



**HAL**  
open science

## Environmental Metabarcoding Reveals Contrasting Belowground and Aboveground Fungal Communities from Poplar at a Hg Phytomanagement Site

Alexis Durand, François Maillard, Julie Foulon, Hyun S. Gweon, Benoit Valot,  
Michel Chalot

► **To cite this version:**

Alexis Durand, François Maillard, Julie Foulon, Hyun S. Gweon, Benoit Valot, et al.. Environmental Metabarcoding Reveals Contrasting Belowground and Aboveground Fungal Communities from Poplar at a Hg Phytomanagement Site. *Microbial ecology*, 2017, 74, pp.795-809. 10.1007/s00248-017-0984-0 . hal-01518636

**HAL Id: hal-01518636**

**<https://hal.science/hal-01518636>**

Submitted on 7 Mar 2024

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

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

*Environmental metabarcoding reveals contrasting belowground and aboveground fungal communities from poplar at a Hg phytomanagement site*

Article

Accepted Version

Durand, A., Maillard, F., Foulon, J., Gweon, H. S. ORCID: <https://orcid.org/0000-0002-6218-6301>, Valot, B. and Chalot, M. (2017) Environmental metabarcoding reveals contrasting belowground and aboveground fungal communities from poplar at a Hg phytomanagement site. *Microbial Ecology*, 74 (4). pp. 795-809. ISSN 1432-184X doi: <https://doi.org/10.1007/s00248-017-0984-0> Available at <https://centaur.reading.ac.uk/75780/>

It is advisable to refer to the publisher's version if you intend to cite from the work. See [Guidance on citing](#).

To link to this article DOI: <http://dx.doi.org/10.1007/s00248-017-0984-0>

Publisher: Springer

All outputs in CentAUR are protected by Intellectual Property Rights law, including copyright law. Copyright and IPR is retained by the creators or other copyright holders. Terms and conditions for use of this material are defined in the [End User Agreement](#).

[www.reading.ac.uk/centaur](http://www.reading.ac.uk/centaur)

**CentAUR**

Central Archive at the University of Reading

Reading's research outputs online

1 **Environmental metabarcoding reveals contrasting belowground and aboveground fungal**  
2 **communities from poplar at a Hg phytomanagement site**

3

4 Alexis Durand<sup>a</sup>, François Maillard<sup>a</sup>, Julie Foulon<sup>a</sup>, Hyun S. Gweon<sup>b</sup>, Benoit Valot<sup>a</sup>, Michel Chalot<sup>a, c\*</sup>

5

6 <sup>a</sup> Laboratoire Chrono-Environnement, UMR 6249, Université de Bourgogne Franche-Comté, Pôle  
7 Universitaire du Pays de Montbéliard, 4 place Tharradin, BP 71427, 25211 Montbéliard, France

8 <sup>b</sup> Centre for Ecology & Hydrology, Maclean Building, Benson Lane, Crowmarsh Gifford,  
9 Wallingford, Oxon OX10 8BB, UK

10 <sup>c</sup> Université de Lorraine, Faculté des Sciences et Technologies, BP 70239, 54506 Vandoeuvre-les-  
11 Nancy, France

12

13 \*Corresponding author: Laboratoire Chrono-Environnement, UMR 6249, Université de Bourgogne  
14 Franche-Comté, Pôle Universitaire du Pays de Montbéliard, 4 place Tharradin, BP 71427, 25211  
15 Montbéliard, France

16

17 Tel: +33 3 81 99 46 76, Email: [michel.chalot@univ-fcomte.fr](mailto:michel.chalot@univ-fcomte.fr)

18

19

20 **Abstract**

21 Characterization of microbial communities in stressful conditions at a field level is  
22 rather scarce, especially when considering fungal communities from aboveground habitats. We  
23 aimed at characterizing fungal communities from different poplar habitats at a Hg-contaminated  
24 phytomanagement site by using Illumina-based sequencing, network analysis approach and  
25 direct isolation of Hg resistant fungal strains. The highest diversity estimated by the Shannon  
26 index was found for soil communities, which was negatively affected by soil Hg concentration.  
27 Among the significant correlations between soil OTUs in the co-occurrence network, 80 %  
28 were negatively correlated revealing dominance of a pattern of mutual exclusion. The fungal  
29 communities associated with *Populus* roots mostly consisted of OTUs from the symbiotic guild,  
30 such as members of the *Thelephoraceae*, thus explaining the lowest diversity found for root  
31 communities. Additionally, root communities showed the highest network connectivity index,  
32 while rarely detected OTUs from the *Glomeromycetes* may have a central role in the root  
33 network. Unexpectedly high richness and diversity were found for aboveground habitats,  
34 compared to the root habitat. The aboveground habitats were dominated by yeasts from the  
35 *Lalaria*, *Davidiella* and *Bensingtonia* genera, not detected in belowground habitats. Leaf and  
36 stem habitats were characterized by few dominant OTUs such as those from the  
37 *Dothideomycete* class producing mutual exclusion with other OTUs. *Aureobasidium pullulans*,  
38 one of the dominating OTUs, was further isolated from the leaf habitat, in addition to  
39 *Nakazawaea populi* species, which were found to be Hg resistant. Altogether, these findings  
40 will provide an improved point of reference for microbial research on inoculation-based  
41 programs of tailings dumps.

42 **Keywords : Hg-enriched tailings dump, Hg resistance, internal transcribed spacer**  
43 **metabarcoding, poplar microbiome, Illumina MiSeq, network.**

## 44 **Introduction**

45 Plants in natural and agricultural settings are colonized by a wide range of microbes on both  
46 their outer and inner surfaces [1]. Nevertheless, the scientific understanding of the microbial  
47 communities of woody species is quite limited, especially for microbes associated with  
48 aboveground tissues. Many tree tissues may indeed represent distinct microbial habitats, such  
49 as the rhizosphere, the root and leaf endospheres, the episphere of the phyllosphere and fruits,  
50 flowers, leaves, buds, stems, branches or even the trunk [2, 3]. Moreover, some of the  
51 microorganisms that colonize these habitats establish strong links with their host, whose  
52 disruption may result in loss of host fitness [4]. For instance, a certain groups of mycorrhizal  
53 fungi that form symbiotic association with the root system, are of a importance for tree nutrition  
54 and health, as they play key roles in the carbon and nitrogen cycles [5]. In the phyllospheric  
55 habitat, archaea, bacteria, filamentous fungi and yeasts have been identified , although less work  
56 has been done on the two latter groups of microorganisms [6]. Studies are increasingly  
57 indicating that fungi influence the fitness of their host plants, either negatively by acting as  
58 pathogens [7], or positively by increasing the stress tolerance of the plant [8], shaping insect  
59 herbivory [9] or reducing the infection of plant tissues by pathogens [10].

60 Trees, as perennials, are made of a variety of habitats that may host contrasted microbial  
61 communities, which composition and diversity may vary with season, age, species, climatic  
62 conditions. Therefore, they are relevant models for studying the structure and composition of  
63 microbial communities, but until recently, our knowledge has been limited due to the difficulty  
64 of adequately describing microbial communities with classical culture-dependent methods. The  
65 recent development of massively parallel 454 pyrosequencing [11, 12] and ion torrent  
66 sequencing [13], combined with DNA multiplexing, provides an opportunity to explore parts  
67 of the microbiome that are otherwise unreachable through culture-dependent approaches [14].  
68 Microbiomes associated with belowground and aboveground tree habitats have been screened

69 at a large spatial scale using these technologies in some cases, and several studies revealed the  
70 potential of the host tree genotype and environmental conditions to induce the establishment of  
71 specific microbial communities [11, 15–17]. More recently, new high-throughput technologies,  
72 such as the Illumina sequencing platform, have become available, showing a far greater  
73 sequencing capacity, producing millions of sequences, which leads to much greater depth of  
74 coverage of microbial [18]. It is now possible to examine the full extent of the richness and  
75 diversity exhibited by microorganisms in different habitats. However, the quantity of data  
76 reaches a critical point at which previous approaches are insufficient to decipher the structure  
77 of complex microbial communities. Network analysis of significant taxon co-occurrence  
78 patterns may help to decipher the structure of complex microbial communities among various  
79 habitats. Software such as CoNet has been developed and optimized specifically to detect  
80 significant non-random patterns of co-occurrence (co-presence and mutual exclusion) using a  
81 Reboot method to determine the significance of each associations in the network [19]. Network  
82 analysis requires lot of samples replication to obtained strong statistical analysis leading to  
83 trustable correlations. This computation method combined with adapted experimental design  
84 and with the tremendous amount of metadata obtained by high throughput sequencing  
85 technologies gives us the opportunity to explore communities with a new global tool to revealed  
86 OTUs importance in the community.

