



HAL
open science

Impact of poplar-based phytomanagement on soil properties and microbial communities in a metal-contaminated site

Julie Foulon, Cyril Zappelini, Alexis Durand, Benoit Valot, Damien Blaudez, Michel Chalot

► To cite this version:

Julie Foulon, Cyril Zappelini, Alexis Durand, Benoit Valot, Damien Blaudez, et al.. Impact of poplar-based phytomanagement on soil properties and microbial communities in a metal-contaminated site. FEMS Microbiology Ecology, 2016, 92 (10), pp.fw163. 10.1093/femsec/fw163 . hal-01512324

HAL Id: hal-01512324

<https://hal.science/hal-01512324v1>

Submitted on 18 Oct 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.

RESEARCH ARTICLE

Impact of poplar-based phytomanagement on soil properties and microbial communities in a metal-contaminated site

Julie Foulon¹, Cyril Zappellini¹, Alexis Durand¹, Benoit Valot¹,
Damien Blaudez^{2,3} and Michel Chalot^{1,4,*}

¹Laboratoire Chrono-Environnement, UMR 6249, Université de Bourgogne Franche-Comté, Pôle Universitaire du Pays de Montbéliard, 4 place Tharradin, BP 71427, 25211 Montbéliard, France, ²CNRS, LIEC UMR7360, Faculté des Sciences et Technologies, BP70239, 54506 Vandoeuvre-lès-Nancy, France, ³Université de Lorraine, LIEC UMR7360, Faculté des Sciences et Technologies, BP 70239, 54506 Vandoeuvre-lès-Nancy, France and ⁴Université de Lorraine, Faculté des Sciences et Technologies, BP 70239, 54506 Vandoeuvre-lès-Nancy, France

*Corresponding author: Laboratoire Chrono-Environnement, UMR 6249, Université de Franche-Comté, Pôle Universitaire du Pays de Montbéliard, 4 place Tharradin, BP 71427, 25211 Montbéliard, France. Tel: +33-3-81-99-46-76; E-mail: michel.chalot@univ-fcomte.fr

One sentence summary: *Laccaria* dominates the poplar root fungal community on a contaminated soil.

Editor: Petr Baldrian

ABSTRACT

Despite a long history of use in phytomanagement strategies, the impacts of poplar trees on the structure and function of microbial communities that live in the soil remain largely unknown. The current study combined fungal and bacterial community analyses from different management regimes using Illumina-based sequencing with soil analysis. The poplar phytomanagement regimes led to a significant increase in soil fertility and a decreased bioavailability of Zn and Cd, in concert with changes in the microbial communities. The most notable changes in the relative abundance of taxa and operational taxonomic units unsurprisingly indicated that root and soil constitute distinct ecological microbial habitats, as exemplified by the dominance of *Laccaria* in root samples. The poplar cultivar was also an important driver, explaining 12% and 6% of the variance in the fungal and bacterial data sets, respectively. The overall dominance of saprophytic fungi, e.g. *Penicillium canescens*, might be related to the decomposition activities needed at the experimental site. Our data further highlighted that the mycorrhizal colonization of poplar cultivars varies greatly between the species and genotypes, which is exemplified by the dominance of *Sclerotinia* under Vesten samples. Further interactions between fungal and bacterial functional groups stressed the potential of high-throughput sequencing technologies in uncovering the microbial ecology of disturbed environments.

Keywords: bacterial 16S; fungal ITS; Illumina-based sequencing; metal-contaminated soils; phytomanagement, poplar cultivars

INTRODUCTION

Since the 1980s, there has been increasing demand for fast-growing, stress-tolerant trees that can be used for the afforestation of postagricultural lands, the recultivation of areas that have been degraded by industry and the production of bioenergy. A short rotation coppice (SRC) is defined as a production system in which fast-growing recoppicing tree and bush species are intensively managed to yield large quantities of woody biomass in rotations of approximately 3–10 years (Šlapokas and Granhall 1991). Phytomanagement of metal-contaminated sites strongly relies on the capacity of the vegetation cover to tolerate high concentrations of metals. Salicaceae species respond to that constraint by accumulating trace elements (TEs) in their above-ground biomass (Migeon et al. 2009). The impact of land management intensity on soil microbial diversity (i.e. richness and evenness) has been recently explored in agricultural soils (Tardy et al. 2015), but the effects of planting trees in former agricultural soils on the diversity of soil microbial communities remain largely unknown. The impact of tree planting needs to be addressed in phytomanaged soils, because the changes in land use induced by fast-growing recoppicing trees may have a strong impact on the structure and function of organism communities living in the system, in addition to modifying soil characteristics. This impact may be particularly true for microorganisms, which are the primary agents responsible for key ecosystem processes (Jeffries et al. 2003; Philippot et al. 2013). Differences between fungal communities in forested and tree-less ecosystems have been recently revealed using a metabarcoding analysis of global soils (Tedersoo et al. 2014), although contaminated soils were not included in that work. As for most trees in temperate and boreal forests, the establishment, growth and survival of trees in large plantations depend strongly on colonization by mycorrhizal fungi and associated bacteria (Smith and Read 2008) and mycorrhizal fungi are crucial for tolerance to various abiotic and biotic stresses (Smith and Read 2008).

Fungal endophytes also represent key microorganisms, as highlighted by our recent study (Lacercat-Didier et al. 2016), where three isolates belonging to the order Helotiales and the species *Serendipita vermifera* were highly tolerant to metals (Cd, Zn, Pb and Cu). Further interactions between bacteria, fungi and plants have been shown to contribute to microbial community stability (Bonfante and Anca 2009; Bell et al. 2014) and many bacterial strains have indeed been reported to promote mycorrhizal symbioses (Frey-Klett, Garbaye and Tarkka 2007; Hryniewicz et al. 2010). Other key soil microorganisms such as saprotrophic fungi are central in forest soils due to their capacity to degrade recalcitrant organic compounds and may strongly interact with soil bacteria (Baldrian 2008). Saprotrophic fungi may also have the potential to mobilize metals from contaminated soils, due to their ability to exude chelating agents (Arwidsson et al. 2009). In addition to estimating the microbial fertility of contaminated soils, knowledge of the associated microbial compartment could be used to help predict the potential recovery of disturbed lands (Kozdrój 2000).

Only recently, high-throughput sequencing technologies have been used to reveal microbial communities' structure and composition in the rhizosphere and endosphere of tree roots in contaminated soils. For instance, metal pollution level significantly impacted bacterial community structure in two metal-polluted areas in Poland (Azarbad et al. 2015). The fungal community composition in a young pine plantation in Zn- and Cd-polluted soil was strongly correlated with Zn, Cd and soil organic matter content (Op De Beeck et al. 2015). We recently em-

ployed physico-chemical analysis, ion torrent sequencing and related bioinformatics tools to explore the characteristics of the soil microbial communities that were found to reside within a tailings dump and adjacent undisturbed soil (Zappelini et al. 2015). This multidisciplinary approach led us to conclude that environmental filtering is more likely to occur through the activity of tree roots than as a result of specific soil characteristics. Some studies based on high-throughput sequencing technologies have recently assessed the microbial communities associated with willows growing, for example, on hydrocarbon-contaminated (Bell et al. 2014; Yergeau et al. 2014; Pagé, Yergeau and Greer 2015; Leewis et al. 2016) and Zn-contaminated (Bell et al. 2015) soils. Among the fast-growing trees, *Populus* is also increasingly employed for the reforestation of post-agricultural lands, the recultivation of areas degraded by industry or environmental pollution, and as a renewable source of biomass for bioenergy production (Capuana 2011). Little is known about the whole microbial communities that are associated with poplar SRC or very short rotation coppice (vSRC), although some attention has been focused on the isolation and characterization of culturable bacterial and fungal endophytes from poplar roots (Van der Lelie et al. 2009; Gottel et al. 2011; Gamalero et al. 2012; Lacercat-Didier et al. 2016).

In this study, we combined highly novel high-throughput sequencing technologies, data analysis approaches and soil parameter analyses to (1) clarify the microbial diversity under different management regimes for contaminated soils, (2) characterize the taxonomic distribution of root and soil microbiomes, and (3) reveal the relations between microbiomes, soils and poplar cultivars.

MATERIALS AND METHODS

Site description, planting, sampling and chemical analysis

The experimental site was located northwest of Paris at Pierrelaye (latitude: 49°12'1' N; longitude: 2°9'1' E), on a plain that was initially dedicated to vegetable gardening. The soil has been irrigated with wastewater from Paris for more than 100 years, which has resulted in the accumulation of trace elements (TEs), mostly Zn, Cd, Pb and Cu. Detailed concentration data are given by Pottier et al. (2015). Crop production for human consumption was prohibited here in the late 1990s (Lamy et al. 2006), and this land had to be devoted to alternative uses, providing other types of ecosystem services. The Pierrelaye region has a continental climate with annual precipitation varying between 43.6 and 62.7 mm and an average mean annual temperature of 10.8°C (the minimum and maximum temperature averages were 6.5 and 15.2°C, respectively).

As part of the Phytopop project (ANR PRECODD), a 5-ha field demonstration trial was implemented in April 2007, with 14 poplar cultivars grown under two management regimes. The regimes consisted in an SRC (at a planting density of 1000 trees/ha) and a vSRC (at a planting density of 10 000 trees/ha). The present work was performed on 8 of the 14 poplar cultivars, which were selected to represent the three poplar families. These families were *P. trichocarpa* × *P. maximowiczii* (Bakan and Skado), *P. deltoides* × *P. nigra* (Muir, I214, Soligo and Vesten), and *P. trichocarpa* (Fritzi Pauley and Trichobel). More details on these genotypes are given in Pottier et al. (2015). The stems in the vSRC plots were cut back in 2010 to elicit the coppice response, which is characterized by the vigorous growth of multiple new stems

in the spring. There was no application of N fertilizer, pesticide or herbicide. Mechanical weed control and irrigation were performed during the first two years. After litter removal, the roots were collected from the upper 20 cm layer of soil from under the tree canopies. The roots were traced to their origin to ensure that they were indeed connected to the selected tree, and they were sampled at a distance of 1 m from the trunk. For each poplar cultivar, five individual trees were randomly sampled, and for each individual tree, three pseudo-replicates were sampled and mixed to obtain root and soil composites. In addition to poplar plots, soil samples were collected from an unplanted plot, which has been naturally recolonized by endogenous herbaceous plant species, and from a maize plot located near the poplar plot. Five bulk soil samples from the poplar plot, the maize plot and the unplanted plots were collected directly below the root system of the poplars and the maize or natural vegetation from the top 20 cm of soil. The poplar roots were carefully washed with tap water to remove visible soil, washed with sterile deionized water three times, and separated from the roots of other plant species by shape and color. The fine roots were then cut off and transferred to Eppendorf tubes and frozen at -20°C .

