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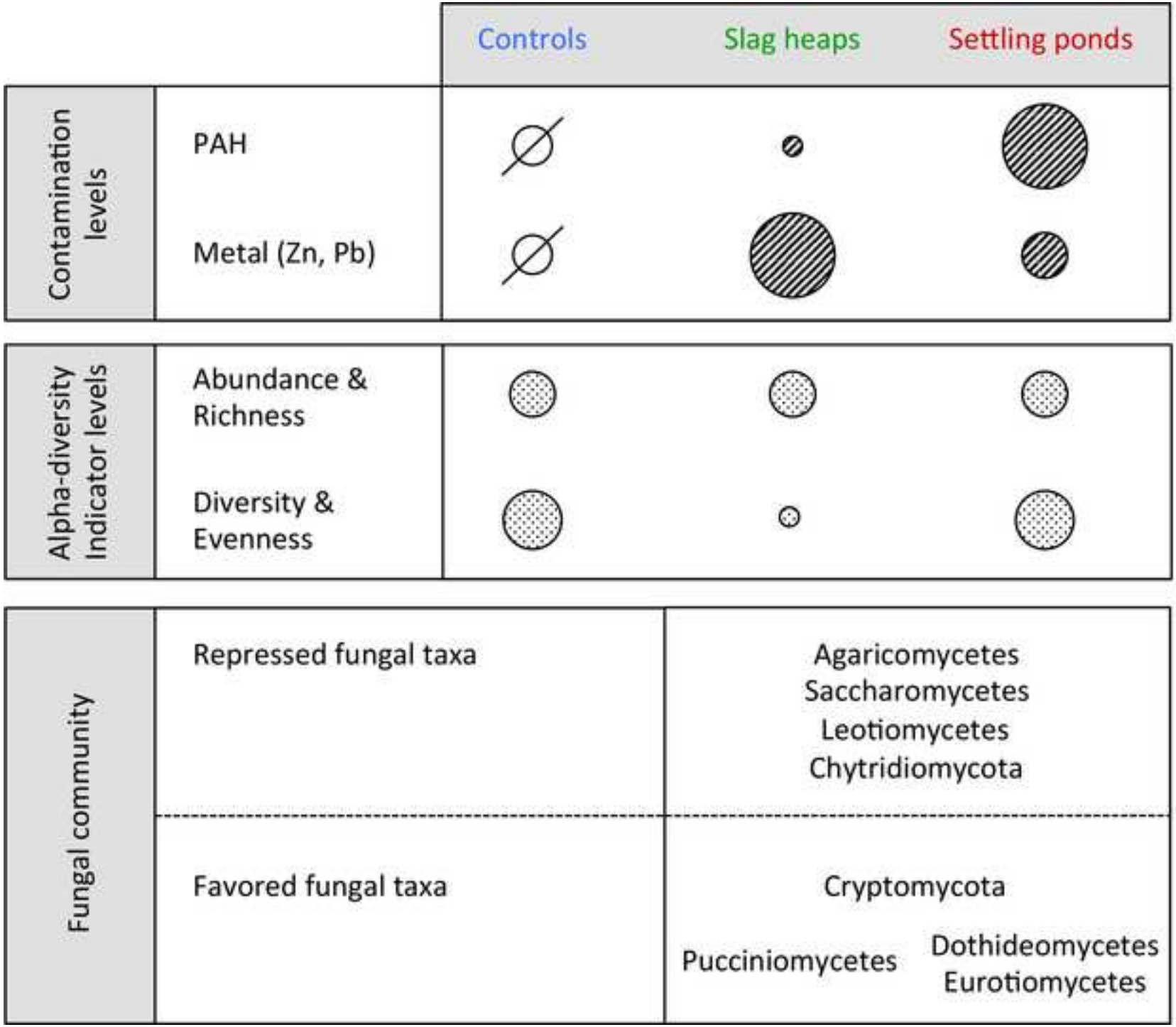
Highlights:

- 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, Leotiomycetes and Agaricomycetes favoured in uncontaminated soils
- Higher relative abundance of Cryptomycota in metal-contaminated soils
- Higher relative abundance of Dothideomycetes in PAH-contaminated soils

Graphical Abstract



Abstract

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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.

Altered fungal communities in contaminated soils from French industrial brownfields

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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.

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Keywords: Fungal communities; 18S rDNA sequencing; Soil pollution; Polycyclic aromatic hydrocarbon; Metals.

1. Introduction

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

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

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

and considered for bioremediation. Additionally, a better understanding of fungal community assemblages living in historically multi-contaminated soils could improve our knowledge of fungal ecology and soil functioning.

The aim of this study was to determine the taxonomic diversity and composition of fungal communities from long-term contaminated soils presenting both PAH and metal contaminations. Slag heap and settling pond soils that evolved from former French steel industries were compared with control uncontaminated soils from the same region. The slag heap sites displayed high PAH and medium metal contamination, while the settling pond sites displayed high metal and low PAH contamination. Taxonomic diversity was assessed through 18S rRNA gene tag-amplicon sequencing (Illumina MiSeq). The links between soil physico-chemical parameters such as contaminants and fungal alpha diversity indices or relative abundance of fungal phyla or OTUs were investigated, and indicator species were identified.