87         Within the growing environmental pollution paradigm, poplar is a keystone tree used as  
88 feedstock for biofuel production and as a biological tool for phytoremediation and revegetation  
89 [20]. The term phytoremediation refers to the use of plants and associated microorganisms to  
90 eliminate, attenuate or restrain environmental damage or threats posed by a contaminant. Hg is  
91 a contaminant classified within the quantitatively most important pollutant groups known as  
92 trace elements (TEs). It is highly persistent in the soil environment and is classified as a  
93 “priority hazardous substance” by the Agency for Toxic Substances and Disease Registry

94 (ATSDR) due to its toxicity, mobility, and long residence time in the atmosphere (*Available*  
95 *online* <http://www.atsdr.cdc.gov/SPL/index.html>). In a previous paper, we focused on  
96 characterizing the microbial communities that had naturally recolonized the sediment of a chlor-  
97 alkali tailings dump after sediment deposition had ceased. We further demonstrated that most  
98 of the Hg detected in the aboveground parts of *Salicaceae* trees collected at that site had entered  
99 the poplar leaves through exclusively through an atmospheric pathway [21]. However, the role  
100 of aboveground and belowground microbial populations in Hg-contaminated environments  
101 remains unknown, and there is a need for a holistic ecosystem-level understanding of microbial  
102 communities associated with poplar [22].

103         In this study, we collected soil and tree samples from the belowground and aboveground  
104 habitats of poplars grown as a short-rotation coppice (SRC) plantation at a Hg-contaminated  
105 site, and performed isolation of fungal strains. We combined fungal community analyses using  
106 Illumina-based sequencing with network analysis to investigate the composition and assembly  
107 of fungal communities in these samples. We expected that we would observe clear differences  
108 in the relative abundance and composition of fungal groups across poplar habitats that may  
109 improve our understanding of the microbial ecology of these environments. Providing key  
110 information on the fungal communities of belowground and aboveground will hopefully be of  
111 use for practitioners of bioremediation approaches who often lack of important information  
112 such as the effect of the pollutants (in this case heavy Hg) on the microbial communities that  
113 surround the flora and fauna. This knowledge may benefit efforts to mitigate the environmental  
114 impact of tailing management facilities.

115

## 116 **Materials and Methods**

### 117 *Site description and sampling design*



118           The location and history of the site have been fully described elsewhere [13, 23] briefly,  
119 the site investigated in the current study was exploited as a sediment storage area from the  
120 1950s to 2003. The sediments originated from effluents produced during electrolytic processes  
121 associated with a Hg cell chlor-alkali process. A poplar monoclonal plantation of the cultivar  
122 Skado (*P. trichocarpa* x *P. maximowiczii*) was implemented in 2011 as a short-rotation coppice  
123 (SRC, 2200 stems/ha). The experimental design thus guaranteed minimum host variation to  
124 focus on interactions between microorganisms in various tree habitats. Sampling was carried  
125 out in summer 2014, consisting of collecting soil, root, stem and leaf samples from six random  
126 trees, selected in three replicated plots (2 trees per plot). Soil samples composed of bulk soils  
127 from under the canopy of the trees were sieved to <4 mm. After the removal of litter, the roots  
128 were collected from the upper 20 cm layer of soil from under the canopy of the trees. They were  
129 separated from the soil via 2 ultra-pure water baths, and the smallest roots were selected and  
130 separated from larger roots by cutting them with a scalpel. Branch samples were collected from  
131 poplar branches of axe 2 (0.8 to 1.2 cm diameter) at an ca. 5 m height, corresponding to the  
132 half-crown of the poplar. Leaf samples were composed of 3 leaves collected from the above  
133 branches. All samples were obtained over a one-day period to reduce any heterogeneity  
134 imparted by climatic conditions. The samples were either freeze-dried and stored at -20°C for  
135 molecular analysis or dried at ambient temperature (24°C ± 1) for physico-chemical analyses.  
136 Thus, we considered the belowground and aboveground habitats to include both endophytic  
137 and epiphytic fungi.

### 138 ***Molecular methods***

139           Within 2 weeks after sampling, the stored samples were freeze-dried (RP2V, Group  
140 S.G.D. France) and ground into a homogenous powder in a Mixer Mill for 3 min at 30 Hz  
141 (model MM400; Retsch Inc., Newtown, Pennsylvania, USA). Environmental DNAs were then  
142 extracted using a modified hexadecyltrimethylammonium bromide (CTAB)

143 chloroform/isoamyl alcohol protocol [24, 25] for root, stem and leaf samples, while  
144 environmental DNA from the soil samples was extracted with the PowerSoil DNA isolation Kit  
145 following the manufacturer's instructions (MoBio Laboratories, Inc., Carlsbad, CA USA). A  
146 purification step was added to all samples using the Power Clean® Pro DNA Clean-Up kit  
147 (MoBio Laboratories, Inc., Carlsbad, CA USA) to improve the quality of the isolated DNA.  
148 DNA quality and quantity were assessed via agarose gel electrophoresis and with the Quant-  
149 iT™ PicoGreen® dsDNA Assay Kit (Invitrogen, Carlsbad, CA, USA) using an FLX-Xenius  
150 spectrofluorometer (SAFAS, Monaco). Equimolar DNA pools were produced and adjusted to  
151 10 ng/μl. Sequencing of the fungal ITS1 region [17, 26] was performed with the Illumina MiSeq  
152 platform (Microsynth AG, Switzerland). PCR amplification of the partial ITS gene was  
153 performed using the fungi-specific primers ITS1F (CTTGGTCATTTAGAGGAAGTAA) and  
154 ITS2 (GCTGCGTTCTTCATCGATGC) [27]. These primers target a short portion of the fungal  
155 ITS region, resulting in an amplicon of small size (~ 300 bp) appropriate for Illumina  
156 sequencing.

157

### 158 *Bioinformatics and statistical analysis of diversity*

159       Sequence de-multiplexing and bioinformatics processing of the datasets were performed  
160 using the PIPITS pipeline [28]. PIPITS is an automated bioinformatics pipeline dedicated for  
161 fungal ITS sequences which incorporates ITSx to extract subregions of ITS and exploits the  
162 latest RDP Classifier to classify sequences against the curated UNITE fungal data set. Briefly,  
163 all raw read pairs were joined at the overlapping region and then quality filtered, chimera  
164 filtered, singleton filtered, contaminant filtered, merged and clustered into operational  
165 taxonomic units (OTUs), defined at 97% sequence similarity. We excluded singleton OTUs to  
166 avoid technical artifacts and overestimation of the number of species [29, 30]. The taxonomic  
167 assignment of OTUs was performed using the UNITE [31] database at a 97% similarity

168 threshold. The samples were rarefied to 26 671 sequences. The Shapiro test and the Bartlett test  
169 were employed to check the normality and homoscedasticity of the data, respectively. Our data  
170 were systematically verified for non-normality and homoscedasticity, and the effects of the  
171 compartment on the alpha diversity estimates for the fungal assemblage were examined using  
172 the Kruskal-Wallis test. A number of alpha diversity indices (OTU richness, Chao estimation,  
173 Shannon diversity index, inverse of the Simpson diversity index, measure of evenness based on  
174 the Shannon index and coverage) were calculated using MOTHUR [32].  
175 The coverage calculator returns Good's coverage for an OTU definition. Coverage was  
176 calculated as:  $C=[1-(n/N)]*100$  (%), where  $n$  is the number of OTUs, and  $N$  is the number of  
177 sequences.

178 A 2-dimensional non-metric multi-dimensional scaling (NMDS) was calculated using  
179 the Bray–Curtis method ( $k=3$ ) on the basis of standardized (Wisconsin double) and square root  
180 transformation of OTU abundance using the “metaMDS” function in the Vegan package in R.  
181 We PERformed a single Multivariate ANalysis Of the VAriance (PERMANOVA), run with  
182 1,000 permutations, using the “anosim” function in the Vegan package in R and employed  
183 ANalysis Of SIMilarities (ANOSIM) to obtain P-values (i.e., significance levels) and the R  
184 value (i.e., the strength of the factors on the samples). These results were paired with a heatmap  
185 of Spearman's correlations between the relative abundances created with “heatmap.2” from the  
186 gplots package. The numbers of OTUs that were shared between habitats were visualized using  
187 Venn diagrams implemented in Mothur with the function “venn”. We considered an OTU to be  
188 present in a compartment if that OTU was present in at least 25% of the samples from the  
189 habitats. Correlations between the diversity parameters and the measured Hg parameters were  
190 calculated based on Spearman’s product moment correlation coefficient ( $R^2$ ). Riverplots were  
191 created with the “riverplot” function in the riverplot R package. Rarefaction curves were  
192 generated with the “rarecurve” function of the Vegan package in R. The bioinformatic analysis

193 was conducted using a computer with the following specifications: Ubuntu, Intel®Core™i7-  
194 4790 CPU @ 3.60GHz x8, 16GB RAM.

195

### 196 *Network analysis*

197 To construct a network and simplify visualization and interpretation, a separate OTU abundance  
198 table was derived using the aforementioned pipeline, but with a different OTU clustering  
199 threshold (90%) [33]. Following Weiss and collaborators [34] extremely rare OTUs were  
200 filtered out; i.e., for each habitat, all OTUs appearing in less than 25% of samples were  
201 discarded, and all OTUs showing a relative abundance of < 0.01% of the total sequences were  
202 also discarded. Network construction was performed with the plugin CoNet (v. 1.1.b) [35] in  
203 Cytoscape software (v. 3.3.0) [36] following the protocol described by Faust and collaborators  
204 [37]. Briefly, for each of the four similarity measures (Bray–Curtis and Kullback–Leibler  
205 dissimilarity, Pearson and Spearman correlation), the distribution of all pair-wise scores was  
206 computed. Given these distributions, initial thresholds were selected such that each measure  
207 contributed 1,000 positive and 1,000 negative edges to the initial network. For each measure  
208 and each edge, 1,000 renormalized permutation and bootstrap scores were computed, followed  
209 by the measure-specific P-value. Any edges exhibiting scores outside the 95% confidence  
210 interval defined by the bootstrap distribution or that were not supported by all measures were  
211 discarded as well [34]. The networks were explored and visualized with Cytoscape. Based on  
212 the results of Berry and Widder (2014)[38], we have chosen to use the degree as a keystone  
213 proxy.