To determine the TE-extractable fractions, 5 g of 2 mm sieved soil was dried at 60°C for 48 h and incubated with 50 ml of 10 mM calcium chloride under agitation (40 r.p.m.) for 2 h at room temperature. The mixture was first filtered using ash-free filters and subsequently passed through a $0.45\text{-}\mu\text{m}$ mesh and acidified at 2% (v/v) with HNO_3 prior to inductively coupled plasma atomic emission spectrometry (ICP-AES, Thermo Fischer Scientific, Inc., Pittsburg, PA, USA) analysis. The methods for measuring the physico-chemical characteristics are described in Zappellini et al. (2015).

DNA extraction and sequencing methods

Total DNA was extracted from 500 mg of soil with a PowerSoil® DNA Isolation Kit (MO-BIO laboratories, Inc., Carlsbad, CA, USA) according to the manufacturer's instructions. To isolate microbial DNA from the roots, plant cell lysis was first performed using 100 mg of frozen root samples that were pulverized with 3-mm tungsten carbide beads (Qiagen S.A.S., Courtaboeuf, France) in a Mixer Mill for 3 min at 30 Hz (model MM400; Retsch Inc., Newtown, PA, USA). Root DNA was then extracted with a PowerSoil® DNA Isolation Kit according to the manufacturer's instructions. The DNA quality and quantity were assessed by agarose gel electrophoresis and with a Quant-iT™ PicoGreen® dsDNA Assay Kit (Invitrogen, Carlsbad, CA, USA) in 96-well microplates using an FLX-Xenius spectrofluorometer (SAFAS, Monaco).

The sequencing of the V3–V5 domains of 16S rRNA genes and the fungal internal transcribed spacer (ITS) 2 was performed on an Illumina MiSeq platform (Microsynth AG, Balgach, Switzerland) according to the standard protocols. PCR amplifications were performed with bacterial primers 341F (5'-CCT ACG GGR SGC AGC AG-3') and 909R (5'-TTT CAG YCT TGC GRC CGT AC-3') and fungal primers ITS3 (5'-GCA TCG ATG AAG AAC GCA GC-3') and ITS4 (5'-TCC GCT TAT TGA TAT GC-3'), using a 600-cycles Miseq Reagent Kit v3.

Data analysis

Reads were assigned to each sample according to a unique barcode, and contigs were then assigned using the Mothur pipeline (Schloss et al. 2009). Raw reads were filtered by length and quality. The reads were pre-clustered at 100% identity for ITS and 99%

identity for 16S using sumaclust (Mercier et al. 2013). Possible artifactual sequences observed with only a few reads (5 for ITS and 10 for 16S) were removed from the following analysis. The 16S sequences were aligned with those in the Silva database to remove non-16S sequences (www.arb-silva.de) (Quast et al. 2013). The 28S conserved regions of ITS sequences were searched using a hidden Markov model (Hartmann et al. 2010), and plant contamination was removed by identifying *Populus* chloroplastic ITS. Taxonomic assignments were performed using a Bayesian approach (Wang et al. 2007) in the Greengene database for bacteria (DeSantis et al. 2006) and the UNITE database for fungi (Kõljalg et al. 2005). Finally, operational taxonomic units (OTUs) were constructed using the Needleman distance and average neighbor clustering at a distance of 0.03. Diversity parameters were calculated on the basis of a data set in which the numbers of reads per sample were rarefied to 2140 and 1000 reads per sample for fungi and bacteria, respectively. We calculated the rarefaction curves at a 97% similarity level to verify that the amount of sequencing reflected the diversity of the original microbial communities. No differences in fungal or bacterial diversity patterns were observed between the rarefied and non-rarefied data sets; therefore, we opted to keep the rarefied data set to control for unequal sampling depths across samples.

Statistical analysis

All statistical analyses were performed using R software v. 3.0.2 (R Development Core Team, Vienna, Austria, 2013). The Shapiro and Bartlett tests were used to verify the normality and homoscedasticity of the data, respectively, and we compared each TE and measured the soil parameters using either ANOVA or a Kruskal-Wallis test (with the 'kruskal.test' function in R). The richness and diversity indices were calculated using the pgrmness and Vegan packages in R. Rarefaction curves were created with the 'rarecurve' function of the Vegan package in R. The 2-dimensional non-metric multi-dimensional scaling (NMDS) was calculated using the Bray-Curtis method ($k = 3$) with the 'metaMDS' function of the Vegan package in R. Analysis of similarity (ANOSIM) was performed using the Vegan package in R. PERMANOVA was run with 10 000 permutations using the 'adonis' function of the Vegan package in R. The resulting clustering trees were paired with a heatmap of Spearman's correlations between the relative abundances created with 'heatmap.2' from the gplots package. The numbers of OTUs that were shared between crops were visualized using Venn diagrams implemented by VennDiagram of the Vegan package in R.

RESULTS AND DISCUSSION

The management regimes greatly impacted the soil properties

The soil at our experimental site contained large amounts of sand (>80%), whereas silt and clay represented less than 10% (Table 1). After 7 years of phytomanagement, the thin sand, CaCO_3 , content, and C/N ratio decreased and the CaCl_2 -extractible K and Mg increased significantly in poplar soils in comparison with the initial 2007 values (Table 1). Conversely, the thin silt, total C content, organic matter, cationic exchangeable capacity (CEC) and CaO decreased only in unplanted soil in comparison with the initial soil values. The pH and coarse sand increased slightly only in planted soils (maize and poplar), whereas large silt and K_2O decreased only in the maize and unplanted plots in comparison with the initial soil. The CaCl_2 -

Table 1. Physico-chemical parameters of Pierreleaye soils. Mean values \pm standard deviation are provided ($n = 5$ for maize, unplanted and initial soil; $n = 8$ for SRC and vSRC physico-chemical parameters; $n = 40$ for SRC and vSRC CaCl_2 -extractible elements). Values designated with the same letters were not significantly different ($P < 0.05$) according to a Kruskal–Wallis test.

	Physical characterization										pH and carbonates				
	Clay (%)	Thin silt (%)	Large silt (%)	Thin sand (%)	Coarse sand (%)	pH	CaCO_3 (%)	Assimilable phosphorus (g/kg)	B (g/kg)						
SRC	3.77 \pm 1.26 (a)	4.4 \pm 1.65 (a)	4.55 \pm 1.59 (a)	39.36 \pm 2.49 (b)	47.94 \pm 4.65 (a)	7.40 \pm 0.00(a)	0.78 \pm 0.27 (c)	1.10 \pm 0.23 (a)	0.83 \pm 0.2 (ab)						
vSRC	3.71 \pm 1.23 (ab)	4.55 \pm 1.89 (a)	4.44 \pm 1.65 (ab)	36.24 \pm 4.01 (b)	51.05 \pm 6.38 (a)	7.41 \pm 0.06 (a)	1.07 \pm 0.30 (b)	1.09 \pm 0.23 (a)	0.74 \pm 0.19 (b)						
Maize	3.03 \pm 0.54 (ab)	3.30 \pm 0.52 (a)	3.05 \pm 0.53 (bc)	44.62 \pm 4.80 (a)	46.00 \pm 4.20 (ab)	7.37 \pm 0.05 (a)	1.62 \pm 0.39 (a)	0.96 \pm 0.06 (a)	0.95 \pm 0.15 (a)						
Unplanted	2.60 \pm 0.42 (b)	2.13 \pm 0.30 (b)	2.85 \pm 0.24 (c)	49.62 \pm 5.18 (a)	42.80 \pm 5.08 (bc)	7.22 \pm 0.08 (b)	1.28 \pm 0.31 (ab)	1.03 \pm 0.08 (a)	0.40 \pm 0.05 (c)						
Initial	2.98 \pm 0.77 (ab)	4.12 \pm 2.17 (a)	4.92 \pm 1.37 (a)	48.78 \pm 4.68 (a)	39.20 \pm 3.90 (c)	7.28 \pm 0.04 (b)	1.48 \pm 0.21 (a)	1.16 \pm 0.21 (a)	0.85 \pm 0.22 (ab)						
Exchangeable oligos															
Organic status															
C org (g/kg)	OM (g/kg)	N tot (g/kg)	C/N ratio	CEC (meq/kg)	K ₂ O (g/kg)	MgO (g/kg)	CaO (g/kg)	K/Mg ratio							
SRC	30.95 \pm 7.75 (a)	53.55 \pm 13.39 (a)	2.94 \pm 0.72 (a)	10.54 \pm 1.16 (c)	81.00 \pm 19.92 (a)	0.19 \pm 0.05 (a)	3.83 \pm 1.05 (a)	1.24 \pm 0.09 (b)							
vSRC	31.68 \pm 9.28 (a)	54.81 \pm 16.03 (a)	2.61 \pm 0.83 (ab)	12.30 \pm 1.97 (b)	84.88 \pm 30.00 (a)	0.17 \pm 0.04 (ab)	4.06 \pm 1.02 (a)	1.40 \pm 0.16 (a)							
Maize	31.52 \pm 3.71 (a)	54.53 \pm 6.44 (a)	2.26 \pm 0.15 (ab)	13.88 \pm 1.63 (a)	86.50 \pm 7.29 (a)	0.14 \pm 0.01 (b)	4.37 \pm 0.35 (a)	0.87 \pm 0.10 (c)							
Unplanted	18.63 \pm 1.84 (b)	32.22 \pm 3.20 (b)	1.23 \pm 0.18 (c)	15.33 \pm 2.22 (a)	55.17 \pm 4.71 (b)	0.10 \pm 0.02 (b)	2.38 \pm 0.20 (b)	0.97 \pm 0.08 (c)							
Initial	32.43 \pm 9.23 (a)	56.12 \pm 15.97 (a)	2.06 \pm 0.48 (b)	15.53 \pm 1.06 (a)	80.50 \pm 24.98 (a)	0.14 \pm 0.03 (b)	3.98 \pm 0.82 (a)	1.38 \pm 0.17 (a)							
CaCl ₂ extractable elements															
Cd ($\mu\text{g/kg}$)	Cu ($\mu\text{g/kg}$)	K ($\mu\text{g/kg}$)	Mg (mg/kg)	Mn (mg/kg)	Na (mg/kg)	P (mg/kg)	Pb ($\mu\text{g/kg}$)	Zn ($\mu\text{g/kg}$)							
SRC	0.05 \pm 0.01 (b)	0.21 \pm 0.21 (a)	143.15 \pm 34.74 (a)	85.73 \pm 17.67 (a)	7.53 \pm 2.38 (b)	8.70 \pm 1.56 (a)	<LQ	2.18 \pm 0.54 (d)							
vSRC	0.04 \pm 0.01 (c)	0.26 \pm 0.21 (a)	147.22 \pm 26.95 (a)	77.83 \pm 16.71 (ab)	8.71 \pm 4.48 (ab)	7.24 \pm 1.30 (bc)	<LQ	2.42 \pm 0.54 (c)							
Maize	0.03 \pm 0.01 (d)	0.15 \pm 0.04 (a)	68.16 \pm 12.23 (b)	65.9 \pm 5.58 (bc)	10.48 \pm 2.79 (a)	6.27 \pm 0.41 (c)	<LQ	2.82 \pm 0.47 (d)							
Unplanted	0.09 \pm 0.02 (a)	0.12 \pm 0.02 (a)	62.40 \pm 10.15 (b)	55.60 \pm 2.59 (c)	4.14 \pm 0.23 (c)	8.25 \pm 0.79 (ab)	<LQ	4.39 \pm 0.56 (a)							
Initial	0.06 \pm 0.01 (a)	0.21 \pm 0.12 (a)	100.36 \pm 26.57 (b)	60.21 \pm 14.84 (c)	9.49 \pm 3.25 (a)	6.95 \pm 1.07 (c)	0.01 \pm 0.03	2.08 \pm 0.31 (b)							

