Over twenty years farmland reforestation decreases fungal diversity of soils, but stimulates the return of ectomycorrhizal fungal communities

Nan Hui[1], Xinxin Liu[1*], Ari Jumpponen[2], Heikki Setälä[1], D. Johan Kotze[1], Liliya Biktasheva[3], Martin Romantschuk[1,3]

1) Faculty of Biological and Environment Sciences, University of Helsinki, Niemenkatu 73, 15140, Lahti, Finland

2) Division of Biology, Kansas State University, Manhattan, KS 66506, USA.

3) Institute of Environmental Sciences, Kazan Federal University, Kazan, 420008, Russian Federation

*** Corresponding author:**

Xinxin Liu

xin.lin@helsinki.fi

+358445876678

Abstract

Background and Aims

Although soil-inhabiting fungi can affect tree health and biomass production in managed and pristine forests, little is known about the sensitivity of the plant-fungal associations to long-term changes in land use. We aimed to investigate how reforestation of farmlands change soil characteristics and affected the recovery of soil fungal functional guilds.

Methods

We examined edaphic conditions and fungal communities (Illumina Sequencing) in three land-use types: primary forests (PF), secondary forests (SF, established over two decades ago) and active farmlands during May, July and September in Wuying, China.

Results

Edaphic conditions and general fungal communities varied with land-use. Interestingly, overall fungal diversity was higher in soils at the farmland than at the forested sites, possibly as a result of recurring disturbances (tilling) allowing competitive release as described by the intermediate disturbance hypothesis. Although ectomycorrhizal fungal diversity and richness were marginally higher in PF than in SF, the latter still hosted surprisingly diverse and abundant ectomycorrhizal fungal communities.

Conclusions

Reforestation largely restored fungal communities that were still in transition, as their composition in SF was distinct from that in PF. Our results highlight the ability of fungi grown in previously strongly managed agricultural land to rapidly respond to reforestation and thus provide support for forest trees.

Key words: Fungal community development, Reforestation, Fungal functional guild, Atrazine, Ectomycorrhizal fungal community

Introduction

Environmental restoration has received considerable attention in the past decades. For example, the Grain for Green (GFG) project, one of the world's largest environmental rehabilitation projects, was launched in China in 1999. The GFG project aimed to convert low-yield farmlands into forests and pastures, thus restoring regional ecosystems (Lei et al. 2012). Recent studies within GFG have shown that implementation of the GFG strategy generally results in favorable ecological outcomes on, for example, carbon sequestration and soil organic carbon storage (Chang et al. 2011; Song et al. 2014). However, much less attention has been paid to the return of soil microorganisms after land-use changes, which are essential in providing ecosystem functions and services in various ecosystems (Fierer 2017). Scattered evidence suggests that site restoration can alter soil microbial communities: in Sharkey County, MS USA, soil bacterial and fungal communities shifted as a result of reforestation of former farmland (Strickland et al. 2017). Our previous studies corroborate: after twenty years of site reforestation, farmland bacterial communities shifted to a state that resembled natural forest soils, although the chemical fingerprints of former farming activities still remained (Liu et al. 2016). In contrast to bacteria, the responses of soil fungi to reforestation of chemically treated farmland remain largely unknown.

Soil-inhabiting fungi are important in managed forest, e.g. through their influence on tree health and biomass production, on nutrient cycling and pedogenesis, thus boosting the reestablishment of biodiversity and functioning of the restored ecosystems (Harris 2009; Jonsson et al. 2001). Ectomycorrhizal (ECM) fungi that form symbiotic relationships with many trees are particularly important as they facilitate host nutrient acquisition (Velmala et al. 2014) and protect them against soil pathogens (Laliberté et al. 2015) and toxic compounds (Luo et al. 2014). Similarly, saprotrophic fungi (SAP), including various litter and wood-decomposing fungi that degrade organic compounds, can improve tree health via improved nutrient cycling (Lindahl et al. 2002). Forest soils also host a diversity of lichenized, root endophytic and pathogenic fungi. Although the interactions between plants and lichenized or endophytic fungi remain poorly understood, the pathogenic fungi likely impact tree health negatively (Higgins et al. 2007).

Soil fungal communities change when land-use practices convert the prevailing ecosystem type to another (Verbruggen et al. 2010). This is because land-use activities are often followed by drastic changes in vegetation and many fungi, especially ECM and pathogenic fungi can be host specific (Hatta et al. 2002; Molina and Horton 2015; Tedersoo et al. 2008). Furthermore, land-use conversion likely impacts on soil characteristics (Setälä et al. 2016), which can alter the communities of SAP and

arbuscular mycorrhizal fungi (Balser et al. 2005). Thus, reforesting repeatedly disturbed farmlands to minimally disturbed forests likely induces changes in soil fungal communities (Verbruggen et al., 2010). In addition to land use and management practices, fungal communities may also vary temporally as a result of, e.g., fluctuating temperature and soil moisture regimes (Kennedy et al. 2006; Schadt et al. 2003).