### 214 *Hg analysis in the substrate and biomass*

215 Hg was measured in the soil and poplar samples with an AMA-254 cold vapor atomic  
216 absorption (CV-AAS) Hg analyzer (Altec Co., Czech Republic), using the standard conditions

217 recommended by the manufacturer (120 s drying, 150 s heating, 45 s cooling). The validity of  
218 the analytical method was checked using the certified reference material (CRM) Oriental  
219 Basma Tobacco Leaves (INCT-OBTL-5), with a certified Hg content of  $20.9 \pm 1.3$  ng/g DM  
220 [39], and quality controls were regularly performed as described elsewhere [23].

### 221 *Hg resistant yeast isolation and Hg resistance*

222 Leaves were collected from the field experimental site described above during summer 2015  
223 and 2016, and immediately brought to the laboratory. The yeast were isolated using an  
224 enrichment technique on a malt extract medium adapted from a previously described method  
225 [40]. Briefly, intact leaves were incubated for 48 hr at 25°C and 200 rpm in an enrichment  
226 medium (at pH 3.7 adjusted with lactic acid) containing 30 g/l malt, 5 g/l peptone, 5 ml/l filtered  
227 leaf extract, and increasing amounts of HgCl<sub>2</sub> at final concentrations of 0, 2, 5, 10 or 20 µM.  
228 Hundred µl of leaf samples were then plated on malt extract agar (12 g/l malt) and PDA (Potato  
229 Dextrose Agar, sigma) media, supplemented with the corresponding HgCl<sub>2</sub> concentrations. The  
230 number of growing yeast was expressed in colony forming units (CFU) per ml. The strains  
231 growing at 10 µM Hg were purified, and resuspended in liquid malt extract or PDA media  
232 supplemented with 35% v/v glycerol and maintained at -80°C for further analysis.

233 Isolated strains were grown in 8 ml of growth medium for 48h at 27°C on a shaker table  
234 (200 rpm). After centrifugation, DNA was extracted from the pellet with the EZNA Bacterial  
235 DNA kit (OMEGA bio-tek, USA) in accordance with the manufacturer's instructions. The  
236 D1/D2 domain of LSU rRNA was amplified using a PCR with the universal primers ITS-1 (59-  
237 TCCGTAGGTGAACCTGCG-39) and NL-4 (59-GGTCCGTGTTTCAAGACGG- 39) [41].  
238 All the ITS PCR products were sequenced by pyrosequencing 454 (Genewiz Beckman Coulter  
239 Cenomics, United Kingdom). DNA sequences were edited with BioEdit software and screened

240 against the GenBank database using BLASTn tool of the NCBI site  
241 (<http://www.ncbi.nlm.nih.gov/>).

242 Minimal inhibitory concentrations (MIC) for Hg were determined for each isolated  
243 strains. Microtitration plates (96 wells) were prepared using two-fold dilutions of Hg in YPD  
244 liquid medium, from a starting concentration of 256  $\mu\text{M}$  down to 0  $\mu\text{M}$ . Growth was measured  
245 by spectrophotometry at  $\text{DO}_{595}$  after 24h and 48h of incubation at 25°C.

246

## 247 **Results**

### 248 *Illumina MiSeq sequencing revealed high diversity of the leaf microbiome*

249 Following total genomic DNA extraction from soil and poplar samples, amplicons of the  
250 ITS1 region were generated, and a total of 8,345,173 paired-end reads were obtained through  
251 Illumina MiSeq sequencing (Table S1). Among the 24 samples from each habitat, those  
252 exhibiting a low sequence count were eliminated from the rest of the analysis. Thus, a total of  
253 7,519,254 filtered and non-chimeric fungal sequences constituted our final processed dataset,  
254 representing 90% of the initial post-sequencing reads, spread among 6,100 non-singleton OTUs  
255 defined by representative DNA sequences with sizes of 101 to 363 bp (mean = 181.9 bp). After  
256 subsampling, our dataset contained 26,672 reads per sample, distributed in 5,565 non-singletons  
257 OTUs.

258 Rarefaction curve analysis, which assesses OTUs richness as a result of sampling, showed  
259 that all samples approached an asymptote, revealing that the overall fungal diversity was well  
260 represented (Fig. S1). Moreover, the measured Good's coverage values (an estimator of  
261 completeness of sampling) were greater than 99% for each sample type (Table S1) and in every  
262 sample, highlighting good overall sampling. Coverage, richness, and diversity, estimates were

263 calculated for each dataset (Table 1). The Chao1 estimator of Mothur, indicated good sample  
264 OTUs richness throughout. The Shannon and Simpson diversity indices, measurements of  
265 overall diversity, indicated a diverse microbiota. More specifically, the diversity and richness  
266 estimates were always significantly higher in the soil samples, followed by the leaf, stem and  
267 root samples (Table 1).

268 A permutation test confirmed that the habitat ( $R^2 = 0.58$ ) explained most of the variance  
269 in the fungal community, whereas variations between plots were negligible and not statistically  
270 significant (Table S2). The importance of the habitat factor was further corroborated through  
271 visualization in a NMDS plot (Fig. 1), and significant dissimilarity between all habitats was  
272 confirmed with the ANOSIM test (Table S3). The Bray-Curtis method indicated that the  
273 belowground and aboveground communities at the Tavaux site were well separated (Fig. 1a).  
274 Considering the global analysis, NMDS plots revealed that root samples exhibited the greatest  
275 between-sample variation (Fig. 1a). Furthermore, we showed that stem and leaf samples  
276 clustered closely together (Fig. 1a), although the NMDS plot of the belowground (Fig. 1b) or  
277 aboveground (Fig. 1c) communities alone showed a net clustering of each sample type in these  
278 two communities. The leaf data presented less scattering than the stem data (see sizes of ellipses  
279 in Fig. 1c). Overall, these data indicated higher homogeneity of the OTUs distribution in  
280 aboveground samples, while the soil and root samples were less homogeneous.

281 In the Venn diagram analysis, the sums of the total observed fungal OTUs in the four  
282 sampled habitats of the Skado plots were 1567, 609, 918 and 948 for the soil, root, stem and  
283 leaf samples, respectively (Fig. 2). Overall, 151 OTUs (5.9%) were shared by all habitats. The  
284 OTUs that were unique represented 52% and 35% of the belowground and aboveground  
285 samples, respectively (Fig. 2). The soil samples exhibited the highest proportion of unique  
286 OTUs (36.6%), followed by the leaf (10.2%) and stem (10.4%) samples. Conversely, the root  
287 samples shared > 97% of OTUs with another habitat, mostly with the soil habitat (>84.7% of

288 the root OTUs were detected in soil samples). Our data also revealed that in the poplar  
289 phyllosphere, 51% of OTUs were shared by the stem and leaf samples, whereas 31% of OTUs  
290 were shared by the soil and root samples.

291 ***Symbiotic fungi dominated the belowground habitats, whereas yeast-like fungi dominated***  
292 ***the aboveground habitats***

293 The fungal communities across all four habitats were dominated by the phylum  
294 *Ascomycota* (54.6% of total relative abundance on average), while *Basidiomycota* represented  
295 a smaller portion of the communities (23.5%) (Fig. 3). However, the  
296 *Ascomycota/Basidiomycota* ratios were significantly higher in the aboveground samples than  
297 the belowground samples (Kruskal-Wallis  $X^2 = 36.7$ ;  $P < 1.4 \times 10^{-9}$ ). The largest proportion of  
298 *Basidiomycota* was found in the root samples (Kruskal-Wallis  $X^2 = 56.1$ ;  $P < 4.0 \times 10^{-12}$ ). These  
299 ratios are very similar to those identified with 454 sequencing technology in fungal  
300 communities associated with broadleaf trees [42]. Few members of the known arbuscular  
301 mycorrhizal fungi (AMF) phylum *Glomeromycota* were detected in the soil (0.25%, 92 OTUs  
302 from the *Entrophospora* and *Rhizophagus* genera) and root (1.30%, 90 OTUs from the  
303 *Entrophospora* and *Rhizophagus* genera) samples collected under poplars. OTUs assigned to  
304 mycorrhizal species were virtually absent from all aboveground samples. Members of the  
305 *Zygomycota* phylum were almost exclusively found in soil samples (5.2%, for 65 OTUs),  
306 mostly associated to *Mortierella* species.