extractible Mn fraction decreased in all the plots in comparison with the initial 2007 values. The most notable changes in TEs were the significant decrease of the CaCl_2 -extractible Cd fraction in planted (with poplar and maize) soils, in comparison with the 2007 values or the unplanted soil. The CaCl_2 -extractible Zn fraction increased significantly in unplanted soils but decreased in all planted soils, compared with the initial soil (Table 1). The total N content and the MgO increased in the SRC plot and decreased in unplanted soil in comparison with the initial 2007 values. The CaCl_2 -extractible P fractions increased, whereas the CaCl_2 -extractible Na decreased in the SRC plot and in unplanted soil compared with the initial soil values. Moreover, the SRC plot contained significantly less CaCO_3 , a lower C/N ratio and lower CaCl_2 -extractible Zn but more CaCl_2 -extractible Cd, Mg, Mn and P relative to the vSRC plot. The total concentrations for all the elements remained unchanged (data not shown). No significant differences in the Cu concentrations and assimilable phosphorus were found between plots or from the initial values.

At our experimental site, there was an earlier suggestion that the clay destruction initiated by excessively large irrigation rates would imply a reduction in the filtering capacity of the horizon, and an increased risk of metal leaching into the groundwater (VanOort et al. 2008). The phytomanagement regime that we chose in 2007 resulted in a slightly but significantly higher pH in comparison with the initial pH or the unplanted option. An acidification risk that was observed in a previous study on poplars (Mertens et al. 2007), which led the authors to conclude that poplar was not a desirable option for their experimental sites, did not occur at our site. This difference is likely related to the different nature of the two soils; our soil contains a large amount of sand (>80%), whereas the soil in Mertens et al. (2007) contained approximately 30% clay. A higher soil fertility (in terms of K, Mg, N and P) was likely caused by the mineralization of organic matter and the higher release of minerals from soil particles, which was also measured at our site. An increase in the bioavailable P content was also found by Gamalero et al. (2012) under poplar plots grown on contaminated soils, which were correlated with an increased in phosphomonoesterase activity. Similarly, an increase in phosphatase activity was found in soil under poplars grown in an SRC plot (Lagomarsino et al. 2008). This mineralization may have also induced increased concentrations of metal-chelating substances in the soil that led to decreased bioavailability and thereby decreased the phytotoxicity of the TEs (notably Zn and Cd) compared with a 'no action (unplanted)' option, which might have led to a decreased risk of metal leaching into the ground water (Baum et al. 2009). Mann and Tolbert (2000) noticed a number of soil ecological benefits from poplar coppices that can be caused by continuous plant cover. Among these benefits, our study confirmed that a reduction in leaching losses and an increase of organic carbon are observed under poplar SRC or vSRC.

Roots were less enriched and less diverse in fungal and bacterial OTUs

A total of 13 088 974 16S bacterial and 38 843 584 ITS fungal raw MiSeq reads were obtained from 90 DNA soil samples and 80 DNA root samples. After quality filtering and target extraction, 1 402 648 bacterial effective sequences and 5 370 652 fungal effective sequences remained for the community analyses. A detailed breakdown of the recovered sequence numbers that was performed sample-by-sample is presented in Supplementary Tables S1 and S2. To determine the rarefaction curves, richness and diversity, 2140 and 1000 reads were randomly selected

from each sample for fungi and bacteria, respectively. Rarefaction analyses were performed to evaluate the depth of sequencing and to compare the relative fungal and bacterial communities in different samples. Our results showed that all the fungal and bacterial libraries from our samples represented the fungal (Supplementary Fig. S1A) and bacterial (Supplementary Fig. S1B) communities well, and the rarefaction curves tended towards saturation. In comparison with the soil samples, all the poplar root samples had lower bacterial and fungal OTU densities.

The OTU richness, Chao1 and ACE estimators, Pielou evenness, and Shannon and Simpson diversity indices for the fungal (Supplementary Table S1) and bacterial (Supplementary Table S2) communities were always significantly higher ($P < 0.05$) in the soil samples (SRC, vSRC, unplanted and maize) relative to the root samples, which is consistent with the rarefaction curves (Supplementary Fig. S1). There was no significant difference for the richness and diversity indices between the poplar SRC and vSRC (soil or root) samples except for the soil fungal communities, which were slightly more diverse under vSRC plots. Bacterial communities in maize and unplanted soils were slightly but significantly less enriched and less diverse than those from poplar-planted soils, whereas the fungal communities were not different. Spearman's correlation coefficients revealed significant, although few, statistical correlations between the richness and diversity indices and soil parameters (Supplementary Table S3). In brief, the fungal diversity was negatively correlated with Cd, and the fungal richness was positively correlated with phosphorus. By contrast, the bacterial richness and diversity indices were more strongly correlated with soil parameters such as the physical (sand) and chemical (C/N ratio, K and Mg, pH and CaCO_3) composition.

We used a β -diversity measure based on OTUs (Bray–Curtis) to compare the fungal (Fig. 1A) and bacterial (Fig. 1B) communities, which quantifies the taxa abundances shared between samples. In the visualized two-dimensional non-metric multidimensional scaling (NMDS) plot, the Bray–Curtis method indicated that the fungal and bacterial communities from SRC or vSRC poplar roots were well separated from those of the different planted and unplanted soils. The NMDS further indicated that maize and unplanted soils were clearly separated from the SRC or vSRC poplar soils for the fungal community, which also showed high fungal heterogeneity in root samples. The scattering of the fungal data was always higher for root samples than for soil samples, which denoted a higher homogeneity of species distribution in the soil samples (see sizes of ellipses in Fig. 1). A permutation test confirmed the separation of the microbial soil and root communities and showed that the habitat (soil in comparison with roots) explained 38% and 49% of the variance in the fungal and bacterial data sets, respectively (Table 2). The vegetation type (eight poplar cultivars, maize and unplanted soil samples) was also an important driver, explaining 12% and 6% of the variance in the fungal and bacterial data sets, respectively. The management regime (vegetation cover by poplars as SRC or vSRC, maize or unplanted soils) had a significant, although limited, impact on the fungal community, whereas bacterial communities were not affected (Table 2). A clear separation of microbiomes was previously reported for the soil and roots of mature poplar sites (Gottel et al. 2011; Shakya et al. 2013) and in an early phase of stand development (Danielsen et al. 2012). In this previous study, the richness and diversity were lower in the roots, and clear separations were observed between the soil and roots, explaining 40 and 50% of the variation for the fungal and bacterial communities, respectively.