The conservation of degraded farmlands to forests may improve ecosystem services and enhance biodiversity, but will not match the composition and structure of the original forest (Chazdon 2008). In this study, we aimed to explore how 20 years of time after reforestation (i) change soil characteristics and (ii) affect the recovery of fungal functional guilds. We sampled soils in the rhizospheres of (i) primary forests, (ii) secondary forests and (iii) farmlands planted with corn during May, July and September of 2014 in Wuying, NE China. We examined whether i) fungal communities are affected by the three land-use types, and ii) these effects are time/season dependent. We also explored iii) which fungal groups (functional guilds) are particularly sensitive to land-use conversion and sampling time. We hypothesized that i) reforestation results in greater fungal diversity in secondary forest than in farmland, because of a greater diversity of plant species/functional groups capable of producing divergent substrates/resources in forests than in farmlands; ii) fungal community composition is not fully restored during the transition from farmland to primary forest in two decades after reforestation, because edaphic conditions, which influence soil microorganisms, restore slowly (Cavagnaro et al. 2016); and, iii) reforestation leads to an increase in the number of fungal functional guilds commonly associated with forest trees, but two decades are not sufficient for a return of diversity and composition comparable to primary forest. This is because fungal community diversity increases steadily towards a climax state in conifer forest soil (Twieg et al. 2007).

Materials and methods

Study area

The study area is described in our previous work (Liu et al. 2016), which investigated bacterial communities in three land-use types on a forested plain in Wuying, northeast China (48.11 N, 129.14 E). Briefly, the town of Wuying is located along the Tangwang River, within the Fenglin National Forest Reserve. Altitude ranges from 400 to 800 m a.s.l. with gentle slopes of 3–7°. Timber harvesting in Wuying was active from the 1950s to the 1990s. Part of the forested land was clear-cut and converted mainly for the cultivation of soybean (*Glycine max*), corn (*Zea mays*) and spring wheat (*Triticum aestivum*). In the 1970s, chemical fertilizers, pesticides and herbicides were commonly

utilized to increase production, and the application of these agrochemicals continues to this day. The gradual reforestation of low-yield farmland started in the 1990s in Wuying.

We resampled the 24 sites used in our previous work (Liu et al. 2016), representing three land-use types (eight sites per type): (i) mixed conifer and broad leaf primary forests (PF), consisting of mainly Korean pine (*Pinus koraiensis*), some birches (*Betula platyphylla*) and other tree species, e.g., *Phellodendron amurense*, *Quercus mongolica*, *Pinus sylvestris* and *Larix gmelinii*; (ii) secondary forests (SF) that were reforested from agricultural fields with mainly Korean pine (planted) in the early 1990s; and (iii) farmlands (FL) that were established in the 1950s or possibly earlier and are still mainly used for the cultivation of corn (in some years soybeans were also cultivated). The dominant understory vegetation in PF and SF comprised of *Acanthopanax senticosus*, *Athyrium spinulosum*, *Corylus mandshurica*, *Leymus secalinus*, *Parasenecio hastatus*, *Rhododendron dauricum*, *Ribes burejense*, *Sipiraea salicifolia*, *Thalictrum fructumcornu*, *Tilia mandshurica* and *Urtica fissa*, with SF containing more grasses than PF. In Wuying, eight PF and eight SF sites, with at least 1 km distance between them, were selected in sixteen discrete forest patches ranging from ca. 0.5 ha to several hectares in size. The farmland sites were selected from eight independent farms belonging to different owners. In the sampling year, corn was cultivated in all FL sampling sites. To determine seasonal dynamics, we sampled three times, in May, July and September at six-week intervals, resulting in a total 72 samples throughout the study.

Soil sampling and analyses

The sampling plots at the two forest categories (PF and SF) were chosen randomly close to Korean pine trees. During each sampling event, one soil sample (pooled by 3 subsampled soil cores) was collected using a stainless steel corer (5 cm diameter, depth 0–15 cm), 2 m from the trunk of a Korean pine in PF and SF and from a random spot in FL. To avoid age effects, the age of the selected trees ranged from 25 to 29 years in both PF and SF. The corer was sterilized between samples using 70% ethanol. In May, trees at the coring locations were marked and galvanized nails were placed in the soil to aid in finding the exact localities in July and September. In FL, these positions were flagged. The samples were stored in Minigrip bags on ice until frozen at −20 °C in the laboratory. The samples were thawed at room temperature and sieved to remove stones, roots and large particles.

Edaphic variables of all samples were measured as described in (Liu et al. 2016). Briefly, soil pH was determined in 0.01 M $CaCl_2$. Dry weight (after drying for 48 h at 105°C) was determined in triplicate from approximately 5 g samples. Organic matter (OM) was determined by loss of ignition in a muffle

oven (5 h at 550°C). Total nitrogen (N), total phosphorus (P), total potassium content (K), and atrazine were extracted from soil and analyzed by high-performance liquid chromatography.

DNA extraction, amplification, and sequencing

Total DNA was extracted from 0.25 g of soil using the DNeasy PowerSoil Kit (Mobio Laboratories, Solana Beach, CA, USA) in triplicate according to the manufacturer's instructions. The DNA yield was confirmed by agarose gel electrophoresis (1.0% 1 × TAE buffer agarose gel run at 120 V for 1 h) and visualized with ethidium bromide. The extracted DNA was stored at −20 °C until PCR amplification.