307 Across all samples, we detected a total of 21 distinct fungal classes, which were  
308 unequally distributed, suggesting substantial differences between sampled habitats (Fig. 4). The  
309 belowground habitats were enriched with *Agaricomycetes* (Kruskal-Wallis  $X^2 = 69.8$ ;  $P < 2.2 \times$   
310  $10^{-16}$ ), *Pezizomycetes* (Kruskal-Wallis  $X^2 = 69.5$ ;  $P < 2.2 \times 10^{-16}$ ) and *Sordariomycetes* (Kruskal-  
311 Wallis  $X^2 = 67.9$ ;  $P < \times 2.2 \times 10^{-16}$ ). Moreover, the root samples contained significantly more  
312 *Agaricomycetes* (Kruskal-Wallis  $X^2 = 16.3$ ;  $P < \times 5.4 \times 10^{-5}$ ), *Pezizomycetes* (Kruskal-Wallis  $X^2 =$



313 25.4;  $P < \times 4.6 \cdot 10^{-7}$ ) and *Glomeromycetes* (Kruskal-Wallis  $X^2= 17.6$ ;  $P < \times 2.8 \cdot 10^{-5}$ ) but  
314 significantly less Sordariomycetes and Zygomycetes than the soil samples. The aboveground  
315 habitats were enriched in *Dothideomycetes* (Kruskal-Wallis  $X^2= 65.7$ ;  $P < 5.4 \times 10^{-16}$ ) and  
316 *Taphrinomycetes* (Kruskal-Wallis  $X^2= 69.8$ ;  $P < 2.2 \times 10^{-16}$ ) from the *Ascomycota* phylum.  
317 While some *Dothideomycetes* members were detected in root and soil samples,  
318 *Taphrinomycetes* were virtually absent from all belowground samples. Although stem and leaf  
319 sample habitats contained members of *Basidiomycota* classes, belonging to  
320 *Agaricostillbomycetes* (Kruskal-Wallis  $X^2= 69.8$ ;  $P < 2.2 \times 10^{-16}$ ), *Exobasidiomycetes* (Kruskal-  
321 Wallis  $X^2= 69.8$ ;  $P < 2.2 \times 10^{-16}$ ), *Microbotryomycetes* (Kruskal-Wallis  $X^2= 69.7$ ;  $P < 2.2 \times 10^{-16}$ )  
322 <sup>16</sup>) and *Tremellomycetes* (Kruskal-Wallis  $X^2= 69.0$ ;  $P < 2.2 \times 10^{-16}$ ), these classes were virtually  
323 absent from all belowground samples. The high proportion of classes of unassigned fungi in the  
324 stem habitat (63.5%) highlights the need for additional investigations of the diversity of the  
325 fungi living in this particular habitat.

326 The assignment tools revealed that root and soil habitats were dominated by OTUs  
327 identified as *Hymenogaster griseus*, *Thelephoraceae* and *Hebeloma hiemale*, all of which  
328 belong to *Agaricomycetes* from the *Basidiomycota* phylum (Figs. 3 and 5). However, *Hebeloma*  
329 (Kruskal-Wallis  $X^2= 8.7$ ;  $P < \times 10^{-10}0.003$ ) and *Thelephoraceae* (Kruskal-Wallis  $X^2= 48.5$ ;  $P <$   
330  $1.7 \times 10^{-10}$ ) OTUs dominated the root samples (Figs. 3 and 5), whereas *Hymenogaster* OTUs  
331 (Kruskal-Wallis  $X^2= 4.3$ ;  $P < 0.04^{-10}$ ) were the most abundant in soil samples (Fig. 3 and Fig.  
332 5). By contrast, the aboveground samples were dominated by *Ascomycota* OTUs, mostly  
333 belonging to 9 genera (*Alternaria*, *Aureobasidium*, *Bensingtonia*, *Lalaria*, *Davidiella*,  
334 *Sphaerulina*, *Rhodotorula*, *Cryptococcus*, *Taphrina*). The *Lalaria* (Kruskal-Wallis  $X^2= 29$ ;  $P <$   
335  $1.7 \times 10^{-8}$ ) and *Davidiella* (Kruskal-Wallis  $X^2= 33$ ;  $P < 9.2 \times 10^{-9}$ ) genera were most abundantly  
336 found in leaves while a Pleosporale OTU was the most abundant in stems (Kruskal-Wallis

337  $\chi^2=3.5$ ;  $P < 0.05$ ) (Fig. 3). The *Basidiomycota* OTUs in aboveground samples were mostly  
338 assigned to the species *Bensingtonia yuccicola* (Figs. 3 and 5).

339 Each fungal OTU was further assigned to functional or morphological groups of fungi  
340 using FUNguild (<http://www.stbates.org/guilds/app.php>) [43] (Fig. 6). For every assignment,  
341 the FUNguild tool provides a confidence ranking, while referring to previously peer reviewed  
342 data (Table S4). To examine the distribution of OTUs within the functional categories, the  
343 abundance of the various OTU groups was set to 100%, and the OTUs were classified into  
344 guilds (Fig. 6A) and morphological categories (Fig. 6B). The investigation of trophic status in  
345 the belowground habitats revealed dominance of symbiotrophs in the root habitat (70.5%),  
346 while the soil community was composed of saprotrophs (45%), symbiotrophs (40%) and  
347 biotrophs (14%). In the aboveground habitats, saprotrophic fungi appeared to be dominant  
348 (stem, 53%; leaf, 65%) (Fig. 6a). The symbiotrophic fungi identified in the aboveground  
349 habitats belong mostly to the lichenized genus *Sphaerulina*, whereas symbiotrophs from the  
350 belowground habitats were identified as ectomycorrhizal fungi from the *Hymenogaster* genus.  
351 Another dichotomy was clearly revealed between the belowground and aboveground habitats  
352 through the analysis of growth form morphology (Fig. 6b). Indeed, as the soil and root habitats  
353 were dominated by gasteroid (soil: 67%; root: 48%) and agaricoid (soil: 21%; root: 45%) fungi,  
354 the fungal communities from the stem and leaf habitats were essentially dominated by yeasts  
355 (stem: 4%; leaf: 11%), dimorphic yeasts (stem: 62%; leaf: 38%), thallus fungi (stem: 27%; leaf:  
356 1%) or rot fungi (stem: 11%; leaf: 37%). The presence of basidiomycetous or ascomycetous  
357 yeast in the phyllosphere has previously been observed in plants from temperate, tropical and  
358 Mediterranean climates [44, 45], in agreement with our results.

### 359 ***Interactions with Hg***

360 The analysis of Hg in the various matrices revealed that the belowground habitats  
361 contained 100 times more Hg compared with the aboveground habitats (Fig. S2). In detail, the  
362 average values of Hg were 42.5 ng/g DM in poplar leaves and 3.6 ng/g DM in poplar stems,  
363 which are within the range of previously published data [21]. The root samples exhibited Hg  
364 concentrations of approximately 2.4 µg/g of DM. The soils exhibited an average Hg  
365 concentration of 5.6 µg/g DM, in agreement with our previous data [13], but ranged from 2.92  
366 to 9.08 µg/g DM within the various harvested soil samples. Given the large variations in Hg  
367 concentrations in each habitat, we analyzed the correlations with Hg concentrations in the  
368 various matrices and found that only soil samples showed significant correlations between the  
369 Hg concentration and the diversity or richness indices. Specifically, we found a significant  
370 negative correlation between the soil Hg content and fungal richness indices (Observed  
371 richness: Spearman correlation coefficient of  $r^2 = -0.68$  and  $p < 0.001$ ; Chao1 index: Spearman  
372 correlation coefficient of  $r^2 = -0.42$ , and  $p < 0.05$ ). The abundance of the two fungal classes,  
373 *Eurotiomycetes* ( $r^2 = 0.63$ , and  $p < 0.001$ ) and *Sordariomycetes* ( $r^2 = 0.41$ ,  $p < 0.05$ ), were  
374 correlated with soil Hg concentrations, as well as the abundance of the two following OTUs,  
375 corresponding to a *Thelephoraceae* ( $r^2 = 0.44$ , and  $p < 0.05$ ) and a *Trichoderma* ( $r^2 = 0.48$ , and  
376  $p < 0.05$ ) species. Conversely, the abundance of an OTU identified as *Hymenogaster griseus*  
377 was significantly negatively correlated with Hg ( $r^2 = -0.46$ , and  $p < 0.05$ ). None of the diversity,  
378 richness or abundance indices were significantly correlated in root, leaf or stem with the Hg  
379 concentrations (data not shown).

380 The number of yeast cells isolated from the phyllosphere was  $2.5 \cdot 10^7$  UFC/ml without  
381 Hg but decreased to  $1.9 \cdot 10^7$  UFC/ml,  $1.2 \cdot 10^6$  UFC/ml,  $1.1 \cdot 10^6$  UFC/ml and  $2.1 \cdot 10^5$  UFC/ml on  
382 media enriched with 2, 5, 10 or 20 µM of HgCl<sub>2</sub>, respectively. At 10 µM HgCl<sub>2</sub>, only 2 species  
383 were isolated, namely *Nakazawaea populi* formerly known as *Candida populi*, and  
384 *Aureobasidium pullulans*, which was one of the most abundant OTU (2.5 % of detected

385 sequences) of the metabarcoding dataset obtained from the leaf habitat. The MIC values for Hg  
386 of the isolated strains were 32  $\mu$ M for *Nakazawaea populi* and 16  $\mu$ M for *Aureobasidium*  
387 *pullulans* (Table 2).