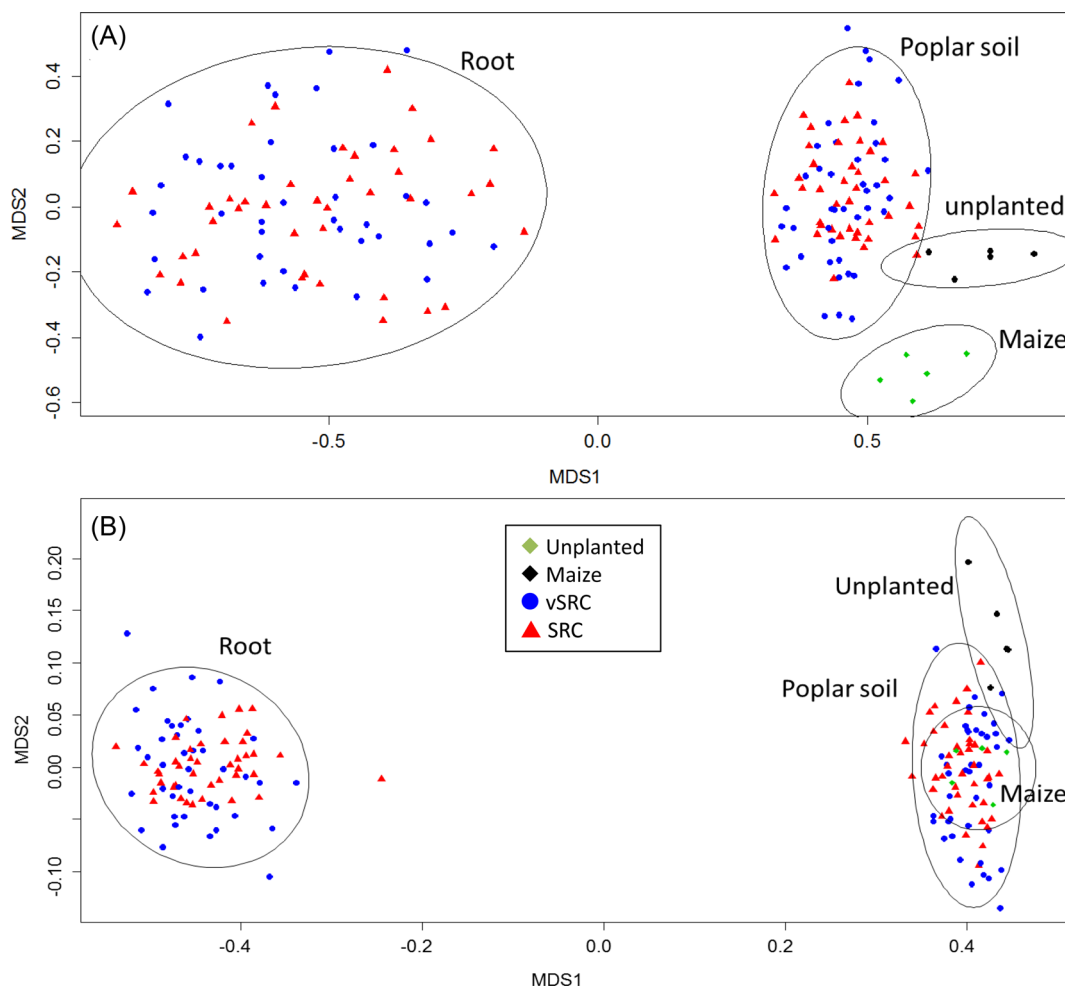


Figure 1. NMDS plot of the fungal (A) and bacterial (B) community structure using the Bray–Curtis dissimilarity measure. Each point represents the fungal or bacterial community of a given sample (vSRC, blue; SRC, red; unplanted, green; maize black). The ellipses clearly separated the sample into two groups as follow: left ellipse, root samples; and right ellipse, soil samples. Confidence area of ellipses = 0.95.

Table 2. PERMANOVA and ANOSIM data set for the bacterial and fungal community structures based on Bray–Curtis distances. Levels of significance are indicated by asterisks: * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$. P-values based on 10 000 permutations. Results are shown for analyses performed with poplar root samples versus all soil samples (habitat); with the eight poplar cultivars, maize and unplanted control samples (vegetation); with SRC, vSRC, maize and unplanted control samples (management regime).

	Fungi			Bacteria		
	PERMANOVA		ANOSIM	PERMANOVA		ANOSIM
	F(p)	R ²	R	F(p)	R ²	R
Habitat	167.9***	0.382	0.945***	198.9***	0.491	1.000***
Vegetation	6.1***	0.124	0.111***	2.9***	0.064	0.071***
Management regime (MR)	9.1***	0.021	0.100***	2.4	0.006	0.053**
Habitat × vegetation	3.2***	0.052		2.1**	0.037	
Habitat × MR	4.2**	0.010		1.6	0.004	
Cultivars × MR	3.6***	0.057		1.8*	0.031	
Habitat × vegetation × MR	2.3***	0.037		1.4	0.025	

In a previous study that was performed at the same experimental site, a *Miscanthus* plantation was shown to stimulate bacterial diversity by approximately 20% (Bourgeois et al. 2015), which is in agreement with the higher bacterial diversity and richness indices in all poplar soils in comparison with the unplanted soils. These authors also found higher fungal diversity

and richness indices under *Miscanthus*, compared with maize plots, which they explained by the intensive use of fungicides (Bourgeois et al. 2015) and soil tillage that may have led to the physical breakdown of fungal mycelia (Lienhard et al. 2014). We did not observe significant effects of poplar plantation on fungal diversity and richness indices. The overall significant decrease

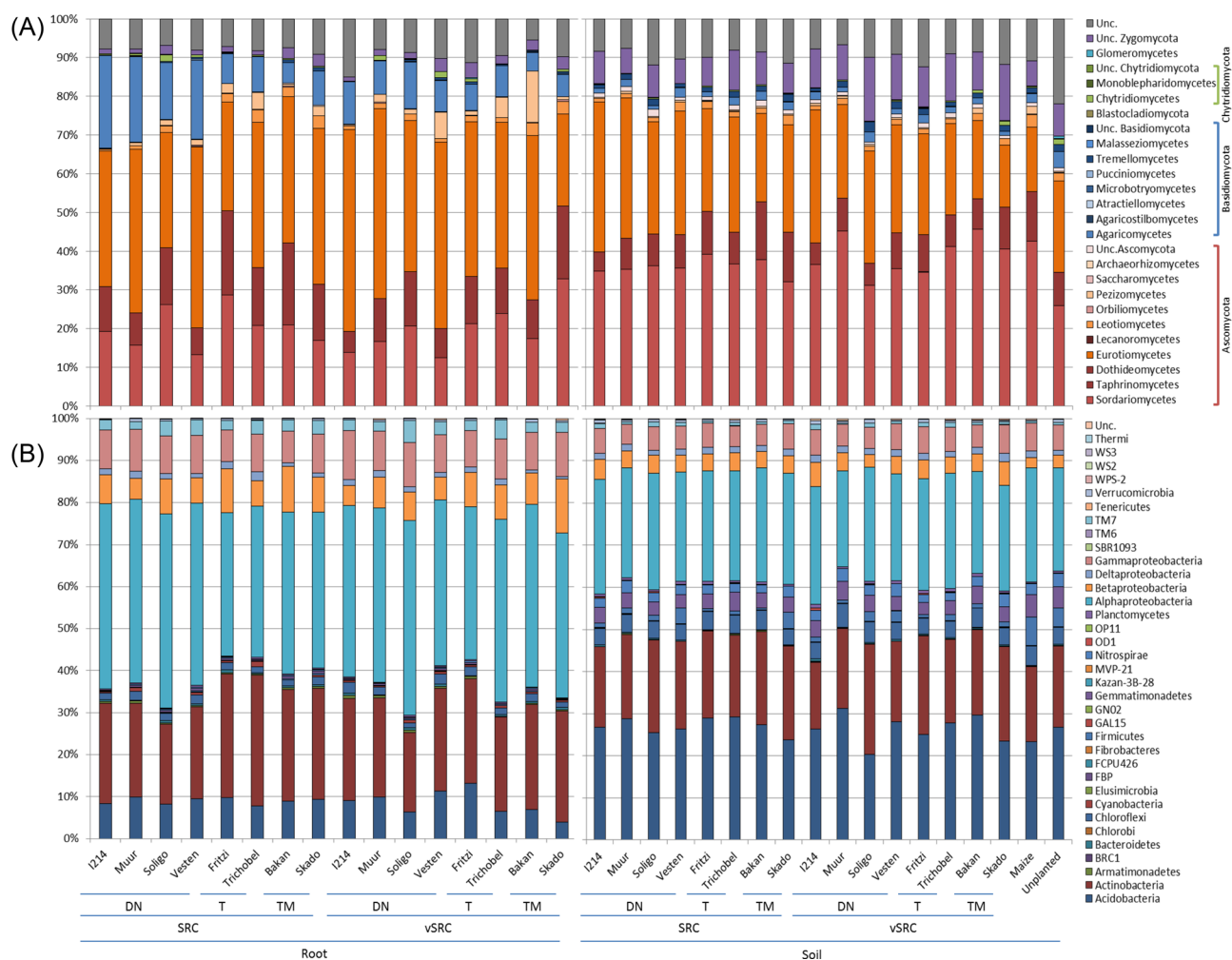


Figure 2. Relative abundance of fungal class (A) and bacterial phylum/class (B) identified in soils (right panels) or roots (left panels) for different poplar cultivars and families (TM, *P. trichocarpa* × *P. maximowiczii*; DN, *P. deltoides* × *P. nigra*; T, *P. trichocarpa*) grown as SRC or vSRC, and maize or unplanted soils. Unc., unclassified.

of fungal diversity and richness in poplar roots may well have impacted the surrounding soil area.

We used a Mantel test to compare the fungal and bacterial community Bray–Curtis dissimilarity matrices within all the experimental plots (Supplementary Fig. S2). The Mantel correlation coefficients showed that significant relations ($P < 0.05$) were found between the fungal and bacterial communities for all the plots. However, the fungal and bacterial communities from the unplanted plot were the highest correlated ones ($R^2 = 0.70$), whereas less correlation was found between fungal and bacterial communities from the roots of the SRC plots ($R^2 = 0.22$).

Root and soil constitute distinct ecological microbial habitats

To identify the phylogenetic diversity of the microbial communities, effective sequences were assigned to phyla, classes or genera. The overall set of reads was associated with seven fungal phyla and 26 fungal classes (Fig. 2A). Across all the root and soil samples, Ascomycota (83 and 77%, respectively) dominated the fungal communities, and in addition, Basidiomycota (4–11%) occurred in the root samples and Zygomycota (2–8%) occurred in the soil samples (Fig. 2A). Within the Ascomycota phylum, the Sordariomycetes (20–35%) dominated all the soil samples, whereas

Eurotiomycetes (23–40%) dominated the root samples. Within the Basidiomycota phyla, the Agaricomycetes (3–11%) were predominant in the roots but accounted for much lower proportions in the soil samples. In more detail, the relative abundance of Agaricomycetes was significantly higher in the roots of the SRC compared with that of the vSRC, whereas unclassified Zygomycota and Pezizomycetes accounted for larger proportions in the roots of vSRC poplars in comparison with the SRC (Supplementary Table S4). In poplar soils, only Eurotiomycetes and unclassified Zygomycota showed significant differences between SRC and vSRC. The unplanted soils contained larger amounts of Glomeromycetes and unclassified fungal OTUs in comparison with other samples (Supplementary Table S4).