The hypervariable Internal Transcribed Spacer (ITS2) region of the fungal rRNA gene was amplified with primers fITS7 5'-GTGARTCATCGAATCTTTG-3' incorporating 5'-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT-3' overhang and ITS4 5'-TCCTCCGCTTATTGATATGC-3' incorporating 5'-ATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT-3' overhang. In the secondary PCR, the full-length P5 and indexed P7 Illumina MiSeq adapters were used. The PCR reactions were performed as in Koskinen *et al*. (2011). Samples were analyzed using the Fragment Analyzer (Advanced Analytical, USA) and amplicons sequenced with Illumina MiSeq (v.3 2x300bp paired-end) at the Institute of Biotechnology, University of Helsinki. The paired fastq files are available in the Sequence Read Archive at NCBI (www.ncbi.nlm.nih.gov) under accession number SRR5961553 - SRR5961624.

Fungal population density by qPCR

We measured fungal population density by qPCR. A DyNAmo SYBR Green QPCR kit (Finnzymes, Espoo, Finland) was used in all runs. Amplification and detection of fungal DNA was performed by using the fITS7 and ITS4 primers, used also for MiSeq amplicon generation. Real-time PCR assays were conducted using a DNA Engine OPTICON 2 (Continuous Fluorescence Detector, MJ Research). Amplification was conducted using 2.0 µl of diluted DNA (dilution of 1:100), 10 µl of 2X DYANAMO Master Mix, 1 µl of each primer (10 µM), and 6 µl of sterile distilled water. The thermal cycling conditions followed (Schmidt et al. 2017). A negative control (dH_2O) and a positive control (*Candida ethanolica*) were included.

Bioinformatics

We processed the paired end sequence data (.fastq) using mothur (version 1.39.5) (Schloss et al. 2009). The fungal .fastq files were contiged and any sequences with ambiguous bases, with more than

one mismatch to the primers, homopolymers longer than 8 bp and any without a minimum overlap of 50 bp were removed. This yielded 7 522 598 fungal sequences. The sequences were screened for chimeras using UCHIME (Edgar et al. 2011) and putative chimeras removed (283 575 sequences removed). To permit pairwise alignment of fungal ITS sequences to calculate a pairwise distance matrix, we omitted sequences that were shorter than 300 bp and truncated the remaining sequences to the first 300 bp (6637 sequences removed). These sequences were assigned to taxa using the Naïve Bayesian Classifier and the UNITE-curated International Nucleotide Sequence Database reference database (Abarenkov et al. 2010). Any sequences not assigned to Kingdom Fungi were removed (4613 sequences). We subsampled the fungal dataset to 20 000 sequences per sample, resulting in a dataset with 1.44 million sequences in total. A pairwise distance matrix was derived from pairwise alignments and sequences clustered to operational taxonomic units (OTU) at a 97% threshold using nearest neighbour joining. All low abundance OTUs were removed ($\leq$ 10 sequences across all experimental units) as they may be PCR or sequencing artifacts (Brown et al. 2015; Oliver et al. 2015; Tedersoo et al. 2010). This resulted in a total of 1 210 579 sequences representing 8102 fungal OTUs. We assigned OTUs into fungal functional guilds using the FUNGuild database (Nguyen et al. 2016). We estimated richness and diversity indices for the fungal communities in mothur. Observed OTU richness (S_{obs}), the complement of Simpson's diversity (1/D: $1/\sum p_i^2$) and Simpson's evenness (E_D: $1/\sum p_i^2/S$), with p_i representing the abundance of each OTU within a sample, were iteratively calculated and subsampled at 15839 sequences per sample. ECM fungal richness and diversity indices were calculated excluding FL samples (due to the low abundance of ECM fungi) and subsampled at 3816 sequences per sample. SAP fungal richness and diversity indices were calculated with all samples and subsampled at 2185 sequences per sample.

Statistical analysis

All statistical analyses were performed in R (version 3.2.1, R Development Core Team, 2015) using various packages. To evaluate the effects of land-use type and sampling time on soil edaphic conditions (pH, OM, N, P, K, atrazine) and fungal population density (qPCR), we conducted two-way ANOVAs, using the *lm* function. We analyzed fungal diversity indices (all fungi, ECM fungi only and SAP fungi only), relative abundances of major fungal phyla and the 10 most abundant fungal functional guilds using generalized linear models (GLM) with the *glm* function in the lme4 package. Variables included land-use type and sampling time as main factors and their interaction, as well as soil pH, OM, N, P, K and atrazine. We performed model selection by removing non-significant terms, starting with the term with the highest p-value. Soil pH, OM, N, P, K and atrazine were initially

subject to model simplification until only terms with p-values < 0.1 remained. If the land-use type by sampling time interaction remained non-significant (p-values > 0.1) after this procedure, it was also removed. However, to remain true to our experimental design, the main effects (land-use type and sampling time) were always retained in the model irrespective of their significance. To approximate normality, the response variables above were Ln-transformed when necessary.

Non-metric multidimensional scaling (NMDS) analyses were performed using the vegan package to visualize the total fungal communities, ECM communities and SAP communities. We evaluated the effects of land-use type and sampling time on community composition based on Bray-Curtis coefficient matrix. We did not include samples of FL in the ECM fungal data due to the low relative abundances of ECM fungi in FL soils. Soil pH, OM, N, P, K and atrazine were correlated with community structure using permutation tests as the vector fitting procedure (the *envfit* function in vegan) and the Bray-Curtis coefficient was used as the dissimilarity measure.