2. Materials and methods

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

The methods used for soil characterisation are described in Lemmel *et al.* (2019). The soil collection presented a gradient of PAH contamination ranging from 0.03 to 1,095.90 mg kg^{-1} dw soil (based on the 16 priority PAHs of the US Environmental Protection Agency, US-EPA) and a gradient of metal contamination mainly represented 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 heap and settling pond soils displayed significantly higher PAH concentrations than the control soils. The settling pond soils displayed significantly higher concentrations of metals (Zn, Cd, and Pb; fluorhydric acid extraction) than the control and slag heap soils. The pH (measured from a soil suspension prepared in distilled 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 soil characteristics, namely cation exchange capacity (CEC), carbonates, total organic carbon (24 to 159 g kg^{-1}), total nitrogen (0.56 to 7.19 g kg^{-1}; Dumas method, ISO 10694), and texture (from silty to sandy; ISO 11277 method) varied among soils, but no significant statistical difference (Kruskal Wallis test, $p>0.05$) was found among the three groups of sites (**Table 1**). To sum up, the slag heap soils were mainly characterised by high PAH contamination and an intermediate level of metal contamination, while the settling pond soils were mainly characterised by high metal contamination and intermediate PAH contamination. The control soils showed the lowest PAH and metal contamination levels, but significantly higher concentrations of major elements (Fe, K, Al) than all contaminated soils.

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

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

Fungal abundance was estimated by real-time PCR quantification (Thion *et al.*, 2012), using the primer sets 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 µl 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

mean community composition *per* site. The carbon to nitrogen concentration ratio (C/N), organic matter (OM), dissolved organic carbon (DOC), phosphorus (P_2O_5), carbonates, PAH concentrations (PAH), total metal concentrations (Cu, Zn, Pb, Cd), and major element concentrations (Al, Ca, Fe, Na, K), pH, CEC and texture (silt and sand percentages) were included to calculate the explained variance in RDA. The relative abundances of fungal phyla and fungal OTUs were normalized (log(x+1)), and the most appropriate statistical model for RDA was determined using forward selection as implemented in vegan's '*ordistep'* and '*anova'* functions. Only significantly explanatory variables (1,000 Monte Carlo permutations, $\alpha < 0.05$) were included in the final selected RDA models.

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

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

3. Results

3.1. Fungal community diversity descriptors

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

3.2. Patterns of fungal phyla and classes

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

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

Redundancy analysis (**Figure 2A**) was performed to identify the soil parameters that influenced the fungal diversity structure at the phylum level. The first two axes of the RDA accounted for 89.4% of variability in the relative abundance of fungal phyla. This variability was best explained by four of the measured soil parameters (silt, p=0.001; Zn, p=0.001; K, p=0.003; PAH, p=0.035; ANOVA). The three main phyla – Ascomycota, Basidiomycota and Cryptomycota – were largely discriminated in the RDA plot. Ascomycota and Cryptomycota were separated on the first axis, mostly explained by soil texture (silt percentage) and metal contamination (total zinc concentration). The plot also showed that the relative abundance of Cryptomycota was positively influenced by zinc concentration, and this observation was confirmed by a significant Pearson correlation between the relative abundance of Cryptomycota and the total zinc and lead concentrations in the soils ($p < 0.001$, r = 0.93 and r = 0.70, respectively; **Table S1**). The relative abundance of Ascomycota was influenced by soil texture (negatively by the presence of silt) and by the PAH concentration (**Figure 2A**). We indeed confirmed a significant Pearson correlation between the relative abundance of Ascomycota and the silt percentage ($p < 0.001$, r = -0.77, **Table S1**). In addition, the relative proportion of fungi affiliated to Dothideomycetes – a class of the Ascomycota phylum – was positively correlated to the PAH concentration (Pearson, $p < 0.001$, r = 0.96, **Table S1**). The relative abundance of Basidiomycota appeared positively influenced by the potassium (K) concentration (**Figure 2A**). The Pearson correlation between the relative abundance of Basidiomycota and the soil characteristics evidenced a negative correlation with the soil pH ($p < 0.001$, r = -84, **Table S1**), and a positive correlation with the dissolved organic carbon content ($p < 0.001$, r = 0.84, **Table S1**), two variables not considered significant in the RDA model.

3.3. Patterns of fungal OTUs and indicator species

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

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

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

4. Discussion

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

4.1. Fungal taxa repressed by contamination

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

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

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

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

4.2. Fungal taxa favoured in slag heap soils

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

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

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

4.3. Fungal taxa favoured in settling pond soils

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

4.4. Fungal taxa favoured in the two contaminated soils

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

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

5. Conclusions

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

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

	Soil site group	Soil classification*	pH	CEC	carbonates	organic C	total N	Texture (%)	Fe	Al	K	Zn	Pb	Cd	Cu	PAH
				(cmol kg^{-1})	(g kg^{-1})			clay:silt:sand	(g kg^{-1})			(mg kg^{-1})				
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]

Table 2. Fungal abundance and taxonomic alpha diversity indices of the soils from the 10 studied sites. Values are means (n = 3) ± standard error of the mean. For soil groups, values are means (n=3 for ctrl and sp soils, and n = 4 for sh soils) ± standard error of the mean. Significant results of Kruskal-Wallis tests ($p < 0.05$), testing differences among the 3 soil groups, are indicated by different letters, and no letter appears when non-significant differences were found.