The dominating genera in the poplar root and soil samples were the two Eurotiomycetes, that is, *Exophiala* and *Penicillium*, respectively (Supplementary Table S5). In the maize and unplanted plots, in addition to these two genera, a *Monodictys* species and a *Metarhizium* species also dominated, respectively. The fungal classes showed variations between poplar cultivars, independent of the management regime. For example, Agaricomycetes represented 20% of the root species of the *P. deltoides* × *P. nigra* poplar hybrids (I214, Muur, Soligo and Vesten) but only 8% of the root species of the other cultivars (Bakan, Skado, Fritzi Pauley and Trichobel) (Fig. 2A). A hierarchical

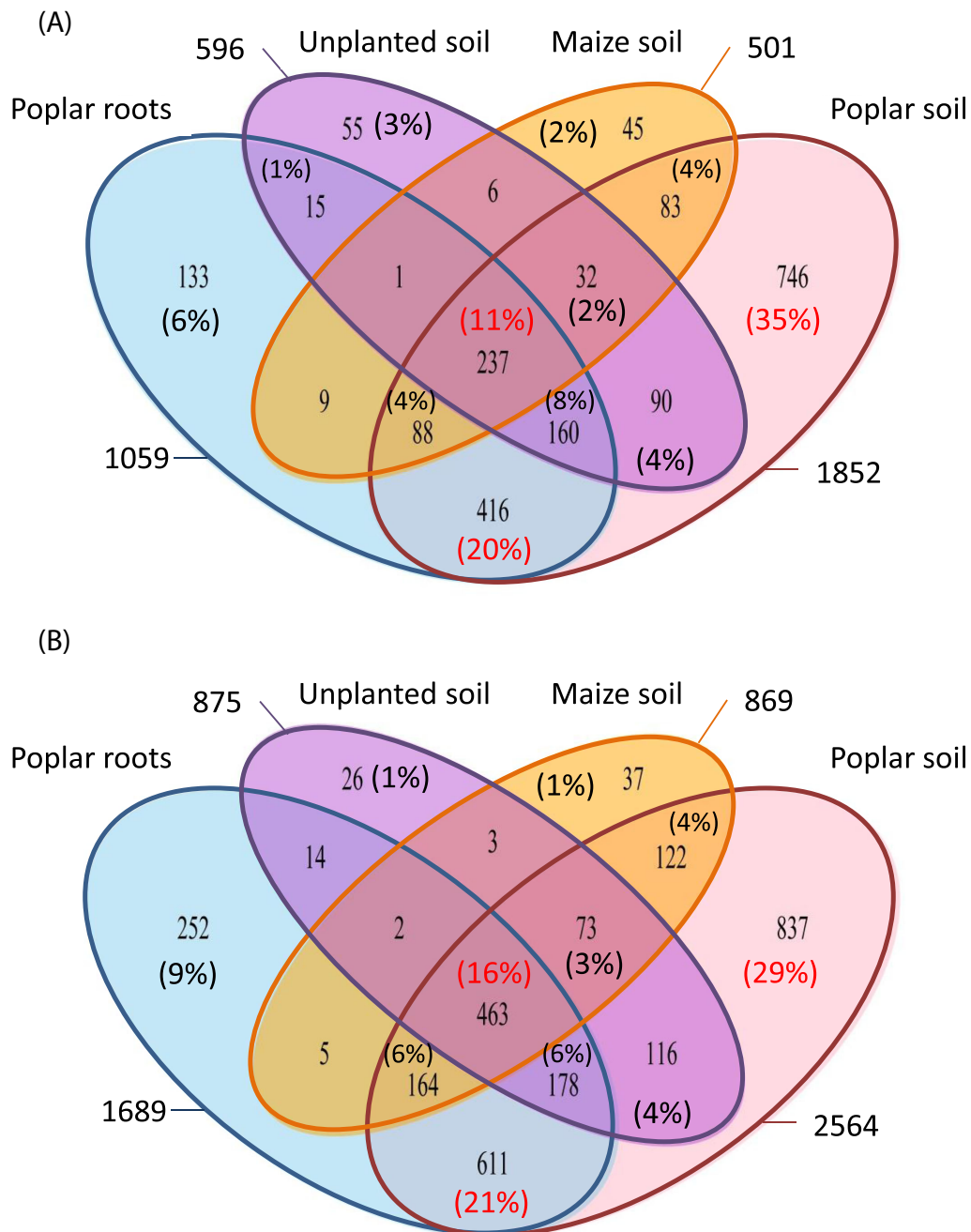


Figure 3. Overlap of the different fungal (A) and bacterial (B) communities from poplar roots, poplar soil, unplanted and maize soils based on OTUs (97% similarity). The numbers of OTUs shared by all the communities were 2116 for fungi and 2903 for bacteria.

cluster analysis of fungal communities at the class level confirmed that root and soil samples have different compositions (Supplementary Fig. S3). In detail, *Sordariomycetes*, unclassified *Zygomycota*, *Tremellomycetes* and some unclassified *Ascomycota* were positively correlated with soil samples, and *Agaricomycetes* and *Eurotiomycetes* were positively correlated with root samples. The total fungal-observed OTUs in the four habitats was 2116, whereas only 237 (11% of the total OTUs) were shared between them (Fig. 3A). Soil and root samples from the poplar plots shared the highest number of fungal OTUs (416 OTUs, that is, 20% of the total OTUs) (Fig. 3A). OTUs that were unique to each community are highlighted, and they represented 746 OTUs for

poplar soil (35% of total OTUs), 133 OTUs for poplar roots (6%), 55 for unplanted soil (3%) and 45 for maize soil (2%).

Each fungal species, genus, family, or class was further assigned to functional groups of fungi. This functional assignment is fully described in Tedersoo et al. (2014), and was performed by a group of taxonomic experts. Taxa were for instance considered to be ectomycorrhizal (ECM) if they best matched any sequences of known ECM lineages. If different functional categories were present within a specific genus, the dominant group was chosen (>75% of species assigned to a specific category) or its ecology considered unknown (<75% of species assignable to a single category). All Glomeromycota were considered to be

Table 3. Relative abundances of fungal OTUs that were assigned to major functional groups. The various OTU groups from soil and root samples that were collected from the SRC, vSRC, maize or unplanted plots were classified as either biotrophic, ectomycorrhizal (ECM), endomycorrhizal (AM), endophytic or saprotrophic. Mean values \pm standard deviation are provided soil or root samples for SRC ($n = 40$) and vSRC ($n = 40$) poplars and control soils (unplanted $n = 5$ and maize $n = 5$). Values designated with the same letters were not significantly different ($P < 0.05$) according to a Kruskal–Wallis test.

	SRC root	vSRC root	SRC soil	vSRC soil	Maize	Unplanted
AM	1.32 \pm 1.79 (b)	1.49 \pm 2.68 (b)	0.66 \pm 1.11 (b)	0.59 \pm 1.18 (b)	1.60 \pm 2.07 (b)	14.60 \pm 8.20 (a)
Biotrophic	381.41 \pm 185.70 (ab)	382.10 \pm 207.19 (b)	399.88 \pm 70.80 (a)	388.49 \pm 83.85 (ab)	366.80 \pm 91.72 (ab)	277.60 \pm 100.31 (b)
ECM	126.54 \pm 124.68 (a)	120.10 \pm 140.76 (a)	8.54 \pm 15.07 (c)	11.56 \pm 12.26 (b)	4.40 \pm 9.29 (c)	1.60 \pm 3.05 (c)
Endophytic	20.61 \pm 22.25 (a)	18.73 \pm 17.24 (a)	3.15 \pm 3.43 (c)	4.80 \pm 4.06 (b)	3.40 \pm 2.07 (bc)	13.00 \pm 17.01 (ab)
Saprotrophic	1155.02 \pm 218.47 (b)	1169.68 \pm 194.41 (b)	1306.85 \pm 117.02 (a)	1295.05 \pm 103.53 (a)	1285.00 \pm 97.78 (ab)	1193.00 \pm 231.56 (ab)
Unknown	455.02 \pm 113.06 (ab)	447.90 \pm 96.89 (ab)	419.76 \pm 71.41 (b)	439.10 \pm 60.27 (ab)	478.00 \pm 108.77 (ab)	639.00 \pm 302.74 (a)

arbuscular mycorrhizal (AM). The OTUs were finally classified as ECM, AM, endophytic, saprotrophic or biotrophic (Table 3). In all of the samples, saprotrophic fungi dominated (54–61%), whereas endophytic and ECM fungi were significantly more abundant in the root samples. The saprotrophic species *Penicillium canescens* that dominated in our samples is known to produce xylanase (Delvigne et al. 2008) and to solubilize phosphate (Mendes et al. 2012) and could therefore have a key role in litter degradation. The other *Penicillium* species present in all the poplar clones (but not in the maize or unplanted soils) of *P. brevicompactum*, although less frequent, can produce plant growth regulators (Kusano et al. 1998). *Penicillium* species have been shown to produce organic acids in the presence of trace elements that can lead to immobilization of metals (Arwidsson et al. 2009).

Although the endophytic OTUs were less abundant, a *Tetracladium maxilliforme* OTU dominated the endophytic OTUs in all the root samples (Supplementary Table S6). *Exophiala* OTUs that dominated in our samples are potential root endophytes, and they help plants to overcome many biotic and abiotic stresses (Zhan et al. 2014; Maciá-Vicente, Glynou and Piepenbring 2016). The *Cyphellophora vermisporea* species detected in the roots of all the poplar clones was previously detected in living plant material such as stems, roots and leaves, and it could behave as a root endophyte (Gao et al. 2015).