In addition, we evaluated the effects of land-use type and sampling time on the relative abundances of major OTUs. To identify fungal OTUs that were over-represented in specific land-use types or sampling time, we conducted indicator OTU analyses with all data using the indicspecies package in R. Since the large divergence between farmland and forest might mask differences between the two forest types, we also conducted indicator OTU analyses including data from PF and SF but not FL.

Results

Changes in edaphic conditions due to land-use type and sampling time

All soil parameters, including pH, OM, N, P, K and atrazine concentration, responded to land-use type, but not to sampling time (Fig. 1, Table S1). Although fertilizers and atrazine were applied to farmlands in late May, i.e. after our first sampling event, we did not observe corresponding changes in soil N, P, K and atrazine concentrations across the growing season. Soil pH was highest in FL, followed by SF and lowest in PF (Fig. 1a). Soil OM content differed among land-use types in the order PF > SF > FL (Fig. 1b). N and K were highest in PF soils (Fig. 1c, e), whereas FL soils were highest in soil P (Fig. 1d). Given that atrazine is commonly used in farmland to control weeds, highest atrazine concentrations were found in FL (Fig. 1f). However, residual atrazine was still detectable in SF, even though the sites were reforested more than two decades ago.

Responses of fungal relative quantities to land-use type and sampling time

We express fungal population density, estimated by qPCR, as the copy number of fungal ITS rRNA gene operons per gram of dry soil. We use this as a proxy of relative fungal abundances when

comparing them among the treatments. Based on these estimates, fungal population density responded to both land-use type ($F_{2.69} = 9.639$, $p = 0.002$) and sampling time ($F_{2.69} = 3.960$, $p = 0.023$), without interaction between the two factors. Among land-use types, PF soils had the highest fungal population density, followed by SF, with FL having the lowest density. Across sampling times, density was highest in July, followed by September and May (Fig. S1).

Responses of fungal richness and diversity to land-use type and sampling time

Funguild database assigned 5402 OTUs (out of 8102) into fungal functional guild, 78.3%, 70.1% and 61.3% in PF, SF and FL, respectively. Soil fungal community diversity (total, ECM, SAP) changed with land-use type and sampling time (Fig. 2, Table S2). Total fungal OTU richness was lowest in SF, followed by PF and highest in FL (Fig. 2a). Fungal diversity and evenness also responded to land-use type with highest values in FL soils, lower values in PF soils and lowest in SF soils (Fig. 2b, c). Fungal community diversity indices also changed with sampling time, with values generally lower in May than in July and September (Fig. 2a, b, c).

Due to their low relative abundance in agricultural sites (less than 1%), we analyzed ECM communities only in the two forested sites. Similarly to the total fungal community, all ECM diversity measures were greater in PF than in SF soils (Fig. 2d, e, f, Table S2). Although we observed greater ECM fungal diversity in PF than in SF soils, these differences were rather small and the SF comparably diverse. Temporal ECM diversity responses differed from those of the total fungal community - all ECM diversity indices were lower in July and September than in May. In contrast, all diversity indices of the SAP fungal community responded to land-use type and seasonal effects similarly to the total fungal community. SAP fungal communities were most diverse in FL soils, followed by PF and SF soils (Fig. 2 g, h, i). All SAP diversity indices increased in Jul and September compared to May.

To evaluate the relationships between diversity metrics and edaphic variables, we included edaphic variables in our GLM models. In these expanded analyses, total fungal richness and SAP fungal diversity indices correlated positively with soil pH. ECM fungal diversity and evenness correlated negatively with soil OM. Total fungal diversity and evenness correlated negatively with N. Total fungal diversity, total fungal evenness, SAP fungal diversity and SAP fungal evenness correlated positively with atrazine, indicating that the FL soils were the highest in atrazine. None of the diversity indices correlated with soil K content (Table S1).

Responses of fungal community composition to land-use type and sampling time

Fungal communities differed across land-use type ($r^2 = 0.633$, $p < 0.001$; Fig. 3a) and sampling time ($r^2 = 0.243$, $p = 0.021$; Fig. 3d). To identify factors that influence community composition of major fungal functional guilds, we analyzed ECM and SAP fungal data separately. Again, FL was excluded from the ECM fungal analyses. ECM fungi responded to both land-use type ($r^2 = 0.358$, $p < 0.001$, Fig. 3b) and sampling time ($r^2 = 0.264$, $p = 0.008$; Fig. 3e). Soil SAP fungal community composition in FL differed from those in PF and SF ($r^2 = 0.587$, $p < 0.001$) and SF differed from PF ($r^2 = 0.447$, $p < 0.001$; Fig. 3c). SAP fungal community composition did not differ across sampling time ($r^2 = 0.636$, $p = 0.118$; Fig. 3f).