	Soil site group	18S rRNA gene copy number (x 10^9 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]

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).

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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.*

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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 *.

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References

Abdel-Azeem, A., El-Morsy, E., Nour El-Dein, M., and Rashad, M. (2015) Occurrence and diversity of mycobiota in heavy metal contaminated sediments of Mediterranean coastal lagoon El-Manzala, Egypt. *Mycosphere* **6**:228-240. https://doi.org/10.5943/mycosphere/6/2/12

Beauregard, M.S., Hamel, C., Atul-Nayyar, A., and St-Arnaud, M. (2010) Long-term phosphorus fertilization impacts soil fungal and bacterial diversity but not AM fungal community in alfalfa. *Microb Ecol* **59**: 379–389. https://doi.org/10.1007/s00248-009-9583-z

Bell, T.H., El-Din Hassan, S., Lauron-Moreau, A., Al-Otaibi, F., Hijri, M., Yergeau, E., and St-Arnaud, M. (2014) Linkage between bacterial and fungal rhizosphere communities in hydrocarbon-contaminated soils is related to plant phylogeny. *ISME J* **8**: 331–343. https://doi.org/10.1038/ismej.2013.149

Bourceret, A., Cébron, A., Tisserant, E., Poupin, P., Bauda, P., Beguiristain, T., and Leyval, C. (2016) The bacterial and fungal diversity of an aged PAH- and heavy metal-contaminated soil is affected by plant cover and edaphic parameters. *Microb Ecol* **71**: 711–724. https://doi.org/10.1007/s00248-015-0682-8

Bourceret, A., Leyval, C., Fouquet, C. de, and Cébron, A. (2015) Mapping the centimeter-scale spatial variability of PAHs and microbial populations in the rhizosphere of two plants. *PLOS ONE* **10**: e0142851. https://doi.org/10.7910/DVN/EFXVQH

Brennecke, D., Duarte, B., Paiva, F., Caçador, I., and Canning-Clode, J. (2016) Microplastics as vector for heavy metal contamination from the marine environment. *Estuar Coast Shelf Sci* **178**: 189–195. https://doi.org/10.1016/j.ecss.2015.12.003

Canini, F., Geml, J., D'Acqui, L.P., Selbmann, L., Onofri, S., Ventura, S., and Zucconi, L. (2020) Exchangeable cations and pH drive diversity and functionality of fungal communities in biological soil crusts from coastal sites of Victoria Land, Antarctica. *Fungal Ecol* **45**: 100923. https://doi.org/10.1016/j.funeco.2020.100923

Chávez-Gómez, B., Quintero, R., Esparza-García, F., Mesta-Howard, A.M., Zavala Díaz de la Serna, F.J., Hernández-Rodríguez, C.H., et al. (2003) Removal of phenanthrene from soil by co-cultures of bacteria and fungi pregrown on sugarcane bagasse pith. *Bioresour Technol* **89**: 177–183. https://doi.org/10.1016/S0960-8524(03)00037-3

Chen, J., He, F., Zhang, X., Sun, X., Zheng, J., and Zheng, J. (2014) Heavy metal pollution decreases microbial abundance, diversity and activity within particle-size fractions of a paddy soil. *FEMS Microbiol Ecol* **87**: 164–181. https://doi.org/10.1111/1574-6941.12212

Clemente, R., Escolar, Á., and Bernal, M.P. (2006) Heavy metals fractionation and organic matter mineralisation in contaminated calcareous soil amended with organic materials. *Bioresour Technol* **97**: 1894–1901. https://doi.org/10.1016/j.biortech.2005.08.018

Cole, M., Lindeque, P., Halsband, C., and Galloway, T.S. (2011) Microplastics as contaminants in the marine environment: A review. *Mar Pollut Bull* **62**: 2588–2597. https://doi.org/10.1016/j.marpolbul.2011.09.025

De Cáceres, M., Legendre, P., and Moretti, M. (2010) De Cáceres M, Legendre P, Moretti M.. Improving indicator species analysis by combining groups of sites. Oikos 119: 1674-1684. *Oikos* **119**: 1674–1684. https://doi.org/10.1111/j.1600-0706.2010.18334.x

De Hoog, G. S., Zeng, J. S., Harrak, M. J., & Sutton, D. A. (2006). Exophiala xenobiotica sp. nov., an opportunistic black yeast inhabiting environments rich in hydrocarbons. *Antonie van Leeuwenhoek*, *90*(3), 257-268. doi: 10.1007/s10482-006-9080-z

Demyttenaere, J., Belleghem, K., and Kimpe, N. (2001) Biotransformation of (R)-(+)- and (S)-(−)-limonene by fungi and the use of solid phase microextraction for screening. *Phytochemistry* **57**: 199–208. https://doi.org/10.1016/S0031-9422(00)00499-4

Dufrene, M. and Legendre, P. (1997) Species assemblages and indicator species: the need for a flexible asymmetrical approach. *Ecol Monogr* **67**: 345–366. https://doi.org/10.1890/0012-9615(1997)067[0345:SAAIST]2.0.CO;2

