 : 20 | 150 ^a | 27 ^b | 3.4 ^b | 68000 ^a | 29533 ^a | 63.9 ^a | 125 | 25.8 ^a |

| | Soil site groups | 18S rRNA gene copy number ($\times 10^9 \text{ g}^{-1}$) | Observed number of OTUs | Chao1 index | |
|--|------------------|--|-------------------------|--------------|--------------|
| | Mo | ctrl | 4.8 ± 0.6 | 162 ± 15 | 215 ± 31 |
| | He | ctrl | 45.0 ± 1.8 | 176 ± 10 | 281 ± 48 |
| | Di | ctrl | 21.8 ± 1.2 | 155 ± 7 | 203 ± 15 |
| | Uc | sh | 5.1 ± 0.3 | 103 ± 4 | 175 ± 7 |
| | Te | sh | 34.2 ± 3.0 | 113 ± 2 | 147 ± 15 |
| | NM | sh | 3.1 ± 0.3 | 150 ± 7 | 179 ± 20 |
| | Ho | sh | 11.8 ± 0.2 | 190 ± 9 | 257 ± 6 |
| | Po | sp | 18.1 ± 1.6 | 196 ± 8 | 317 ± 22 |
| | MsM | sp | 10.3 ± 0.9 | 148 ± 7 | 186 ± 9 |
| | RM | sp | 8.5 ± 0.7 | 101 ± 5 | 158 ± 15 |
| | Mean per group | ctrl | 23.8 ± 5.9 | 164 ± 6 | 233 ± 21 |
| | | sh | 13.5 ± 3.8 | 139 ± 11 | 190 ± 14 |
| | | sp | 12.3 ± 1.6 | 148 ± 14 | 220 ± 26 |

| Shannon's diversity index H' | Pielou's evenness index J' |
|-----------------------------------|---------------------------------|
| 3.40 ± 0.02 | 0.669 ± 0.009 |
| 3.08 ± 0.04 | 0.596 ± 0.018 |
| 2.77 ± 0.09 | 0.549 ± 0.014 |
| 1.25 ± 0.05 | 0.271 ± 0.011 |
| 2.08 ± 0.06 | 0.440 ± 0.011 |
| 2.92 ± 0.03 | 0.581 ± 0.002 |
| 2.75 ± 0.01 | 0.529 ± 0.005 |
| 3.14 ± 0.02 | 0.593 ± 0.006 |
| 3.11 ± 0.02 | 0.623 ± 0.002 |
| 1.46 ± 0.05 | 0.313 ± 0.007 |

| | |
|--------------------------|----------------------------|
| 3.08 ± 0.10 ^a | 0.605 ± 0.019 ^a |
| 2.25 ± 0.20 ^b | 0.455 ± 0.036 ^b |
| 2.57 ± 0.28 ^a | 0.510 ± 0.049 ^a |