388 ***The co-occurrence network revealed rare fungal OTUs with a high level of interaction in the***  
389 ***community***

390 We built co-occurrence networks to further assess the links within the fungal  
391 communities of the four habitats (Fig. 8 and Table 3). After network calculations, some  
392 topological properties that are commonly used in network analysis were completed to reveal  
393 complex patterns [46]. The root and soil habitats harbored the highest network connectivity, as  
394 exemplified by the highest number of edges and nodes (Fig. 8, Table 3). The co-presence and  
395 mutual exclusion of OTUs in the whole dataset were equally well distributed in the leaf, stem  
396 and root, habitats, whereas soil showing the highest mutual exclusion percentage (Table 3).

397 The network indices allowed us to define the 10 dominant keystone OTUs for each  
398 habitat (Table S5), which were defined as being important to maintain the function and structure  
399 of the microbial community and were arbitrarily identified here based on the number of  
400 connections established with the rest of the network [47]. Some taxa can be less abundant but  
401 highly connected with other taxa (as shown by the number of degrees within the node). These  
402 keystone OTUs can be divided in two groups: those generating positive connections (co-  
403 presence) and those generating negative connections (mutual exclusion). Both (+ and -) groups  
404 were evident in this subset of keystone OTUs in the leaf, stem and root habitats, whereas the  
405 soil contained mostly OTUs exhibiting negative connections, as observed for the whole soil  
406 dataset. The tendency of OTUs to cluster is revealed by the clustering coefficient, which was  
407 two-fold higher for the root habitat. In the leaf habitat, OTUs from the genus *Myrothecium* and  
408 from the class *Dothideomycetes* (unassigned genus) produced the highest number of negative

409 connections. The genus *Myrothecium*, previously detected in mulberry, has been identified as  
410 a foliar pathogen producing mycotoxins [48]. Our work revealed that OTUs from this genus  
411 found on poplar leaves had an overall negative impact on other microbes from the leaf  
412 community. In the stem habitat, an OTU from the *Exobasidiomycetes* class showed only  
413 negative connections with all other fungal OTUs. The other keystone OTUs from the stem  
414 belonged to the *Dothideomycetes* class and exhibited mostly negative connections. Similarly,  
415 in the root habitat, two keystone OTUs belonging to *Glomeromycetes* exhibited mostly negative  
416 connections with other OTUs. In contrast to the leaf and stem habitats, other keystone OTUs  
417 presented mostly positive connections, constituting a cluster highlighted in Figure 8.  
418 *Rhodotorula* and *Lalaria* OTUs from this cluster were rather rare in the root habitat but were  
419 frequently encountered in the leaf habitat (Fig. 3). The soil habitat was characterized by  
420 keystone OTUs exhibiting mostly negative connections. The *Peziza* OTU (ITS-75-68665)  
421 displayed the greatest number of connections among all keystone OTUs by far (Table S5).

422

## 423 **Discussion**

424 We used the Illumina MiSeq sequencing platform to characterize fungal communities  
425 from a poplar plantation at a Hg-contaminated site. It is important to bear in mind that we were  
426 unable to distinguish between endophytes and epiphytes in each of the three plant habitats (root,  
427 stem and leaf) and instead considered the fungal communities in these habitats in their entirety.  
428 Although we did not set out to study seasonal dynamics of the belowground and aboveground  
429 fungal communities, we should bear in mind that differing seasonal patterns between  
430 belowground [49] and aboveground [50, 51] fungal taxa have been described previously. It was  
431 concluded that the variation of foliar chemistry across growing seasons should not be  
432 considered a major driver of the observed fungal dynamics.

433 Rarefaction analyses and richness estimators indicate that much of the total diversity  
434 detectable with the Illumina-based sequencing was obtained. The finding of higher richness and  
435 diversity in aboveground habitats compared with the root habitat was poorly predictable, as  
436 there are considerably fewer available studies on aboveground communities, compared with  
437 belowground communities. A previous study revealed a low percentage of fungal OTUs shared  
438 by leaf and root samples in *Fagus sylvatica* trees [42], while another study showed that a  
439 majority of aboveground OTUs were also present in the belowground compartment of agave  
440 plants [3]. Our dataset unequivocally revealed that i) less than 6% of the OTUs were detected  
441 in all four habitats, and ii) the aboveground fungal communities from poplar leaves were  
442 extremely diverse, although they were represented by only a few abundant taxa and numerous  
443 rare taxa [15, 52]. Overall, our results strongly indicate that belowground habitats host fungal  
444 communities almost completely isolated from from the aboveground habitats communities in  
445 terms of taxonomy, growth morphology, and relationship with trees or microbial interactions.  
446 Considering previous studies, the finding of lower richness and diversity in the root compared  
447 with the soil habitat was expected [3, 11, 53]. Clear separation of microbiomes has been  
448 reported for soil and root samples from mature poplars [54] and 2-year-old poplars [11]. In our  
449 study, we showed that 87% of the detectable OTUs of the roots habitat were also found in the  
450 soil habitat but few taxa were strongly associated to root. Thus, the root fungal communities  
451 also displayed lower homogeneity of the species distribution compared with soil communities.

452 We demonstrated that the fungal communities associated with *Populus* roots mostly  
453 consisted of ectomycorrhizal fungi, which are known to develop mutually beneficial  
454 interactions with their hosts. These plant-microorganism interactions in the root compartment  
455 are probably one of the factors explaining the reasonable adaptation of *Populus skado* to this  
456 particular soil. The *Thelephorales* OTUs in the root samples accounted for the main  
457 contribution to the dominance of symbiotrophs in roots, as most members of this order are

458 known to be ectomycorrhizal and to live in symbiosis with various host plants across Northern  
459 America and Europe. *Thelephoraceae* are indeed abundant colonizers of *Salix caprea* or  
460 *Populus tremula* roots in TE-contaminated soils [55–57]. Additionally, OTUs corresponding to  
461 the *Hebeloma*, *Cortinarius* and *Geopora* genera were also detected in our root and soil samples,  
462 in agreement with previous studies [55, 56]. Members of the *Hebeloma* mycorrhizal genus  
463 (notably *H. mesophaeum*) are frequently found within unvegetated soils [55, 56] and have been  
464 shown to promote the growth of host trees in soils contaminated with metals [58]. At the family  
465 level, both this and a previous study by our group [13] identified *Agaricomycetes* and  
466 *Pezizomycetes* as the most frequent fungal families in the belowground compartment. Similarly,  
467 five of the 6 most common genera (*Hebeloma*, *Mortierella*, *Tuber*, *Geopora* and *Cortinarius*,  
468 but not *Hymenogaster*) identified in this study were among the top five detected previously.

469         The analysis of growth morphology clearly resulted in clustering of the belowground  
470 and aboveground habitats. The soil and root habitats were dominated by agaricoid and gasteroid  
471 fungi, as highlighted by the presence of *Hebeloma* and *Hymenogaster* species, respectively.  
472 The fungi from stem and leaf communities were essentially identified as yeasts or facultative  
473 yeast morphotypes (Fig. 6b), as exemplified by *Lalaria* OTUs [59]. Abundance of *Lalaria*  
474 OTUs in the phyllosphere has previously been reported on the leaves of *Fagus sylvatica* [60]  
475 and in the *Quercus* phyllosphere [15]. *Davidiella tassiana*, also known as *Mycosphaerella*  
476 *tassiana* or *Cladosporium herbarum*, is a leaf pathogenic fungus from the Helotiales order that  
477 is commonly encountered in the phyllosphere of trees [61]. Reader should keep in mind that  
478 many fungi have several names, which can lead to mistakes and thus have always to be taken  
479 in consideration when using dated data. Standardization at a global scale of fungal names should  
480 only profit to fungal ecology. The stem tissues were enriched in *Pleosporales* sp. and  
481 *Sphaerulina pseudovirgaureae* OTUs. Previous studies have shown that phyllosphere

482 endophytic fungi can play an important role in enhancing plant health [20], acting as biocontrol  
483 agents against other plants, insects and pathogens.

484 Hg is known to be a toxic element, but only few studies have explored the impact of Hg on  
485 fungal communities in field trials. Müller (2001) showed that the soil fungal biomass was not  
486 affected by the Hg along a Hg gradient ranging from 7-522 mg THg/kg of soil. Over a narrow  
487 gradient in terms of the Hg concentration, only a negative correlation between arbuscular  
488 mycorrhizal fungi and Hg was observed in the literature, while no correlation was found  
489 between ectomycorrhizal (ECM) fungi and Hg [63]. Therefore, this study is the first to  
490 describe a significant negative effect of Hg on soil fungal richness and diversity under long-  
491 term, natural Hg exposure. In contrast, Hg exposure was not a major driver of the root, stem  
492 and leaf communities, probably due to the limited variations and the limited impact these  
493 variations may have on cellular processes. Nevertheless, we were able to isolate some Hg  
494 resistant yeast strains from the leaf habitat. Resistance here refer to the fact that these strains  
495 were isolated on Hg-enriched growth media, and to the MIC measured for these strains, which  
496 are comparable to previously published data [64]. As most of the Hg detected in poplar leaves  
497 entered through the atmospheric pathway [21], we indeed focused on the isolation of Hg  
498 resistant fungi from this habitat. We thus isolated *Aureobasidium pullulans* Hg resistant  
499 strains, also highly represented in the leaf metabarcoding dataset (Fig. 3). This species is  
500 recognized as an active phylloplane colonizer [65], which showed some capacity to bind  
501 metals to the cell surfaces [66]. Other, authors previously revealed that melanized fungi such  
502 as *Aureobasidium pullulans*, *Cladosporium spp.* and *Alternaria alternate* have been isolated  
503 from soil samples treated with toxic industrial wastes containing high concentrations of  
504 copper and mercury and may also be dominant members of the mycobiota of metal-  
505 contaminated phylloplanes [67]. We also isolated *Nakazawaea populi* Hg resistant strains,  
506 that were not detected in our metabarcoding dataset, probably due to an uncomplete



507 assignment, and that may be part of the unassigned *Ascomycota* cluster (Fig. 3). This strain  
508 has been recently assigned to the *Nakazawaea* genus, and was previously known as a member  
509 of the *Candida* genus [68]. *C. populi* was indeed isolated from poplar sap exudate [69]. These  
510 strains will be further used in inoculation experiments, to better understand the role of leaf  
511 yeast communities on the overall Hg cycle between soil, atmospheric and leaf compartments.