Edgar, R.C., Haas, B.J., Clemente, J.C., Quince, C., and Knight, R. (2011) UCHIME improves sensitivity and speed of chimera detection. *Bioinforma Oxf Engl* **27**: 2194–2200. https://doi.org/10.1093/bioinformatics/btr381

Ferrari, B.C., Zhang, C., and van Dorst, J. (2011) Recovering greater fungal diversity from pristine and diesel fuel contaminated sub-Antarctic soil through cultivation using both a high and a low nutrient media approach. *Front Microbiol* **2**:217. https://doi.org/10.3389/fmicb.2011.00217

Gueidan, C., Aptroot, A., Cáceres, M., Badali, H., and Stenroos, S. (2014) A reappraisal of orders and families within the subclass Chaetothyriomycetidae (Eurotiomycetes, Ascomycota). *Mycol Prog* **13**:990. https://doi.org/10.1007/s11557-014-0990-2

Hannula, S.E., de Boer, W., and van Veen, J. (2012) A 3-year study reveals that plant growth stage, season and field site affect soil fungal communities while cultivar and GM-trait have minor effects. *PloS One* **7**: e33819. https://doi.org/10.1371/journal.pone.0033819

Harms, H., Schlosser, D., and Wick, L.Y. (2011) Untapped potential: exploiting fungi in bioremediation of hazardous chemicals. *Nat Rev Microbiol* **9**: 177–192. https://doi.org/10.1038/nrmicro2519

Hill, T.C.J., Walsh, K.A., Harris, J.A., and Moffett, B.F. (2003) Using ecological diversity measures with bacterial communities. *FEMS Microbiol Ecol* **43**: 1–11. https://doi.org/10.1111/j.1574-6941.2003.tb01040.x

Hoffman, M.T. and Arnold, A.E. (2010) Diverse bacteria inhabit living hyphae of phylogenetically diverse fungal endophytes. *Appl Environ Microbiol* **76**: 4063–4075. https://doi.org/10.1128/AEM.02928-09

Horwath, W. (2007). Carbon cycling and formation of soil organic matter. In *Soil microbiology, ecology and biochemistry* (pp. 303-339). Academic Press.

Jones, M.D.M., Forn, I., Gadelha, C., Egan, M.J., Bass, D., Massana, R., and Richards, T.A. (2011) Discovery of novel intermediate forms redefines the fungal tree of life. *Nature* **474**: 200–203. https://doi.org/10.1038/nature09984

Kalbitz, K., Kaiser, K., Bargholz, J., and Dardenne, P. (2006) Lignin degradation controls the production of dissolved organic matter in decomposing foliar litter. *Eur J Soil Sci* **57**: 504–516. https://doi.org/10.1111/j.1365-2389.2006.00797.x

Kettner, M.T., Rojas-Jimenez, K., Oberbeckmann, S., Labrenz, M., and Grossart, H.-P. (2017) Microplastics alter composition of fungal communities in aquatic ecosystems. *Environ Microbiol* **19**: 4447–4459. https://doi.org/10.1111/1462-2920.13891

Kozich, J.J., Westcott, S.L., Baxter, N.T., Highlander, S.K., and Schloss, P.D. (2013) Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the MiSeq Illumina sequencing platform. *Appl Environ Microbiol* **79**: 5112–5120. https://doi.org/10.1128/AEM.01043-13

Koziol, L. and Bever, J. (2016) AMF, phylogeny, and succession: Specificity of response to mycorrhizal fungi increases for late-successional plants. *Ecosphere* **7**:e01555. https://doi.org/10.1002/ecs2.1555

Kunze, G., Gaillardin, C., Czernicka, M., Durrens, P., Martin, T., Böer, E., ... & Goffeau, A. (2014). The complete genome of Blastobotrys (Arxula) adeninivorans LS3-a yeast of biotechnological interest. *Biotechnology for biofuels*, *7*(1), 66. https://doi.org/10.1186/1754-6834-7-66

Lara, E., Moreira, D., and López-García, P. (2010) The environmental clade LKM11 and Rozella form the deepest branching clade of fungi. *Protist* **161**: 116–121. https://doi.org/10.1016/j.protis.2009.06.005

Lauber, C.L., Strickland, M.S., Bradford, M.A., and Fierer, N. (2008) The influence of soil properties on the structure of bacterial and fungal communities across land-use types. *Soil Biol Biochem* **40**: 2407–2415. https://doi.org/10.1016/j.soilbio.2008.05.021

Lazarus, K. L., & James, T. Y. (2015). Surveying the biodiversity of the Cryptomycota using a targeted PCR approach. *Fungal Ecology*, *14*, 62-70. http://dx.doi.org/10.1016/j.funeco.2014.11.004

Lemmel, F., Maunoury-Danger, F., Fanesi, A., Leyval, C., and Cébron, A. (2019) Soil properties and multi-pollution affect taxonomic and functional bacterial diversity in a range of French soils displaying an anthropisation gradient. *Microb Ecol* **77**: 993–1013. https://doi.org/10.1007/s00248-018-1297-7

Lenart-Boroń, A., & Boroń, P. (2014). The effect of industrial heavy metal pollution on microbial abundance and diversity in soils—a review. In *Environmental risk assessment of soil contamination*. IntechOpen. DOI: 10.5772/57406