Fungal OTUs were classified into five phyla: Basidiomycota dominated (57.7% of all sequences), followed by Ascomycota (25.2%), basal clades formerly assigned to Zygomycota (12.5%), Glomeromycota (3.1%) and Chytridiomycota (<1%). At the phylum level, 1.3% of fungal sequences were unclassified. Basidiomycota tended to be less abundant in FL soils compared to PF and SF, whereas Ascomycota showed the opposite trend (Fig. 4a, b, Table S1). In July and September, these trends were more pronounced. Zygomycota were constantly more abundant in FL soils than in PF and SF soils throughout the sampling period (Fig. 4c). The relative abundance of Basidiomycota correlated negatively with soil pH, but positively with soil OM, whereas the relative abundances of Ascomycota responded to these soil variables in an opposite way (Table S1). The relative abundance of Zygomycota correlated positively with atrazine. The relative abundance of Glomeromycota correlated positively with P.

To determine factors that may affect fungal trophic guild composition, we classified OTUs into trophic mode and analyzed the 10 most abundant fungal functional guilds (Fig. 4f-o). ECM fungi dominated in the two forest (PF and SF) soils (Fig. 4f). The relative abundance of these fungi in PF soils (50.1±3.9%; mean ± SE) was greater than in SF soils (36.2 ± 3.2%). SAP, arbuscular mycorrhizal (AM), plant pathogenic and root endophytic fungi were more abundant in FL soils than in the two forest soils (Fig. 4, Table S1). Lichenized fungi were detected but were rare in PF and virtually absent in SF and FL. Interestingly, SF soils hosted the lowest abundance of plant pathogenic fungi, but the highest ECM and SAP fungi among the three land-use types. In addition, except for ECM fungi, sampling time did not affect the abundances of fungi. ECM fungi were most abundant in May, less in September and least in July in the forest soils (Fig. 4, Table S1). The relative abundances of two fungal functional guilds correlated with soil pH (both positive), four groups with soil OM (2 positive, 2 negative), one group with soil total N (negative), one group with soil total P (positive), one group with soil K (negative) and two groups with atrazine (both positive) (Table S1).

Responses of fungal OTUs and genera to land-use type and sampling time

To determine land-use type and seasonal effects on fungal OTUs, we conducted indicator species analysis for the 200 most abundant OTUs, representing 63.9% of the sequence data. We found 26 indicator OTUs for FL (3 AM fungal OTUs, 15 SAP fungal OTUs and 8 pathogenic fungal OTUs). Only 1 indicator ECM fungal OTU was found for PF (*Amphinema* sp.), and none for SF (Table S3). Sampling time revealed no indicator OTUs. Because of the large distinction between farmland and forest, FL soils may mask differences between the two forest types. As a result, we excluded FL data from additional analyses. In these analyses, we identified five fungal indicator OTUs between the two forest types (Table 1), 3 OTUs (1 ECM, 1 SAP and 1 plant pathogen) for PF and 2 OTUs (2 SAP) for SF. In addition we identified 9 indicator OTUs across sampling time (Table 1), 5 OTUs in May (4 SAP and 1 plant pathogen), 2 OTUs in July (2 SAP) and 2 OTUs in September (1 SAP and 1 plant pathogen).

Discussion

Effects of land-use type on fungal communities

Land-use can affect soil fungal communities in several ways - indirectly through, e.g. changes in edaphic conditions or directly via host plant selection and exposure to agro-chemical additives (Emurotu and Anyanwu 2016; Fu et al. 2015; Huang et al. 2009; Hui et al. 2017a; Prescott and Grayston 2013; Schmidt et al. 2017). Here we showed that fungal population densities, based on fungal ITS copy numbers, were higher in PF and SF than in FL, indicating that reforestation resulted in the partial restoration of soil fungal populations. However in contrast to our first hypothesis and despite the qPCR-inferred population restoration, soil fungal communities were more diverse in FL than in the two forested sites (SF and PF), suggesting that reforestation has a negative influence on fungal diversity. It is surprising that, when compared to FL, the more diverse and permanent forest vegetation including trees, shrubs, ferns and herbs with potentially more diverse resource input did not result in higher soil fungal diversity. In our study, FL soils experience more frequent disturbances (e.g. tilling) than forest soils. Such disturbances fragment the existing fungal mycelial network, thus resulting in smaller and distinct genets in farmland soil (Helgason et al. 1998; Verbruggen et al. 2010). Tilling may generally benefit communities by broadening the range of environmental conditions, preventing dominance of some taxa and allowing a greater number of species to persist due to diminished competitive exclusion (Connell 1978). The richness of soil SAP fungi in this and other studies (Santalahti et al. 2016; Schmidt et al. 2017) is generally many-fold greater than that of ECM

fungi. Although we observed high farmland fungal diversity, it might not necessarily serve as a positive outcome to farming. This is because farming is culturing high abundance of parasitic fungi, e.g. pathogenic fungi, which do not promote plant growth.

Although relatively similar in richness, the community composition of total fungi and major functional guilds (ECM and SAP) in SF were still divergent from those in PF after 20 years of reforestation. These findings support our second hypothesis, and agree with Xiao et al. (2016) who showed that soil microbial communities do not fully recover 30 years after the reforestation of farmland in Shanxi China. In contrast, based on fungal sporocarp data, Oria-de-Rueda et al. (2010) showed that abandoned farmland dominantly reforested with *Pinus* spp. in arid Mediterranean systems can develop fungal communities as productive and diverse as those in natural stands. However, due to the high year-to-year variation in fungal sporocarp production (Boddy et al. 2014), molecular analyses – such as those used in the current study – likely produce more reliable results in expounding the effects of land-use conversion on fungal communities.