512 This study is the first to explore the organization of the fungal communities of soil,  
513 roots, stems and leaves using a co-occurrence approach at the scale of a clonal tree stand.  
514 Indeed, previous studies have focused on species abundance and diversity, but not on the  
515 interactions among species, which could be more important to ecosystem functioning [70]. Co-  
516 occurrence networks represent individual microbes (operational taxonomic units (OTUs)) as  
517 nodes and feature–feature pairs as edges, where an edge may imply a biologically or  
518 biochemically meaningful relationship between features, and are based on correlations [34].  
519 For instance, one may expect that mutualistic microbes, or those that benefit each other, will  
520 co-occur across samples. In contrast, antagonistic relationships between microbes, such as  
521 competition for the same niche, result in a mutual exclusion. It has been observed that  
522 phylogenetically related microbes have a tendency to positively co-occur [71]. In practice,  
523 microbes may exhibit positive or negative correlations for indirect reasons, based on their  
524 environmental preferences. The overall dataset revealed that non-abundant OTUs might play a  
525 significant role in the network of interactions. Co-occurrence network analysis of the fungal  
526 communities from the four habitats established a clear dichotomy between soil and the three  
527 other habitats, where the soil community was dominated by negative edges, known as mutual  
528 exclusion. It should be noted that sequencing depth impacts the percentage of positive edges in  
529 the network, with a low depth resulting in spurious positive correlations [37]. Thus, the large  
530 number of negative correlations found in our study can be correlated with our extremely high  
531 sequencing depth. This dominance of negative degrees found in the soil (80%), but not in the

532 roots nor in the aboveground habitats, could reflect a high degree of competition between fungi  
533 in the soil due to a lack of nutrient availability in the absence of tree exudates. It is also possible  
534 that the soil microorganisms under the canopy were in competition with the plants for nutrients  
535 such as nitrogen, exacerbating the nutrient competition between microorganisms [72]. The  
536 network obtained from the fungal sequencing data also revealed that the root compartment  
537 present the highest number of interactions between fungi and the highest clustering coefficient,  
538 with predominance of *Glomeromycetes*, showing a great number of interactions with other  
539 fungi. Arbuscular mycorrhizas (AM) formed by *Glomeromycetes* are widespread in living  
540 plants, supporting the ancestral origin of the plant–*Glomeromycetes* symbiosis, as fully  
541 supported by the literature [73]. We noted that the class *Glomeromycetes* produced many  
542 degrees of mutual exclusion with other classes and between the most interactive  
543 *Glomeromycetes* themselves. The hub in the root compartment network typical of the leaf  
544 compartment could correspond to the transfer of microorganisms during leaf fall in the root  
545 area, but the real explanation is still unclear.

546 We may conclude that each habitat that we studied represents a unique niche for the  
547 fungal communities in a monoclonal plantation of the cultivar Skado (*P. trichocarpa* x *P.*  
548 *maximowiczii*) implemented in 2011 as a short-rotation coppice (SRC, 2200 stems/ha).  
549 Aboveground and belowground poplar habitats host completely different fungal communities,  
550 as highlighted by the core microbiome of the four habitats that represent only reduced to 5.9%  
551 of the total OTUs. We will further explore the role of fungal organisms in the Hg cycle, which  
552 deserves attention. We believe that our findings will be instructive for the design of future  
553 ecological restoration practices.

554

555 **Acknowledgments**

556 This work was supported by the French National Research Agency [ANR BIOFILTREE  
557 2010-INTB-1703-01], the ADEME (French Environment and Energy Management Agency)  
558 [PROLIPHYT 1172C0053], the Région Franche-Comté [Environnement-Homme-Territoire  
559 2014-069], the Pays de Montbéliard Agglomération [13/070-203-2015], and the French  
560 national program EC2CO- MicrobiEen FREIDI-Hg. A.D. received a PhD grant from the  
561 Région Franche-Comté.

562 A.D and F.M. contributed equally to this work.

563

564

565 **References**

- 566 1. van der Heijden MGA, Hartmann M (2016) Networking in the Plant Microbiome. *PLOS Biol*  
567 14:e1002378. doi: 10.1371/journal.pbio.1002378
- 568 2. Leff JW, Tredici P Del, Friedman WE, Fierer N (2014) Spatial structuring of bacterial  
569 communities within individual *Ginkgo biloba* trees. *Environ Microbiol* 17:2352–2361. doi:  
570 10.1111/1462-2920.12695
- 571 3. Coleman-Derr D, Desgareignes D, Fonseca-Garcia C, et al (2015) Biogeography and cultivation  
572 affect microbiome composition in the drought-adapted plant Subgenus *Agave*. *New Phytol*  
573 209:798–811. doi: 10.1111/nph.13697
- 574 4. Balint M, Bartha L, O'Hara RB, et al (2015) Relocation, high-latitude warming and host  
575 genetic identity shape the foliar fungal microbiome of poplars. *Mol Ecol* 24:235–248. doi:  
576 10.1111/mec.13018
- 577 5. Lenoir I, Fontaine J, Lounès-Hadj Sahraoui A (2016) Arbuscular mycorrhizal fungal responses  
578 to abiotic stresses: A review. *Phytochemistry* 123:4–15. doi: 10.1016/j.phytochem.2016.01.002
- 579 6. Whipps JM, Hand P, Pink D, Bending GD (2008) Phyllosphere microbiology with special  
580 reference to diversity and plant genotype. *J Appl Microbiol* 105:1744–55. doi: 10.1111/j.1365-  
581 2672.2008.03906.x
- 582 7. Ritpitakphong U, Falquet L, Vimoltust A, et al (2016) The microbiome of the leaf surface of  
583 *Arabidopsis* protects against a fungal pathogen. *New Phytol* 210:1033–1043. doi:  
584 10.1111/nph.13808
- 585 8. Waller F, Achatz B, Baltruschat H, et al (2005) The endophytic fungus *Piriformospora indica*  
586 reprograms barley to salt-stress tolerance, disease resistance, and higher yield. *Proc Natl Acad*  
587 *Sci U S A* 102:13386–13391. doi: 10.1073/pnas.0504423102
- 588 9. Hartley SE, Gange AC (2009) Impacts of plant symbiotic fungi on insect herbivores:

- 589 mutualism in a multitrophic context. *Annu Rev Entomol* 54:323–42. doi:  
590 10.1146/annurev.ento.54.110807.090614
- 591 10. Martínez-Álvarez P, Fernández-González RA, Sanz-Ros AV, et al (2016) Two fungal  
592 endophytes reduce the severity of pitch canker disease in *Pinus radiata* seedlings. *Biol Control*  
593 94:1–10. doi: 10.1016/j.biocontrol.2015.11.011
- 594 11. Danielsen L, Thürmer A, Meinicke P, et al (2012) Fungal soil communities in a young  
595 transgenic poplar plantation form a rich reservoir for fungal root communities. *Ecol Evol*  
596 2:1935–1948. doi: 10.1002/ece3.305
- 597 12. Tedersoo L, Bahammad B, Polme S, et al (2014) Global diversity and geography of soil fungi.  
598 *Science* (80- ) 346:1256688.
- 599 13. Zappelini C, Karimi B, Foulon J, et al (2015) Diversity and complexity of microbial  
600 communities from a chlor-alkali tailings dump. *Soil Biol Biochem* 90:101–110. doi:  
601 10.1016/j.soilbio.2015.08.008
- 602 14. Yergeau E, Lawrence JR, Sanschagrín S, et al (2012) Next-generation sequencing of microbial  
603 communities in the athabasca river and its tributaries in relation to oil sands mining activities.  
604 *Appl Environ Microbiol* 78:7626–7637. doi: 10.1128/AEM.02036-12
- 605 15. Jumpponen A, Jones KL (2009) Massively parallel 454 sequencing indicates hyperdiverse  
606 fungal communities in temperate *Quercus macrocarpa* phyllosphere. *New Phytol* 184:438–448.  
607 doi: 10.1111/j.1469-8137.2009.02990.x
- 608 16. Knief C, Delmotte N, Chaffron S, et al (2012) Metaproteogenomic analysis of microbial  
609 communities in the phyllosphere and rhizosphere of rice. *ISME J* 6:1378–1390. doi:  
610 10.1038/ismej.2011.192
- 611 17. Bonito G, Reynolds H, Robeson MS, et al (2014) Plant host and soil origin influence fungal  
612 and bacterial assemblages in the roots of woody plants. *Mol Ecol* 23:3356–70. doi:  
613 10.1111/mec.12821