Li, X., Song, Y., Yao, S., Bian, Y., Gu, C., Yang, X., et al. (2019) Can biochar and oxalic acid alleviate the toxicity stress caused by polycyclic aromatic hydrocarbons in soil microbial communities? *Sci Total Environ* **695**: 133879. https://doi.org/10.1016/j.scitotenv.2019.133879

Longcore, J.E. and Simmons, D.R. (2020) Chytridiomycota. In *eLS*. American Cancer Society. https://doi.org/10.1002/9780470015902.a0000349.pub4

Lueders, T., Wagner, B., Claus, P., and Friedrich, M.W. (2004) Stable isotope probing of rRNA and DNA reveals a dynamic methylotroph community and trophic interactions with fungi and protozoa in oxic rice field soil. *Environ Microbiol* **6**: 60–72. https://doi.org/10.1046/j.1462-2920.2003.00535.x

Maharachchikumbura, S.S.N., Hyde, K.D., Jones, E.B.G., McKenzie, E.H.C., Bhat, J.D., Dayarathne, M.C., et al. (2016) Families of Sordariomycetes. *Fungal Divers* **79**:1-317. https://doi.org/10.1007/s13225-016-0369-6

de Mattos-Shipley, K.M.J., Ford, K.L., Alberti, F., Banks, A.M., Bailey, A.M., and Foster, G.D. (2016) The good, the bad and the tasty: The many roles of mushrooms. *Stud Mycol* **85**: 125–157. https://doi.org/10.1016/j.simyco.2016.11.002

Mendiburu, F. de (2013) agricolae: Statistical Procedures for Agricultural Research. R Package Version 1.3-2. http://CRAN.R-project.org/package=agricolae

Michán, C., Martínez, J. L., Alvarez, M. C., Turk, M., Sychrova, H., & Ramos, J. (2013). Salt and oxidative stress tolerance in Debaryomyces hansenii and Debaryomyces fabryi. *FEMS yeast research*, *13*(2), 180-188. https://doi.org/10.1111/1567-1364.12020

Narendrula, R. and Nkongolo, K. (2017) Bacterial and fungal community structure and diversity in a mining region under long-term metal exposure revealed by metagenomics sequencing. *Ecol Genet Genomics* **2**: 13–24. https://doi.org/10.1016/j.egg.2016.11.001

Nassiri, N. and Faramarzi, M. (2015) Recent developments in the fungal transformation of steroids. *Biocatal Biotransformation* **33**:1-28. https://doi.org/10.3109/10242422.2015.1022533

Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., et al. (2017) Vegan: Community Ecology Package. https://CRAN.R-project.org/package=vegan

Op De Beeck, M., Lievens, B., Busschaert, P., Rineau, F., Smits, M., Vangronsveld, J., and Colpaert, J.V. (2015) Impact of metal pollution on fungal diversity and community structures. *Environ Microbiol* **17**: 2035–2047. https://doi.org/10.1111/1462-2920.12547

Pöggeler, S. and Wöstemeyer, J. eds. (2011) Evolution of Fungi and Fungal-Like Organisms, Berlin Heidelberg: Springer-Verlag.

Prenafeta-Boldú, F. X., Ballerstedt, H., Gerritse, J., & Grotenhuis, J. T. C. (2004). Bioremediation of BTEX hydrocarbons: effect of soil inoculation with the toluene-growing fungus Cladophialophora sp. strain T1. *Biodegradation*, *15*(1), 59-65. https://doi.org/10.1023/B:BIOD.0000009973.53531.96

Rachwał, M., Magiera, T., and Wawer, M. (2015) Coke industry and steel metallurgy as the source of soil contamination by technogenic magnetic particles, heavy metals and polycyclic aromatic hydrocarbons. *Chemosphere* **138**: 863–873. https://doi.org/10.1016/j.chemosphere.2014.11.077

Rojas-Jimenez, K., Wurzbacher, C., Bourne, E.C., Chiuchiolo, A., Priscu, J.C., and Grossart, H.-P. (2017) Early diverging lineages within Cryptomycota and Chytridiomycota dominate the fungal communities in ice-covered lakes of the McMurdo Dry Valleys, Antarctica. *Sci Rep* **7**: 15348. https://doi.org/10.1038/s41598-017-15598-w

Salvo, V.S., Gallizia, I., Moreno, M., and Fabiano, M. (2005) Fungal communities in PAH-impacted sediments of Genoa-Voltri Harbour (NW Mediterranean, Italy). *Mar Pollut Bull* **50**: 553–559. https://doi.org/10.1016/j.marpolbul.2005.01.001

Schloss, P.D., Westcott, S.L., Ryabin, T., Hall, J.R., Hartmann, M., Hollister, E.B., et al. (2009) Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Appl Environ Microbiol* **75**: 7537–7541. https://doi.org/10.1128/AEM.01541-09

Seyedmousavi, S., Badali, H., Chlebicki, A., Zhao, J., Prenafeta-Boldu, F. X., & De Hoog, G. S. (2011). Exophiala sideris, a novel black yeast isolated from environments polluted with toxic alkyl benzenes and arsenic. *Fungal biology*, *115*(10), 1030-1037. doi: 10.1016/j.funbio.2011.06.004.