Tuber and *Laccaria* OTUs (and to a lesser extent an unclassified *Sebacinaceae* OTU, a *Geopora*, *Tomentella* and a *Hebeloma*) were significantly more frequently represented in root samples than in soil samples. *Tuber* OTUs also dominated in vSRC soil and root samples but were less frequently represented in SRC soil samples, from which *T. rufum*, *T. scruposum* and *T. rapaeodorum* were missing. The *Laccaria* OTUs largely dominated in SRC root samples and to a lesser extent in vSRC root samples, whereas they were virtually absent from the soil samples (Fig. 4A and Supplementary Table S6). Interestingly, a *Scleroderma* OTU dominated in all Vesten poplar samples, and it was almost absent from all the other (root, soil, SRC or vSRC) poplar samples (Fig. 4A), which also explained the high variability observed in the SRC root samples (Supplementary Table S6). Conversely, *Geopora* OTUs were detected in almost all soil and root poplar samples, but they were absent from maize and unplanted soil samples. Our results confirmed those of a previous study (Hryniewicz et al. 2010) that determined that selective promotion occurred for some Pezizales (*Geopora*, *Tuber* and *Peziza* spp.) and some Agaricales (*Laccaria* spp.) under short rotation regimes, and a selective promotion of other Agaricales (*Inocybe* sp.) and Boletales (*Scleroderma* spp.) because of the longer rotations. The clear dominance of *Tuber* species in vSRC in comparison with SRC is consistent with this observation. Using an ITS sequencing approach, we also detected *Tuber*, *Geopora* and *Hebeloma* species at our experimental site (Lacercat-Didier et al. 2016). The *Geopora* species found

in the present work and in previous studies (Hryniewicz et al. 2008) formed ECM in various early and late successional woodlands in acidic to alkaline forest soils as well as in nutrient-poor volcanic cinder soils (Gehring et al. 1998; Fujimura et al. 2004). The high adaptation ability of these organisms to diverse soil conditions and their long survival as spores might explain the successful spread of *Geopora* (Hryniewicz et al. 2010). *Laccaria* spp. were found on other *Populus* species (*P. trichocarpa* and *P. nigra* \times *maximowiczii*) during pot and field experiments (Baum and Makeschin 2000; Hryniewicz et al. 2010). Our data confirmed that the mycorrhizal colonization of salicaceous cultivars varies greatly between the species and genotypes (Khasa et al. 2002; Puttsepp, Rosling and Taylor 2004), which is exemplified specifically by the detection of *Scleroderma* on Vesten roots, *Laccaria* on all but Skado cultivars, and *Geopora* on all cultivars.

Bacterial composition and interactions with fungal communities

The overall set of reads was associated with 27 bacterial phyla and 83 bacterial classes (Fig. 2B). Proteobacteria (mostly Alphaproteobacteria followed by Beta- and Gammaproteobacteria) and Actinobacteria dominated the root microbiome, whereas Alphaproteobacteria, Acidobacteria and Actinobacteria were the three major bacterial groups in the soil samples (Fig. 2B and Supplementary Table S7). Nitrospirae and Chloroflexi, which were also less abundant than the 3 former groups, accounted for significantly much larger proportions of the soil samples compared with the root samples (Supplementary Table S7). Firmicutes and Gemmatimonadetes were highly represented in maize and unplanted soils, whereas they accounted for significantly much lower proportions of poplar root or soil samples. Conversely, Cyanobacteria were highly represented in root samples in comparison with soil samples (Supplementary Table S7). A hierarchical cluster analysis of bacterial communities at the phylum and class levels confirmed that the root and soil samples have different compositions (Supplementary Fig. S4). The soil samples contained mostly Acidobacteria, Chloroflexi, Gemmatimonadetes, Nitrospirae and Firmicutes for the most abundant phylum, whereas the root samples were enriched in Alphaproteobacteria, Actinobacteria, Gammaproteobacteria, Betaproteobacteria, TM7, Cyanobacteria, Armatimonadetes and Bacteroidetes. The total for observed bacterial OTUs in the four habitats was 2903, whereas only 463 (16% of the total OTU) were shared between them (Fig. 3B). Soil and root samples from the poplar plots shared 611 bacterial OTUs, that is, 21% of the total OTUs (Fig. 3B). OTUs that were unique to each community are highlighted, and they represented 29% of the total OTUs for poplar soil, 9% for poplar roots, and 1% for unplanted and maize soils.



Figure 4. Relative proportions of (A) fungal genera that were assigned to ECM fungal and (B) bacterial genera in which PGPBs are currently recognized. The abundances of the various OTU groups are shown for soils (right panels) or roots (left panels) for different poplar cultivars and families (TM, *P. trichocarpa* × *P. maximowiczii*; DN, *P. deltoides* × *P. nigra*; T, *P. trichocarpa*) that were grown in SRC or vSRC, and maize or unplanted soils. Unc., unclassified.

Table 4. Relative abundances of bacterial OTUs that were assigned to bacterial genera, in which plant growth promoting-bacteria (PGPB) are currently recognized. Soil and root samples were collected from the SRC, vSRC, maize or unplanted plots. Mean values ± standard deviation are provided for soil or root samples for SRC ($n = 40$) and vSRC ($n = 40$) poplars and unplanted ($n = 5$) and maize ($n = 5$) soils. Values designated with the same letters were not significantly different ($P < 0.05$) according to a Kruskal–Wallis test.

	SRC root	vSRC root	SRC soil	vSRC soil	Maize	Unplanted
<i>Bacillus</i>	1.00 ± 0.92 (c)	0.93 ± 1.27 (c)	8.29 ± 8.08 (b)	6.33 ± 5.63 (b)	17.6 ± 7.99 (a)	18.00 ± 8.72 (a)
<i>Flavobacterium</i>	1.20 ± 1.40 (a)	1.20 ± 1.65 (a)	0.27 ± 0.45 (b)	0.20 ± 0.52 (b)	0	0.40 ± 0.89 (b)
<i>Mesorhizobium</i>	11.61 ± 4.57 (a)	14.68 ± 10.61 (a)	3.54 ± 1.52 (b)	3.92 ± 2.27 (b)	1.60 ± 1.14 (c)	1.60 ± 1.14 (c)
<i>Microbacterium</i>	1.85 ± 1.82 (bc)	2.32 ± 2.35 (b)	3.98 ± 2.53 (a)	4.40 ± 4.18 (a)	0.40 ± 0.55 (c)	0.80 ± 0.84 (bc)
<i>Pseudomonas</i>	6.80 ± 9.27 (a)	11.71 ± 15.27 (a)	2.00 ± 1.55 (b)	1.65 ± 1.58 (bc)	0.20 ± 0.45 (c)	2.60 ± 3.21 (bc)
<i>Rhizobium</i>	43.22 ± 16.00 (a)	53.20 ± 23.83 (a)	1.95 ± 1.79 (b)	2.10 ± 1.89 (b)	1.80 ± 0.84 (b)	2.80 ± 1.30 (b)
<i>Rhodococcus</i>	0.51 ± 0.78 (b)	0.44 ± 0.63 (b)	1.32 ± 1.08 (a)	1.30 ± 1.26 (a)	0.60 ± 0.89 (ab)	1.00 ± 0.71 (ab)
<i>Sphingomonas</i>	6.07 ± 2.85 (a)	7.41 ± 3.97 (a)	3.29 ± 2.78 (b)	2.95 ± 2.31 (b)	1.60 ± 1.34 (b)	1.60 ± 1.52 (b)
<i>Streptomyces</i>	29.49 ± 15.87 (a)	27.02 ± 11.04 (a)	5.02 ± 2.83 (c)	4.90 ± 3.18 (c)	8.00 ± 2.12 (b)	8.00 ± 3.16 (b)
<i>Variovorax</i>	27.93 ± 13.26 (a)	27.98 ± 15.24 (a)	8.24 ± 3.83 (b)	8.05 ± 4.65 (b)	1.00 ± 0.71 (c)	3.20 ± 2.28 (c)

As described for fungal OTUs, bacterial OTUs that could be classified to phylum/class or genera were further assigned to categories in which plant growth-promoting bacteria (PGPB) are currently recognized, i.e. symbiotic nitrogen-fixing bacteria and mycorrhiza helper bacteria solely based on a literature search (Frey-Klett, Garbaye and Tarkka 2007; Hryniewicz *et al.* 2008; Singh, Pandey and Singh 2011; Trujillo *et al.* 2015; Ullah *et al.* 2015). Relative abundances of bacterial OTUs that were classified at the genus or at the phylum/class level, in which PGPB are currently recognized, are provided in Table 4 and Supplementary

Table S7, respectively. Overall, 56 OTUs were assigned to a hypothetical PGPB, and they were associated with 14 bacterial genera, representing 14% of the total OTUs in the root samples but only 4% of the total OTU in the soil samples. The *Rhizobium*, *Streptomyces*, *Mesorhizobium* and *Variovorax* OTUs were most abundant in root samples, whereas *Microbacterium*, *Rhodococcus* and *Bacillus* OTUs dominated the soil samples (Fig. 4B and Table 4). The relative abundance of a *Bradyrhizobiaceae* OTU was significantly higher in root samples compared with soil samples (Supplementary Table S5). Overall, the OTUs from nitrogen-fixing

bacteria in the *Bradyrhizobiaceae* and *Rhizobiaceae* families clearly dominated (>15%) the root samples (Table 4 and Supplementary Table S5). There was otherwise much less variation in the OTU profiles compared with the fungal OTUs between management regimes and cultivars (Fig. 4A and B). Although the planted trees are not nitrogen fixers, the *Rhizobium* and *Mesorhizobium* genera were frequently encountered in non-nitrogen-fixer tree microbiomes (Shakya et al. 2013; Bonito et al. 2014) and can promote symbiosis (Frey-Klett, Garbaye and Tarkka 2007). Many soil bacteria affiliated with the dominating genus *Variovorax* have shown resistance to various TEs and are able to adapt to a wide variety of soil environments (Gamalero et al. 2012; Durand et al. 2015). The *Variovorax paradoxus* species also had PGPB properties (Jiang et al. 2012). Our results also showed that *Sphingomonas wittichii* organisms were present in the roots of all the poplar cultivars, but not in the maize or unplanted soils. *Sphingomonas wittichii* was found in a variety of habitats including soil, sediment, oceans and lakes, where it had a profound role in the degradation of large, complex aromatic compounds associated with decaying plant matter and chemical pollution such as dioxin (Miller et al. 2010).