- 614 18. Foulon J, Zappelini C, Durand A, et al (2016) Impact of poplar-based phytomanagement on  
615 soil properties and microbial communities in a metal-contaminated site. *FEMS Microbiol Ecol*  
616 92:fiw163. doi: 10.1093/femsec/fiw163
- 617 19. Faust K, Sathirapongsasuti JF, Izard J, et al (2012) Microbial co-occurrence relationships in the  
618 Human Microbiome. *PLoS Comput Biol* 8:e1002606. doi: 10.1371/journal.pcbi.1002606
- 619 20. Xiang L, Gong S, Yang L, et al (2015) Biocontrol potential of endophytic fungi in medicinal  
620 plants from Wuhan Botanical Garden in China. *Biol Control* 94:47–55. doi:  
621 10.1016/j.biocontrol.2015.12.002
- 622 21. Assad M, Parelle J, Cazaux D, et al (2015) Mercury uptake into poplar leaves. *Chemosphere*  
623 146:1–7. doi: 10.1016/j.chemosphere.2015.11.103
- 624 22. Hacquard S, Schadt CW (2015) Towards a holistic understanding of the beneficial interactions  
625 across the *Populus* microbiome. *New Phytol* 205:1424–30. doi: 10.1111/nph.13133
- 626 23. Maillard F, Girardclos O, Assad M, et al (2016) Dendrochemical assessment of mercury  
627 releases from a pond and dredged-sediment landfill impacted by a chlor-alkali plant. *Environ*  
628 *Res* 148:122–126. doi: 10.1016/j.envres.2016.03.034
- 629 24. Lefort F, Douglas GC (1999) An efficient micro-method of DNA isolation from mature leaves  
630 of four hardwood tree species *Acer*, *Fraxinus*, *Prunus* and *Quercus*. *Ann For Sci* 56:259–263.  
631 doi: 10.1051/forest:19990308
- 632 25. Healey A, Furtado A, Cooper T, Henry RJ (2014) Protocol: a simple method for extracting  
633 next-generation sequencing quality genomic DNA from recalcitrant plant species. *Plant*  
634 *Methods* 10:21. doi: 10.1186/1746-4811-10-21
- 635 26. Huang CL, Jian FY, Huang HJ, et al (2014) Deciphering mycorrhizal fungi in cultivated  
636 *Phalaenopsis* microbiome with next-generation sequencing of multiple barcodes. *Fungal Divers*  
637 66:77–88. doi: 10.1007/s13225-014-0281-x

- 638 27. Smith DP, Peay KG (2014) Sequence depth, not PCR replication, improves ecological  
639 inference from next generation DNA sequencing. *PLoS One* 9:e90234. doi:  
640 10.1371/journal.pone.0090234
- 641 28. Gweon HS, Oliver A, Taylor J, et al (2015) PIPITS: An automated pipeline for analyses of  
642 fungal ITS sequences from the Illumina sequencing platform. *Methods Ecol Evol* 6:973–980.  
643 doi: 10.1111/2041-210X.12399
- 644 29. Dickie IA (2010) Insidious effects of sequencing errors on perceived diversity in molecular  
645 surveys. *New Phytol* 188:916–918. doi: 10.1111/j.1469-8137.2010.03473.x
- 646 30. Tedersoo L, May TW, Smith ME (2010) Ectomycorrhizal lifestyle in fungi: global diversity,  
647 distribution, and evolution of phylogenetic lineages. *Mycorrhiza* 20:217–263. doi:  
648 10.1007/s00572-009-0274-x
- 649 31. Kõljalg U, Nilsson RH, Abarenkov K, et al (2013) Towards a unified paradigm for sequence-  
650 based identification of fungi. *Mol Ecol* 22:5271–5277. doi: 10.1111/mec.12481
- 651 32. Schloss PD, Westcott SL, Ryabin T, et al (2009) Introducing mothur: Open-Source, Platform-  
652 Independent, Community-Supported Software for Describing and Comparing Microbial  
653 Communities. *Appl Environ Microbiol* 75:7537–7541. doi: 10.1128/AEM.01541-09
- 654 33. Bell TH, El-Din Hassan S, Lauron-Moreau A, et al (2014) Linkage between bacterial and  
655 fungal rhizosphere communities in hydrocarbon-contaminated soils is related to plant  
656 phylogeny. *ISME J* 8:331–43. doi: 10.1038/ismej.2013.149
- 657 34. Weiss S, Van Treuren W, Lozupone C, et al (2016) Correlation detection strategies in  
658 microbial data sets vary widely in sensitivity and precision. *ISME J*. doi:  
659 10.1038/ismej.2015.235
- 660 35. Faust K, Raes J (2012) Microbial interactions: from networks to models. *Nat Rev Microbiol*  
661 10:538–550. doi: 10.1038/nrmicro2832

- 662 36. Christmas R, Avila-Campillo I, Bolouri H, et al (2005) Cytoscape: a software environment for  
663 integrated models of biomolecular interaction networks. *Am Assoc Cancer Res Educ B*  
664 13:2498–2504. doi: 10.1101/gr.1239303.metabolite
- 665 37. Faust K, Lima-Mendez G, Lerat JS, et al (2015) Cross-biome comparison of microbial  
666 association networks. *Front Microbiol* 6:1–13. doi: 10.3389/fmicb.2015.01200
- 667 38. Berry D, Widder S (2014) Deciphering microbial interactions and detecting keystone species  
668 with co-occurrence networks. *Front Microbiol* 5:1–14. doi: 10.3389/fmicb.2014.00219
- 669 39. Samczyński Z, Dybczyński RS, Polkowska-Motrenko H, et al (2012) Two new reference  
670 materials based on tobacco leaves: certification for over a dozen of toxic and essential  
671 elements. *ScientificWorldJournal* 2012:216380. doi: 10.1100/2012/216380
- 672 40. Fu S-F, Sun P-F, Lu H-Y, et al (2016) Plant Growth–Promoting Traits of Yeasts Isolated from  
673 the Phyllosphere and Rhizosphere of *Drosera spatulata* Lab. *Fungal Biol* 120:433–448. doi:  
674 10.1016/j.funbio.2015.12.006
- 675 41. Sun PF, Fang WT, Shin LY, et al (2014) Indole-3-Acetic Acid-Producing Yeasts in the  
676 Phyllosphere of the Carnivorous Plant. 1–22. doi: 10.1371/journal.pone.0114196
- 677 42. Counce A, Cordier T, Lengellé J, et al (2014) Leaf and root-associated fungal assemblages do  
678 not follow similar elevational diversity patterns. *PLoS One* 9:e100668. doi:  
679 10.1371/journal.pone.0100668
- 680 43. Nguyen NH, Song Z, Bates ST, et al (2015) FUNGuild: An open annotation tool for parsing  
681 fungal community datasets by ecological guild. *Fungal Ecol* 20:241–248. doi:  
682 10.1016/j.funeco.2015.06.006
- 683 44. Inacio J, Portugal L, Spencer-Martins I, Fonseca A (2005) Phylloplane yeasts from Portugal:  
684 Seven novel anamorphic species in the Tremellales lineage of the Hymenomycetes  
685 (Basidiomycota) producing orange-coloured colonies. *FEMS Yeast Res* 5:1167–1183. doi:  
686 10.1016/j.femsyr.2005.05.007



- 687 45. Limtong S, Koowadjanakul N (2012) Yeasts from phylloplane and their capability to produce  
688 indole-3-acetic acid. *World J Microbiol Biotechnol* 28:3323–3335. doi: 10.1007/s11274-012-  
689 1144-9
- 690 46. Barberán A, Bates ST, Casamayor EO, Fierer N (2012) Using network analysis to explore co-  
691 occurrence patterns in soil microbial communities. *ISME J* 6:343–351. doi:  
692 10.1038/ismej.2011.119
- 693 47. Lupatini M, Suleiman AK a., Jacques RJS, et al (2014) Network topology reveals high  
694 connectance levels and few key microbial genera within soils. *Front Environ Sci* 2:1–11. doi:  
695 10.3389/fenvs.2014.00010
- 696 48. Murakami R, Kawakita H, Shirata A (2002) Infection behaviour of conidia of *Myrothecium*  
697 *roridum* on mulberry leaf and cytological changes of leaf cells infected with the fungus.  
698 *Séricologia* 42:19–38.
- 699 49. Shakya M, Gottel N, Castro H, et al (2013) A multifactor analysis of fungal and bacterial  
700 community structure in the root microbiome of mature *Populus deltoides* trees. *PLoS One*  
701 8:e76382. doi: 10.1371/journal.pone.0076382
- 702 50. Glushakova AM, Chernov IY (2004) Seasonal Dynamics in a Yeast Population on Leaves of  
703 the Common Wood Sorrel *Oxalis acetosella* L . *Microbiology* 73:184–188. doi:  
704 10.1023/B:MICL.0000023987.40253.2d
- 705 51. Jumpponen A, Jones KL (2010) Seasonally dynamic fungal communities in the *Quercus*  
706 *macrocarpa* phyllosphere differ between urban and nonurban environments. *New Phytol*  
707 186:496–513. doi: 10.1111/j.1469-8137.2010.03197.x
- 708 52. Balint M, Tiffin P, Hallstrom B, et al (2013) Host Genotype Shapes the Foliar Fungal  
709 Microbiome of Balsam Poplar (*Populus balsamifera*). *PLoS One* 8:e53987. doi:  
710 10.1371/journal.pone.0053987
- 711 53. Foulon J, Zappellini C, Durand A, et al (2016) Environmental metabarcoding reveals