Tedersoo, L., Bahram, M., Põlme, S., Kõljalg, U., Yorou, N.S., Wijesundera, R., et al. (2014) Global diversity and geography of soil fungi. *Science* **346**: 1256688. https://doi.org/10.1126/science.1256688

Thion, C., Cébron, A., Beguiristain, T., and Leyval, C. (2012) Long-term in situ dynamics of the fungal communities in a multi-contaminated soil are mainly driven by plants. *Fems Microbiol Ecol* **82**: 169–181. https://doi.org/10.1111/j.1574-6941.2012.01414.x

Thomas, F. and Cébron, A. (2016) Short-term rhizosphere effect on available carbon sources, phenanthrene degradation, and active microbiome in an aged-contaminated industrial soil. *Front Microbiol* 92. https://doi.org/10.3389/fmicb.2016.00092

US EPA, R. (2014) Polycyclic Aromatic Hydrocarbons (PAHs) Fact Sheet. *US EPA*.

Vainio, E.J. and Hantula, J. (2000) Direct analysis of wood-inhabiting fungi using denaturing gradient gel electrophoresis of amplified ribosomal DNA. *Mycol Res* **104**: 927–936. https://doi.org/10.1017/S0953756200002471

Ventorino, V., Pascale, A., Adamo, P., Rocco, C., Fiorentino, N., Mori, M., et al. (2018) Comparative assessment of autochthonous bacterial and fungal communities and microbial biomarkers of polluted agricultural soils of the Terra dei Fuochi. *Sci Rep* **8**: 14281. https://doi.org/10.1038/s41598-018-32688-5

Vetterlein D, Hüttl RF (1999) Can applied organic matter fulfilsimilar functions as soil organic matter? Risk-benefit analysisfor organic matter application as a potential strategy for reha-bilitation of disturbed ecosystems. Plant Soil 213, 1–10. https://doi.org/10.1023/A:1004681506901

Wang, S., Gutell, R. R., & Miranker, D. P. (2007). Biclustering as a method for RNA local multiple sequence alignment. *Bioinformatics*, *23*(24), 3289-3296. https://doi.org/10.1093/bioinformatics/btm485

Yun, H.Y., Hong, S.G., Rossman, A., Lee, S., Lee, K., and Bae, K.S. (2009) The rust fungus Gymnosporangium in Korea including two new species, G. monticola and G. unicorne. *Mycologia* **101**: 790–809. https://doi.org/10.3852/08-221

Zarei, M., Hempel, S., Wubet, T., Schäfer, T., Savaghebi, G., Jouzani, G.S., et al. (2010) Molecular diversity of arbuscular mycorrhizal fungi in relation to soil chemical properties and heavy metal contamination. *Environ Pollut* **158**: 2757–2765. https://doi.org/10.1016/j.envpol.2010.04.017

Zhang, C., Sirijovski, N., Adler, L., & Ferrari, B. C. (2019). Exophiala macquariensis sp. nov., a cold adapted black yeast species recovered from a hydrocarbon contaminated sub-Antarctic soil. *Fungal biology*, *123*(2), 151-158. DOI: 10.1016/j.funbio.2018.11.011

Zhao, J., Zeng, J., De Hoog, G. S., Attili-Angelis, D., & Prenafeta-Boldú, F. X. (2010). Isolation and identification of black yeasts by enrichment on atmospheres of monoaromatic hydrocarbons. *Microbial Ecology*, *60*(1), 149-156. DOI: 10.1007/s00248-010-9651-4

Zhou, Z.-F., Wang, M.-X., Zuo, X.-H., and Yao, Y.-H. (2017) Comparative investigation of Bacterial, Fungal, and Archaeal community structures in soils in a typical oilfield in Jianghan, China. *Arch Environ Contam Toxicol* **72**: 65–77. https://doi.org/10.1007/s00244-016-0333-1