Community differences among land-use types are largely attributable to differences in the relative abundances of major fungal functional guilds: AM, ECM, lichenized and SAP fungi. Although there were an array of understory plants, such as deciduous shrubs (e.g. *Spiraea salicifolia)*, as well as various grass and herb taxa that can be colonized by AM fungi in our forest stands, these AM fungi were more frequent in the farmland than in the two forested sites. Three AM OTUs (*Rhizophagus*) were FL indicators, indicating that AM fungal community in farmland were distinct from forest stand. In forests, despite of many plants capable of forming AM associations, ECM plants are vastly more abundant and common than plants producing AM (Nan H., personal observations). Another fungal functional guild, the lichenized fungi, is common in coniferous forest soils (Wedin et al. 2004). Yet, in our study, twenty years of reforestation did not restore this guild in the forest soil. This is likely because of the slow colonization by lichenized fungi (Ketner-Oostra et al. 2006) and their competition between mosses and vascular plants (Motiejūnaitė et al. 2014). In addition, the relative abundance of SAP fungi was the highest in FL, even though this fungal guild was sensitive to disturbances and require abundant OM to survive (Aliasgharzad et al. 2010; Schnoor et al. 2011). In arable systems, the removal of aboveground biomass and generation of greater amounts of labile C would lead SAP communities to be diverse and abundant. In general, these results suggest that reforestation restored a large part of fungal populations, but communities were still in transition, as fungal community composition in SF was distinct from those in PF.

Reforestation aids the establishment of ECM fungi

In the secondary forest, most soil fungal communities were still in transition: for example soil ECM guilds had nearly comparable richness and diversity in PF and SF, but were compositionally distinct. Reforestation strongly facilitates the establishment of some fungal functional guilds (e.g. ECM, ericoid-mycorrhizal and ECM-SAP fungi), which were nearly absent in FL soils, suggesting a trajectory towards the successful restoration. The secondary forests were established using mainly seedlings. It is worth noting that tree seedlings often associate with ECM fungi in the nursery prior to being outplanted. However these fungi typically decrease in abundance along time and can be persistence for only a few years (Gagné et al. 2006; Jones et al. 2003), suggesting that the nursery induced fungi had minor influence on the observed fungal community in the over 20 years old secondary forests. Twenty years after reforestation, the difference in soil ECM fungal richness between primary and secondary forest was small, but corroborated the results by Hui et al. (2017b) who reported surprisingly similar soil ECM fungal richness in old natural forest stands and newly established (10-15 years old) urban parks. The relatively similar ECM diversity that we observed in the secondary and primary forests with overlapping, yet partially divergent, vegetation (N. Hui, personal observations) was likely a result of rapid ECM colonization of the young tree roots (Jones et al. 2003). Although PF stands have interacted with their fungal communities over extended periods of time, the short SF reforestation period seems not to restrict ECM colonization, suggesting ubiquitous ECM inoculum in SF. We conclude that trees, and other vegetation, recruit diverse ECM fungi within two decades after farmland reforestation, highlighting the ability of ECM fungi to reestablish after a prolonged alternate ecosystem state (FL).

Effect of land-use conversion on soil properties and soil fungi

The conversion of forest to agricultural land has led to a global average loss of 24% of soil organic carbon and 15% of total nitrogen (Yan et al. 2012). On the other hand, Cavagnaro et al. (2016) found that edaphic conditions changed slowly during a change from pasture to forest in Australia. Jangid et al. (2011) estimated that the recovery of a disturbed ecosystem to its native state can occur within a human life span. Although we observed that reforestation falls short of restoring edaphic conditions in 20 years, our results indicate positive land-use conversion impacts, e.g. decreases in soil atrazine concentrations and increases in soil OM.

Although many studies show direct and indirect land-use effects on soil fungal communities (Francini et al. 2018; Jangid et al. 2011), few have attempted to quantify the contribution of reforestation and/or

cessation of herbicide use on soil fungal communities. In our study, FL soil contained the highest amount of atrazine as a result of its continual use, followed by SF where its application had been discontinued more than twenty years ago. Atrazine applied to soil is largely biologically degradable, whereas the residuals may linger and leach to the ground water (Vonberg et al. 2014). The half-life of atrazine in top soils ranges from 4 to 6 weeks (Kruger et al. 1993; Miller et al. 1997). Thus, it is unsurprising that atrazine concentrations have declined in SF soils. However, a small amount of atrazine still remained in SF soils after two decades of cessation of atrazine use, even though taxa capable of its degradation were previously detected (Liu et al. 2016). *Schizophyllum commune*, a fungus common in rotting wood and able to degrade atrazine via the Fenton mechanism (Khromonygina et al. 2004), was enriched in SF soil based on our indicator taxa analyses. The presence of potential atrazine degraders indicate that potential for degradation remains in SF soils. Supporting our results, (Nousiainen et al. 2014) detected atrazine degrading genes in boreal forest soil in Finland even though the use of atrazine has been banned for more than twenty years.