Spearman's correlation coefficients revealed statistically significant and positive correlations between PGPB and ECM OTUs ($P < 0.001$, $\rho = 0.50$), between PGPB and fungal endophytic ones ($P < 0.001$, $\rho = 0.50$) and between ECM and fungal endophytic OTUs ($P < 0.001$, $\rho = 0.41$). Conversely, saprotrophic OTUs were negatively correlated with PGPB OTUs ($P < 0.001$, $\rho = -0.41$). In more detail, the bacterial *Rhizobium* and *Streptomyces* OTUs were positively correlated with one another ($\rho = 0.79$) and with the bacterial OTUs *Variovorax paradoxus* ($\rho = 0.74$ and 0.69 , respectively), *Mesorhizobium* sp. ($\rho = 0.74$ and 0.70 , respectively), *Flavobacterium succinicans* ($\rho = 0.48$ and 0.41 , respectively), *Sphingomonas* sp. ($\rho = 0.60$ and 0.58 , respectively), and *Pseudomonas* sp. ($\rho = 0.53$ and 0.46 , respectively), and the fungal OTUs *Tetracladium maxilliforme* ($\rho = 0.59$ and 0.57 , respectively), *Sebacina* ($\rho = 0.41$ and 0.47 , respectively) and *Laccaria* ($\rho = 0.42$ and 0.44 , respectively). Consistent with our data, many bacterial strains have indeed been reported to be able to promote ECM symbioses (Frey-Klett, Garbaye and Tarkka 2007). Hryniewicz et al. (2008) showed that the inoculation of willows with *Sphingomonas* sp. improved nitrogen uptake by plants, increased plant growth and stimulated the formation of *Geopora* sp. ectomycorrhizae. *Pseudomonas* and *Bacillus* species were found to have a helper effect on *Tuber*, *Pisolithus*, *Laccaria* and *Suillus* species (Frey-Klett, Garbaye and Tarkka 2007). Because many *Streptomyces* sp. produce secondary metabolites with antifungal properties or because they secrete chitinolytic enzymes, they are generally considered antagonists of both plant pathogenic and saprophytic soil fungi (Reddy and Satyanarayana 2006; Lehr et al. 2007; Tarkka and Hampp 2008). However, some cases have been described in which *Streptomyces* promoted the growth of rhizospheric fungi, notably those that are involved in forming ectomycorrhizae with plants (Seipke, Kaltenpoth and Hutchings 2012; Bontemps et al. 2013). Similarly, *Streptomyces* and *Pseudomonas* species that were positively correlated in our study with *Laccaria* sp., *Sebacina* sp. and *Tetracladium maxilliforme* could be considered as helper bacteria.

Bacteria can be photoautotrophs, heterotrophs or chemoautotrophs, and many can fix atmospheric nitrogen (Nemergut et al. 2007; Duc et al. 2008; Schmidt et al. 2008), whereas all fungi are heterotrophs. However, fungi are the primary decomposers of recalcitrant components in plant litter because they can produce lignin-modifying enzymes (Lundell, Mäkelä and Hildén 2010). These two microbial populations may therefore have complementary functions.

CONCLUSIONS

From the analysis of rRNA gene amplicon MiSeq sequencing data from 7-year-old poplars, we may conclude that the implementation of poplar SRC or vSRC led to a significant increase in soil fertility and a decrease in the bioavailability of Zn and Cd that accompanied changes in microbial communities. Planting poplars increased the proportion of symbiotic/endophytic fungi in the rhizosphere, although at lower proportions than those frequently observed in forest stands. This finding might be explained by the overall decomposition activities needed at the Pierrelaye site, where the accumulation of large amounts of organic carbon from raw wastewater occurred for more than one century, which is revealed by the dominance of saprotrophic fungi in all the samples. We further showed that roots and soil constitute distinct habitats for fungal and bacterial communities, independent of the poplar management regimes (SRC and vSRC) and of the cultivars in use.

Finally, we reveal that planting poplar crops on contaminated soils that have been irrigated with raw but contaminated wastewaters can be considered as a relevant strategy to stabilize the metal elements and produce biomass on an otherwise unused agricultural soil. Poplar biomass production has not been discussed here, and it will be fully detailed in a further paper. We may, however, reveal that the biomass yield was indeed rather high in comparison with the production of the same poplar cultivars in non-contaminated areas, producing between 12 and 16 t DW/ha/year. We have addressed this question in a previous study based on a decision tool, in which we did conclude that poplar plantations may reduce the risk of dusting and leaching (Andersson-Sköld et al. 2014).

SUPPLEMENTARY DATA

Supplementary data are available at FEMSEC online.

ACKNOWLEDGEMENTS

The authors wish to thank Isabelle Lamy (INRA, Versailles) for providing them with access to the Pierrelaye site.

FUNDING

This work was supported by the French National Research Agency [ANR PHYTOPOP 06-ECOT-O15-01, ANR BIOFILTREE 2010-INTB-1703-01], the French Environment and Energy Management Agency [PROLIPHYT 1172C0053], the Région Franche-Comté [Environnement-Homme-Territoire 2014-069] and the Pays de Montbéliard Agglomération [13/070-203-2015].

Conflict of interest. None declared.

REFERENCES

- Andersson-Sköld Y, Bardos P, Chalot M et al. Developing and validating a practical decision support tool (DST) for biomass selection on marginal land. *J Environ Manage* 2014;**145**: 113–21.
- Arwidsson Z, Johansson E, von Kronhelm T et al. Remediation of metal contaminated soil by organic metabolites from fungi I—Production of organic acids. *Water Air Soil Pollut* 2009;**205**:215–26.

- Azarbad H, Niklińska M, Laskowski R et al. Microbial community composition and functions are resilient to metal pollution along two forest soil gradients. *FEMS Microbiol Ecol* 2015;**91**:1–11.
- Baldrian P. Wood-inhabiting ligninolytic basidiomycetes in soils: Ecology and constraints for applicability in bioremediation. *Fungal Ecol* 2008;**1**:4–12.
- Baum C, Leinweber P, Weih M et al. Effects of short rotation coppice with willows and poplar on soil ecology. *Landbauforsch Volkenrode* 2009;**59**:183–96.
- Baum C, Makeschin F. Effects of nitrogen and phosphorus fertilization on mycorrhizal formation of two poplar clones (*Populus trichocarpa* and *P. tremula* x *tremuloides*). *J Plant Nutr Soil Sci* 2000;**163**:491–7.
- Bell TH, Cloutier-Hurteau B, Al-Otaibi F et al. Early rhizosphere microbiome composition is related to the growth and Zn uptake of willows introduced to a former landfill. *Environ Microbiol* 2015;**17**:3025–38.
- Bell TH, Hassan SE-D, Lauron-Moreau A et al. Linkage between bacterial and fungal rhizosphere communities in hydrocarbon-contaminated soils is related to plant phylogeny. *ISME J* 2014;**8**:331–43.
- Bonfante P, Anca I-A. Plants, mycorrhizal fungi, and bacteria: a network of interactions. *Annu Rev Microbiol* 2009;**63**:363–83.
- Bonito G, Reynolds H, Robeson MS et al. Plant host and soil origin influence fungal and bacterial assemblages in the roots of woody plants. *Mol Ecol* 2014;**23**:3356–70.
- Bontemps C, Toussaint M, Revol P-V et al. Taxonomic and functional diversity of Streptomyces in a forest soil. *FEMS Microbiol Lett* 2013;**342**:157–67.
- Bourgeois E, Dequiedt S, Lelièvre M et al. Positive effect of the Miscanthus bioenergy crop on microbial diversity in wastewater-contaminated soil. *Environ Chem Lett* 2015;**13**:495–501.
- Capuana M. Heavy metals and woody plants – biotechnologies for phytoremediation. *Iforest Biogeosciences For* 2011;**4**:7–15.
- Danielsen L, Thürmer A, Meinicke P et al. Fungal soil communities in a young transgenic poplar plantation form a rich reservoir for fungal root communities. *Ecol Evol* 2012;**2**:1935–48.
- Delvigne F, Aldric J-M, Destain J et al. Improvement of xylanase production by *Penicillium canescens* 10-10c in solid-state fermentation. *Biotechnol Agron Société Environ Biotechnol Agron Soc Environ BASE* 2008;**12**:111–8.
- DeSantis TZ, Hugenholtz P, Larsen N et al. Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. *Appl Environ Microbiol* 2006;**72**:5069–72.
- Duc L, Noll M, Meier BE et al. High Diversity of Diazotrophs in the Forefield of a Receding Alpine Glacier. *Microb Ecol* 2008;**57**:179–90.
- Durand A, Piutti S, Rue M et al. Improving nickel phytoextraction by co-cropping hyperaccumulator plants inoculated by plant growth promoting rhizobacteria. *Plant Soil* 2015;**399**:179–92.
- Frey-Klett P, Garbaye J, Tarkka M. The mycorrhiza helper bacteria revisited. *New Phytol* 2007;**176**:22–36.
- Fujimura KE, Smith JE, Horton TR et al. Pezizalean mycorrhizas and sporocarps in ponderosa pine (*Pinus ponderosa*) after prescribed fires in eastern Oregon, USA. *Mycorrhiza* 2004;**15**:79–86.
- Gamalero E, Cesaro P, Ciatelli A et al. Poplar clones of different sizes, grown on a heavy metal polluted site, are associated with microbial populations of varying composition. *Sci Total Environ* 2012;**425**:262–70.
- Gao L, Ma Y, Zhao W et al. Three new species of Cyphellophora (Chaetothyriales) associated with sooty blotch and flyspeck. *PLoS One* 2015;**10**:e0136857.
- Gehring CA, Theimer TC, Whitham TG et al. Ectomycorrhizal fungal community structure of pinyon pines growing in two environmental extremes. *Ecology* 1998;**79**:1562–72.
- Gottel NR, Castro HF, Kerley M et al. Distinct microbial communities within the endosphere and rhizosphere of populus deltoides roots across contrasting soil types. *Appl Environ Microbiol* 2011;**77**:5934–44.
- Hartmann M, Howes CG, Abarenkov K et al. V-Xtractor: An open-source, high-throughput software tool to identify and extract hypervariable regions of small subunit (16 S/18 S) ribosomal RNA gene sequences. *J Microbiol Methods* 2010;**83**:250–3.
- Hrynkiwicz K, Baum C, Leinweber P et al. The significance of rotation periods for mycorrhiza formation in Short Rotation Coppice. *For Ecol Manag* 2010;**260**:1943–9.
- Hrynkiwicz K, Baum C, Niedojadło J et al. Promotion of mycorrhiza formation and growth of willows by the bacterial strain *Sphingomonas* sp. 23L on fly ash. *Biol Fertil Soils* 2008;**45**:385–94.
- Jeffries P, Gianinazzi S, Perotto S et al. The contribution of arbuscular mycorrhizal fungi in sustainable maintenance of plant health and soil fertility. *Biol Fertil Soils* 2003;**37**:1–16.
- Jiang F, Chen L, Belimov AA et al. Multiple impacts of the plant growth-promoting rhizobacterium *Variovorax paradoxus* 5C-2 on nutrient and ABA relations of *Pisum sativum*. *J Exp Bot* 2012;**63**:6421–30.
- Khasa PD, Chakravarty P, Robertson A et al. The mycorrhizal status of selected poplar clones introduced in Alberta. *Biomass Bioenergy* 2002;**22**:99–104.
- Köljalg U, Larsson K-H, Abarenkov K et al. UNITE: a database providing web-based methods for the molecular identification of ectomycorrhizal fungi. *New Phytol* 2005;**166**:1063–8.
- Kozdrój J. Microflora of technogenous wastes characterised by fatty acid profiling. *Microbiol Res* 2000;**155**:149–56.
- Kusano M, Sotoma G, Koshino H et al. Brevicompanines A and B: new plant growth regulators produced by the fungus, *Penicillium brevicompactum*. *J Chem Soc [Perkin 1]* 1998:2823–6.
- Lacercat-Didier L, Berthelot C, Foulon J et al. New mutualistic fungal endophytes isolated from poplar roots display high metal tolerance. *Mycorrhiza* 2016, DOI: 10.1007/s00572-016-0699-y.
- Lagomarsino A, Moscatelli MC, Hoosbeek MR et al. Assessment of soil nitrogen and phosphorous availability under elevated CO₂ and N-fertilization in a short rotation poplar plantation. *Plant Soil* 2008;**308**:131–47.
- Lamy I, Van Oort F, Dère C et al. Use of major- and trace-element correlations to assess metal migration in sandy Luvisols irrigated with wastewater. *Eur J Soil Sci* 2006;**57**:731–40.
- Leewis M-C, Uhlik O, Fraraccio S et al. Differential impacts of willow and mineral fertilizer on bacterial communities and biodegradation in diesel fuel oil-contaminated soil. *Front Microbiol* 2016;**7**:837.
- Lehr NA, Schrey SD, Bauer R et al. Suppression of plant defence response by a mycorrhiza helper bacterium. *New Phytol* 2007;**174**:892–903.
- Lienhard P, Terrat S, Prévost-Bouré NC et al. Pyrosequencing evidences the impact of cropping on soil bacterial and fungal diversity in Laos tropical grassland. *Agron Sustain Dev* 2014;**34**:525–33.
- Lundell TK, Mäkelä MR, Hildén K. Lignin-modifying enzymes in filamentous basidiomycetes – ecological, functional and phylogenetic review. *J Basic Microbiol* 2010;**50**:5–20.