Figure1

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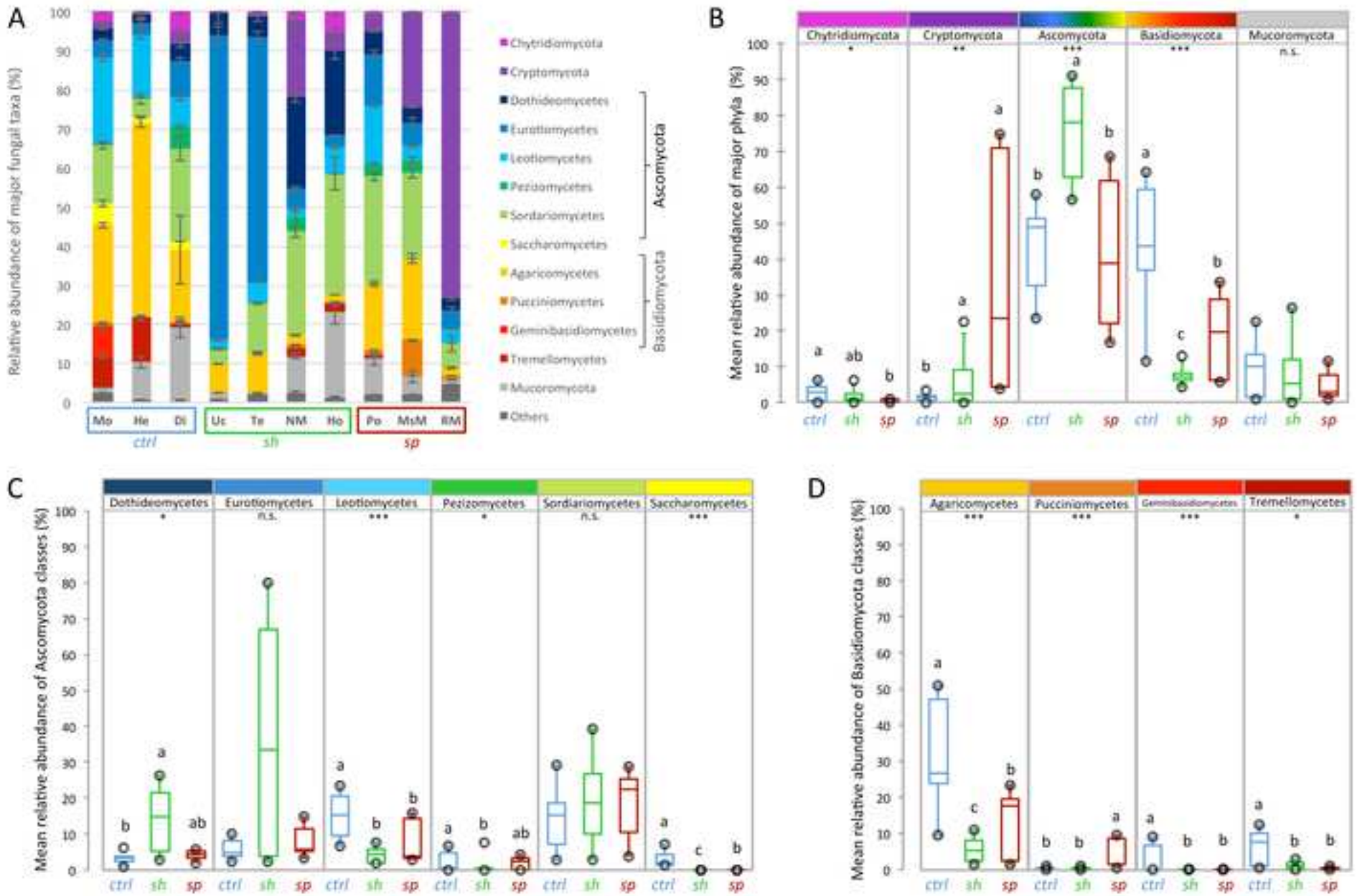

Figure2

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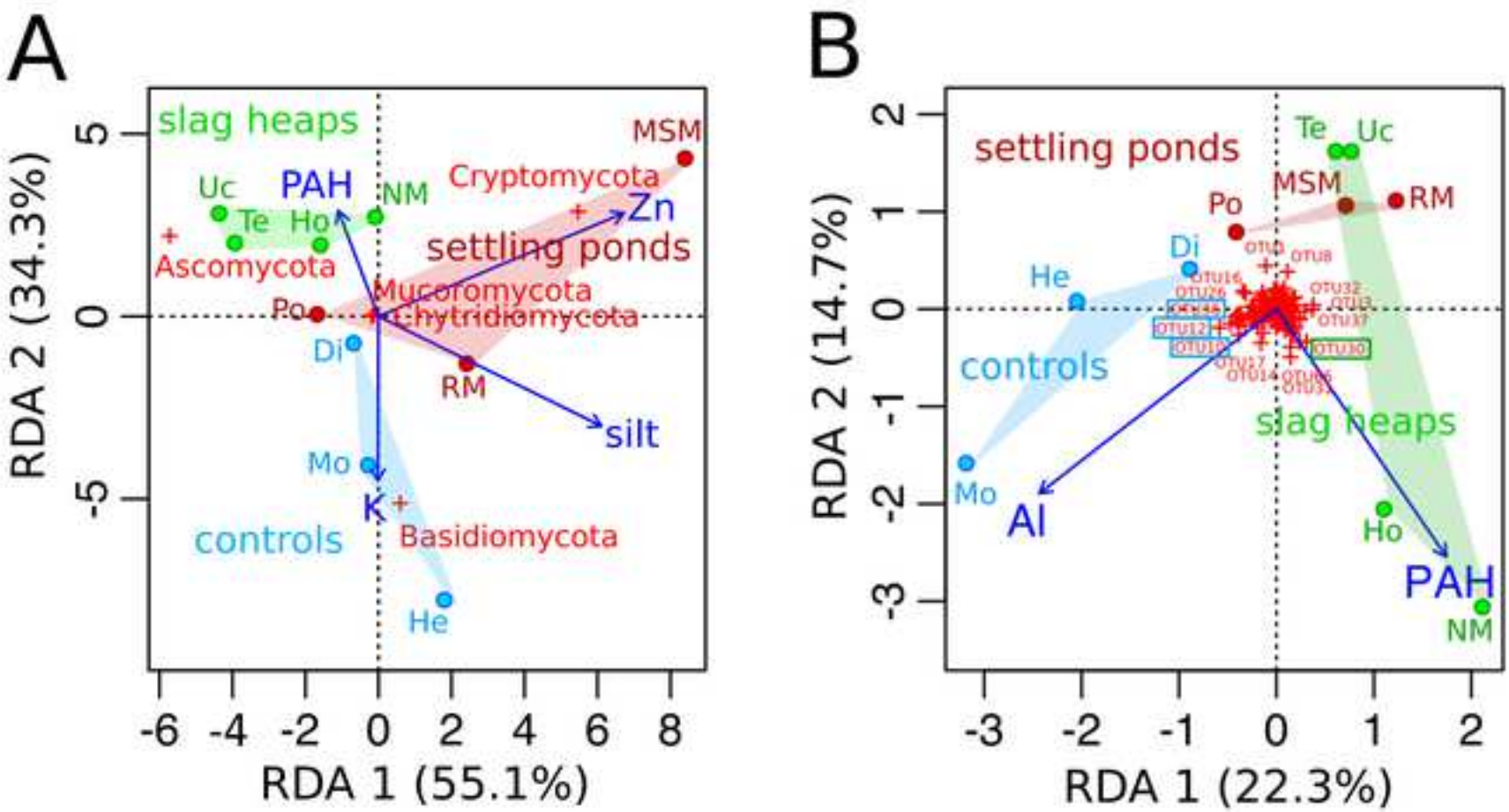