Conclusion

Our results show that edaphic conditions and fungal communities respond to historic land-use. Introducing trees to farmland increased fungal population density, but communities remained in transition, as indicated by the distinct fungal communities in secondary forests and primary forests. It is commonly believed that soil fungal diversity is higher in forest stands than in farmlands, because of a greater diversity of plant species/functional groups capable of producing divergent substrates/resources in forests than in farmlands. However, this classic ecological "wisdom" may not always hold. Interestingly, our results showed that fungal diversity was higher in farmland soils than forest soils, possibly as a result of recurring disturbances (tilling) allowing competitive release as described by the intermediate disturbance hypothesis. We also showed that, although ectomycorrhizal fungal diversity and richness were marginally higher in primary forests than in secondary forests, the latter still hosted surprisingly diverse and abundant ectomycorrhizal fungal communities. Our results highlight the ability of fungi grown in previously strongly managed agricultural land to rapidly respond to reforestation and thus provide support for forest trees. Despite the fact that pesticide contamination and altered edaphic fingerprints of remain, ectomycorrhizal fungal diversity in secondary forests was surprisingly similar to primary forests and distinct from farmlands twenty years of reforestation. Finally, continuing studies on soil microbial communities in land-use conversion is crucial as these diverse soil microbiomes contribute to many soil processes, e.g. removal of historically applied agrochemicals.

Acknowledgments

We are grateful to Mr. Quanyou Zhang and Mr. Minghao Zhang for their help with field sampling. Prof. Changlin Hui provided information on land-use history in Wuying. Mr. Lars Paulin provided advice and services in DNA sequencing. The study was supported by Tekes (project number 2975/31/2015).

Reference

Abarenkov K, Henrik Nilsson R, Larsson KH, Alexander IJ, Eberhardt U, Erland S, Høiland K, Kjøller R, Larsson E, Pennanen T (2010) The UNITE database for molecular identification of fungi–recent updates and future perspectives. New Phytologist 186: 281-285.

Aliasgharzad N, Mårtensson L-M, Olsson PA (2010) Acidification of a sandy grassland favours bacteria and disfavours fungal saprotrophs as estimated by fatty acid profiling. Soil Biology and Biochemistry 42: 1058-1064. doi: https://doi.org/10.1016/j.soilbio.2010.02.025.

Balser T, Treseder K, Ekenler M (2005) Using lipid analysis and hyphal length to quantify AM and saprotrophic fungal abundance along a soil chronosequence. Soil Biology and Biochemistry 37: 601-604.

Boddy L, Büntgen U, Egli S, Gange AC, Heegaard E, Kirk PM, Mohammad A, Kauserud H (2014) Climate variation effects on fungal fruiting. Fungal Ecology 10: 20-33.

Brown SP, Veach AM, Rigdon-Huss AR, Grond K, Lickteig SK, Lothamer K, Oliver AK, Jumpponen A (2015) Scraping the bottom of the barrel: are rare high throughput sequences artifacts? Fungal Ecology 13: 221-225.

Cavagnaro T, Cunningham S, Fitzpatrick S (2016) Pastures to woodlands: changes in soil microbial communities and carbon following reforestation. Applied soil ecology 107: 24-32.

Chang R, Fu B, Liu G, Liu S (2011) Soil carbon sequestration potential for "Grain for Green" project in Loess Plateau, China. Environmental management 48: 1158-1172.

Chazdon RL (2008) Beyond deforestation: restoring forests and ecosystem services on degraded lands. science 320: 1458-1460.

Connell JH (1978) Diversity in tropical rain forests and coral reefs. Science 199: 1302-1310.

Edgar RC, Haas BJ, Clemente JC, Quince C, Knight R (2011) UCHIME improves sensitivity and speed of chimera detection. Bioinformatics 27: 2194-2200.

Emurotu M, Anyanwu C (2016) Effect of atrazine and butachlor on soil microflora in agricultural farm in Anyigba, Nigeria. European Journal of Experimental Biology 6: 16-20.

Fierer N (2017) Embracing the unknown: disentangling the complexities of the soil microbiome. Nature Reviews Microbiology 15: 579.

Francini G, Hui N, Jumpponen A, Kotze DJ, Romantschuk M, Allen JA, Setälä H (2018) Soil biota in boreal urban greenspace: Responses to plant type and age. Soil Biology and Biochemistry 118: 145-155. doi: https://doi.org/10.1016/j.soilbio.2017.11.019.

Fu X, Yang F, Wang J, Di Y, Dai X, Zhang X, Wang H (2015) Understory vegetation leads to changes in soil acidity and in microbial communities 27years after reforestation. Science of The Total Environment 502: 280-286. doi: http://dx.doi.org/10.1016/j.scitotenv.2014.09.018.

Harris J (2009) Soil microbial communities and restoration ecology: facilitators or followers? Science 325: 573-574.

Hatta R, Ito K, Hosaki Y, Tanaka T, Tanaka A, Yamamoto M, Akimitsu K, Tsuge T (2002) A conditionally dispensable chromosome controls host-specific pathogenicity in the fungal plant pathogen Alternaria alternata. Genetics 161: 59-70.