- Maciá-Vicente JG, Glynou K, Piepenbring M. A new species of *Exophiala* associated with roots. *Mycol Prog* 2016;**15**:1–12.
- Mann L, Tolbert V. Soil sustainability in renewable biomass plantings. *AMBIO J Hum Environ* 2000;**29**:492–8.
- Mendes GO, Dias CS, Silva IR et al. Fungal rock phosphate solubilization using sugarcane bagasse. *World J Microbiol Biotechnol* 2012;**29**:43–50.
- Mercier C, Boyer F, Bonin A et al. SUMATRA and SUMACLUST: fast and exact comparison and clustering of sequences. *Programs and Abstracts of the SeqBio 2013 Workshop*, 27–9.
- Mertens J, Van Nevel L, De Schrijver A et al. Tree species effect on the redistribution of soil metals. *Environ Pollut* 2007;**149**:173–81.
- Migeon A, Richaud P, Guinet F et al. Metal accumulation by woody species on contaminated sites in the north of France. *Water Air Soil Pollut* 2009;**204**:89–101.
- Miller TR, Delcher AL, Salzberg SL et al. Genome sequence of the dioxin-mineralizing bacterium *Sphingomonas wittichii* RW1. *J Bacteriol* 2010;**192**:6101–2.
- Nemergut DR, Anderson SP, Cleveland CC et al. Microbial community succession in an unvegetated, recently deglaciated soil. *Microb Ecol* 2007;**53**:110–22.
- Op De Beeck M, Lievens B, Busschaert P et al. Impact of metal pollution on fungal diversity and community structures. *Environ Microbiol* 2015;**17**:2035–47.
- Pagé AP, Yergeau É, Greer CW. *Salix purpurea* stimulates the expression of specific bacterial xenobiotic degradation genes in a soil contaminated with hydrocarbons. *PLoS One* 2015;**10**:e0132062.
- Philippot L, Raaijmakers JM, Lemanceau P et al. Going back to the roots: the microbial ecology of the rhizosphere. *Nat Rev Microbiol* 2013;**11**:789–99.
- Pottier M, García de la Torre VS, Victor C et al. Genotypic variations in the dynamics of metal concentrations in poplar leaves: A field study with a perspective on phytoremediation. *Environ Pollut* 2015;**199**:73–82.
- Puttsepp U, Rosling A, Taylor AFS. Ectomycorrhizal fungal communities associated with *Salix viminalis* L. and *S. dasyclados* Wimm. clones in a short-rotation forestry plantation. *For Ecol Manag* 2004;**196**:413–24.
- Quast C, Pruesse E, Yilmaz P et al. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Res* 2013;**41**:D590–6.
- Reddy MS, Satyanarayana T. Interactions between ectomycorrhizal fungi and rhizospheric microbes. In: Mukerji KG, Manoharachary C, Singh J (eds). *Microbial Activity in the Rhizosphere*. Berlin, Heidelberg: Springer, 2006, 245–63.
- Schloss PD, Westcott SL, Ryabin T et al. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Appl Environ Microbiol* 2009;**75**:7537–41.
- Schmidt S, Reed SC, Nemergut DR et al. The earliest stages of ecosystem succession in high-elevation (5000 metres above sea level), recently deglaciated soils. *Proc R Soc B Biol Sci* 2008;**275**:2793–802.
- Seipke RF, Kaltenpoth M, Hutchings MI. *Streptomyces* as symbionts: an emerging and widespread theme? *FEMS Microbiol Rev* 2012;**36**:862–76.
- Shakya M, Gottel N, Castro H et al. A multifactor analysis of fungal and bacterial community structure in the root microbiome of mature *Populus deltoides* trees. *PLoS One* 2013;**8**:e76382.
- Singh JS, Pandey VC, Singh DP. Efficient soil microorganisms: A new dimension for sustainable agriculture and environmental development. *Agric Ecosyst Environ* 2011;**140**:339–53.
- Šlapokas T, Granhall U. Decomposition of willow-leaf litter in a short-rotation forest in relation to fungal colonization and palatability for earthworms. *Biol Fertil Soils* 1991;**10**:241–8.
- Smith SE, Read DJ. *Mycorrhizal Symbiosis*. Amsterdam, Boston: Academic Press, 2008.
- Tardy V, Spor A, Mathieu O et al. Shifts in microbial diversity through land use intensity as drivers of carbon mineralization in soil. *Soil Biol Biochem* 2015;**90**:204–13.
- Tarkka M, Hampp R. Secondary metabolites of soil streptomycetes in biotic interactions. In: Karlovsky PDP (ed). *Secondary Metabolites in Soil Ecology*. Berlin, Heidelberg: Springer, 2008, 107–26.
- Tedersoo L, Bahram M, Polme S et al. Global diversity and geography of soil fungi. *Science* 2014;**346**:1256688.
- Trujillo ME, Riesco R, Benito P et al. Endophytic Actinobacteria and the interaction of *Micromonospora* and nitrogen fixing plants. *Front Microbiol* 2015;**6**:1341.
- Ullah A, Heng S, Munis MFH et al. Phytoremediation of heavy metals assisted by plant growth promoting (PGP) bacteria: A review. *Environ Exp Bot* 2015;**117**:28–40.
- Van der Lelie D, Taghavi S, Monchy S et al. Poplar and its bacterial endophytes: coexistence and harmony. *Crit Rev Plant Sci* 2009;**28**:346–58.
- VanOort F, Jongmans AG, Lamy I et al. Impacts of long-term waste-water irrigation on the development of sandy Luvisols: consequences for metal pollutant distributions. *Eur J Soil Sci* 2008;**59**:925–38.
- Wang Q, Garrity GM, Tiedje JM et al. Naïve Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Appl Environ Microbiol* 2007;**73**:5261–7.
- Yergeau E, Sanschagrin S, Maynard C et al. Microbial expression profiles in the rhizosphere of willows depend on soil contamination. *ISME J* 2014;**8**:344–58.
- Zappellini C, Karimi B, Foulon J et al. Diversity and complexity of microbial communities from a chlor-alkali tailings dump. *Soil Biol Biochem* 2015;**90**:101–10.
- Zhan F, He Y, Li T et al. Tolerance and antioxidant response of a dark septate endophyte (DSE), *Exophiala pisciphila*, to cadmium stress. *Bull Environ Contam Toxicol* 2014;**94**:96–102.