- 712 contrasting microbial communities at two poplar phytomanagement sites. *Sci Total Environ*  
713 571:1230–1240. doi: 10.1016/j.scitotenv.2016.07.151
- 714 54. Gittel NR, Castro HF, Kerley M, et al (2011) Distinct Microbial Communities within the  
715 Endosphere and Rhizosphere of *Populus deltoides* Roots across Contrasting Soil Types. *Appl*  
716 *Environ Microbiol* 77:5934–5944. doi: 10.1128/AEM.05255-11
- 717 55. Regvar M, Likar M, Piltaver A, et al (2010) Fungal community structure under goat willows  
718 (*Salix caprea* L.) growing at metal polluted site: the potential of screening in a model  
719 phytostabilisation study. *Plant Soil* 330:345–356. doi: 10.1007/s11104-009-0207-7
- 720 56. Krpata D, Peintner U, Langer I, et al (2008) Ectomycorrhizal communities associated with  
721 *Populus tremula* growing on a heavy metal contaminated site. *Mycol Res* 112:1069–1079. doi:  
722 10.1016/j.mycres.2008.02.004
- 723 57. Hryniewicz K, Haug I, Baum C (2008) Ectomycorrhizal community structure under willows  
724 at former ore mining sites. *Eur J Soil Biol* 44:37–44. doi: 10.1016/j.ejsobi.2007.10.004
- 725 58. Hryniewicz K, Dabrowska G, Baum C, et al (2012) Interactive and Single Effects of  
726 Ectomycorrhiza Formation and *Bacillus cereus* on Metallothionein MT1 Expression and  
727 Phytoextraction of Cd and Zn by Willows. *Water Air Soil Pollut* 223:957–968. doi:  
728 10.1007/s11270-011-0915-5
- 729 59. Inácio J, Rodrigues MG, Sobral P, Fonseca Á (2004) Characterisation and classification of  
730 phylloplane yeasts from Portugal related to the genus *Taphrina* and description of five novel  
731 *Lalaria* species. *FEMS Yeast Res* 4:541–555. doi: 10.1016/S1567-1356(03)00226-5
- 732 60. Cordier T, Stan, Robin C, et al (2012) The composition of phyllosphere fungal assemblages of  
733 European beech (*Fagus sylvatica*) varies significantly along an elevation gradient. *New Phytol*  
734 196:510–519.
- 735 61. Jakuschkin B, Fievet V, Schwaller L, et al (2016) Deciphering the Pathobiome: Intra- and  
736 Interkingdom Interactions Involving the Pathogen *Erysiphe alphitoides*. *Environ Microbiol* 1–

- 737 11. doi: 10.1007/s00248-016-0777-x
- 738 62. Müller A (2001) The effect of long-term mercury pollution on the soil microbial community.  
739 FEMS Microbiol Ecol 36:11–19.
- 740 63. Jean-Philippe SR, Franklin JA, Buckley DS, Hughes K (2011) The effect of mercury on trees  
741 and their mycorrhizal fungi. Environ Pollut 159:2733–2739. doi: 10.1016/j.envpol.2011.05.017
- 742 64. Moller AK, Barkay T, Hansen M a., et al (2014) Mercuric reductase genes (*merA*) and mercury  
743 resistance plasmids in High Arctic snow, freshwater and sea-ice brine. FEMS Microbiol Ecol  
744 87:52–63. doi: 10.1111/1574-6941.12189
- 745 65. Andrews JH, Harris RF (2000) The ecology and biogeography of microorganisms on plant  
746 surfaces.
- 747 66. Gadd geoffrey M, Mowll JL (1985) Copper uptake by yeast-like cells, Hyphae and  
748 chlamydospores of *Aureobasidium pullulans*. Exp Mycobiology 9:230–240.
- 749 67. Gadd GM (2007) Geomycology: biogeochemical transformations of rocks, minerals, metals  
750 and radionuclides by fungi, bioweathering and bioremediation. Mycol Res 111:3–49. doi:  
751 10.1016/j.mycres.2006.12.001
- 752 68. Kurtzman CP, Robnett CJ (2014) Description of *Kuraishia piskuri* f.a., sp. nov., a new  
753 methanol assimilating yeast and transfer of phylogenetically related *Candida* species to the  
754 genera *Kuraishia* and *Nakazawaea* as new combinations. FEMS Yeast Res 14:1028–1036. doi:  
755 10.1111/1567-1364.12192
- 756 69. Hagler AN, Mendonca-Hagler LC, Phaff HJ (1989) *Candida populi*, a new species of yeast  
757 occurring in exudates of *Populus* and *Betula* species. Int J Syst Evol Microbiol 39:97–99. doi:  
758 10.1099/00207713-39-2-97
- 759 70. Lu L, Yin S, Liu X, et al (2013) Fungal networks in yield-invigorating and -debilitating soils  
760 induced by prolonged potato monoculture. Soil Biol Biochem 65:186–194. doi:

761 10.1016/j.soilbio.2013.05.025

762 71. Lozupone C, Faust K, Raes J, et al (2012) Identifying genomic and metabolic features that can  
763 underlie early successional and opportunistic lifestyles of human gut symbionts. *Genome Res*  
764 22:1974–1984. doi: 10.1101/gr.138198.112

765 72. Kaye JP, Hart SC (1997) Competition for nitrogen between plants and soil microorganisms.  
766 *Trends Ecol Evol* 12:139–143. doi: 10.1016/S0169-5347(97)01001-X

767 73. Selosse MA, Strullu-Derrien C, Martin FM, et al (2015) Plants, fungi and oomycetes: A 400-  
768 million year affair that shapes the biosphere. *New Phytol* 206:501–506. doi:  
769 10.1111/nph.13371

770

771

772

773

774 **Figure legends**

775

776 **Figure 1.** Non-parametric multidimensional scaling (NMDS) plot of fungal communities associated  
777 with the four poplar habitats, using the Bray-Curtis dissimilarity measure. Each point represents the  
778 fungal community of a given sample. Each color represents one of the 6 trees sampled. Confidence area  
779 of ellipses = 0.95. (a) All habitats, (b) belowground habitats, and (c) aboveground habitats.

780 **Figure 2.** Venn diagram showing the overlap of the fungal communities from the four poplar habitats,  
781 based on OTUs. OTU delineation was based on a threshold of < 97% sequence similarity

782 **Figure 3.** Proportion and taxonomic assignment of abundant and rare (< 0.5% relative abundance)  
783 operational taxonomic units (OTUs) from the various poplar habitats. The assignments are given at the  
784 lowest taxonomic level possible, with relative proportions presented in parentheses. The abundance of  
785 the major phyla and the total number of reads are provided on the left side of each graph, color coded  
786 as follows: *Ascomycota* (red), *Basidiomycota* (blue), *Zygomycota* (yellow), *Chytridiomycota* (brown),  
787 *Glomeromycota* (green) and unassigned fungi (grey).

788 **Figure 4.** Composition of the fungal communities from the various poplar habitats at the class level.  
789 The data were derived from MiSeq sequencing of the ITS1 region.

790 **Figure 5.** Heat map and hierarchical cluster analysis of the relative abundance of fungal OTUs from the  
791 various poplar habitats. Letters indicate significantly different abundances at  $p < 0.05$  (Kruskal-Wallis  
792 comparison test),  $n=23$  (root and leaf) or  $n=24$  (soil and stem). The dendrogram represents linkage  
793 clustering using Euclidean distance measures. OTU delineation was based on a threshold of < 97%  
794 sequence similarity. The number associated with the OTU corresponds to the relative abundance rank  
795 of that OTU in the total dataset. Assignments between brackets show the lowest taxonomic level  
796 associated with the OTU using the UNITE database, k: kingdom, p: phylum, o: order, c: class, f: family,  
797 s: genus\_species.

798 **Figure 6.** Relative proportions of fungal sequences from the various poplar habitats assigned to major  
799 fungal guilds (a) and morphological groups (b).

800 **Figure 7.** Box plots of the Hg concentration (ng/mg DM) in (a) belowground habitats, and (b)  
801 aboveground habitats. Letters indicate significant differences between habitats ( $p$ -value < 0.05).

802 **Figure 8.** Co-occurrence network of microbial taxa detected in the four habitats via a high-throughput  
803 DNA sequencing (Illumina MiSeq). Nodes represent fungal OTUs, whereas edges represent  
804 significant positive correlations between pairs of OTUs. The node size corresponds to the number of  
805 connections, and taxa with many correlations are within densely connected areas of the network.  
806 Green edges between nodes represent co-presence, while red edges represent mutual exclusion.

807

808