Figure3

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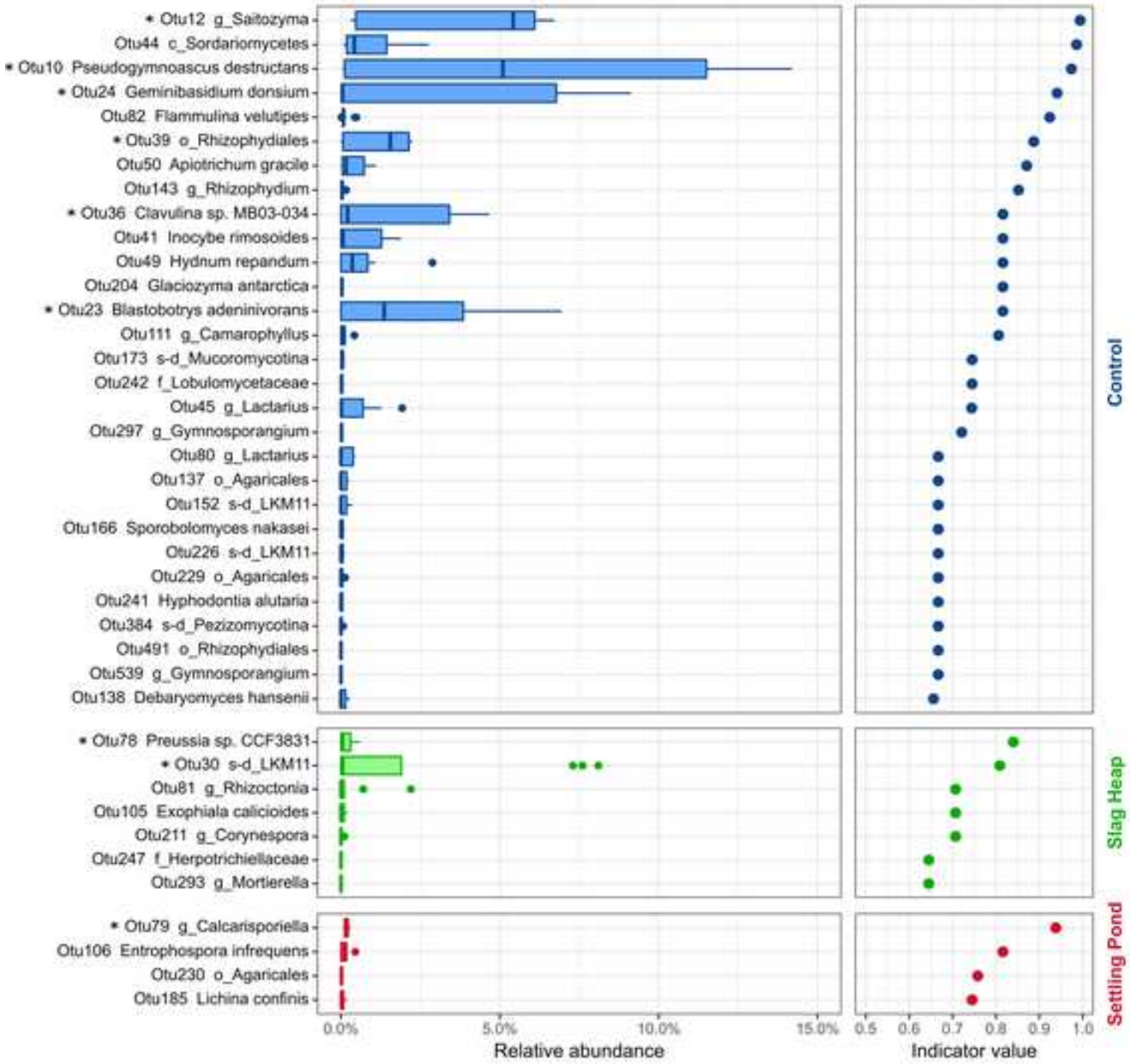

Table1&2

	Soil site group	Soil classification	pH	CEC	carbonates	organic C	total N
				(cmol kg^{-1})	(g kg^{-1})		
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 (x 10^9 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]