Helgason T, Daniell T, Husband R, Fitter A, Young J (1998) Ploughing up the wood-wide web? Nature 394: 431-431.
Higgins KL, Arnold AE, Miadlikowska J, Sarvate SD, Lutzoni F (2007) Phylogenetic relationships, host affinity, and geographic structure of boreal and arctic endophytes from three major plant lineages. Molecular Phylogenetics and Evolution 42: 543-555. doi: https://doi.org/10.1016/j.ympev.2006.07.012.
Horner-Devine MC, Carney KM, Bohannan BJ (2004) An ecological perspective on bacterial biodiversity. Proceedings of the Royal Society of London B: Biological Sciences 271: 113-122.
Huang H, Zhang S, Wu N, Luo L, Christie P (2009) Influence of Glomus etunicatum/Zea mays mycorrhiza on atrazine degradation, soil phosphatase and dehydrogenase activities, and soil microbial community structure. Soil Biology and Biochemistry 41: 726-734.
Hui N, Jumpponen A, Francini G, Kotze DJ, Liu X, Romantschuk M, Strommer R, Setala H (2017a) Soil microbial communities are shaped by vegetation type and park age in cities under cold climate. Environ Microbiol 19: 1281-1295. doi: 10.1111/1462-2920.13660.
Hui N, Liu X, Kotze DJ, Jumpponen A, Francini G, Setälä H (2017b) Ectomycorrhizal fungal communities in urban parks are similar to those in natural forests but shaped by vegetation and park age. Applied and Environmental Microbiology: e01797-01717.
Jangid K, Williams MA, Franzluebbers AJ, Schmidt TM, Coleman DC, Whitman WB (2011) Land-use history has a stronger impact on soil microbial community composition than aboveground vegetation and soil properties. Soil Biology and Biochemistry 43: 2184-2193. doi: http://dx.doi.org/10.1016/j.soilbio.2011.06.022.
Jones MD, Durall DM, Cairney JW (2003) Ectomycorrhizal fungal communities in young forest stands regenerating after clearcut logging. New Phytologist 157: 399-422.
Jonsson LM, Nilsson MC, Wardle DA, Zackrisson O (2001) Context dependent effects of ectomycorrhizal species richness on tree seedling productivity. Oikos 93: 353-364.
Kennedy N, Brodie E, Connolly J, Clipson N (2006) Seasonal influences on fungal community structure in unimproved and improved upland grassland soils. Canadian journal of microbiology 52: 689-694.
Ketner-Oostra R, Van der Peijl M, Sýkora K (2006) Restoration of lichen diversity in grass-dominated vegetation of coastal dunes after wildfire. Journal of vegetation science 17: 147-156.
Khromonygina V, Saltykova A, Vasil'chenko L, Kozlov YP, Rabinovich M (2004) Degradation of the herbicide atrazine by the soil mycelial fungus INBI 2-26 (–), a producer of cellobiose dehydrogenase. Applied Biochemistry and Microbiology 40: 285-290.
Kruger EL, Somasundaram L, Coats JR, Kanwar RS (1993) Persistence and degradation of [14C] atrazine and [14C] deisopropylatrazine as affected by soil depth and moisture conditions. Environmental Toxicology and Chemistry 12: 1959-1967.
Laliberté E, Lambers H, Burgess TI, Wright SJ (2015) Phosphorus limitation, soil-borne pathogens and the coexistence of plant species in hyperdiverse forests and shrublands. New Phytologist 206: 507-521.
Lei D, Shangguan Z-P, Rui L (2012) Effects of the grain-for-green program on soil erosion in China. International Journal of Sediment Research 27: 120-127.
Lindahl BO, Taylor AF, Finlay RD (2002) Defining nutritional constraints on carbon cycling in boreal forests–towards a lessphytocentric'perspective. Plant and soil 242: 123-135.
Liu X, Hui C, Bi L, Romantschuk M, Kontro M, Strömmer R, Hui N (2016) Bacterial community structure in atrazine treated reforested farmland in Wuying China. Applied Soil Ecology 98: 39-46.
Luo Z-B, Wu C, Zhang C, Li H, Lipka U, Polle A (2014) The role of ectomycorrhizas in heavy metal stress tolerance of host plants. Environmental and Experimental Botany 108: 47-62.

Mackey RL, Currie DJ (2001) The diversity–disturbance relationship: is it generally strong and peaked? Ecology 82: 3479-3492. doi: 10.1890/0012-9658(2001)082[3479:TDDRII]2.0.CO;2.
Miller J, Wollum A, Weber J (1997) Degradation of carbon-14-atrazine and carbon-14-metolachlor in soil from four depths. Journal of environmental quality 26: 633-638.
Molina R, Horton TR (2015) Mycorrhiza Specificity: Its Role in the Development and Function of Common Mycelial Networks. In: TR Horton (ed) Mycorrhizal Networks. Springer Netherlands, Dordrecht.
Motiejūnaitė J, Adamonytė G, Iršėnaitė R, Juzėnas S, Kasparavičius J, Kutorga E, Markovskaja S (2014) Early fungal community succession following crown fire in Pinus mugo stands and surface fire in Pinus sylvestris stands. European journal of forest research 133: 745-756.
Nguyen NH, Song Z, Bates ST, Branco S, Tedersoo L, Menke J, Schilling JS, Kennedy PG (2016) FUNGuild: an open annotation tool for parsing fungal community datasets by ecological guild. Fungal Ecology 20: 241-248.
Nousiainen AO, Björklöf K, Sagarkar S, Mukherjee S, Purohit HJ, Kapley A, Jørgensen KS (2014) Atrazine degradation in boreal nonagricultural subsoil and tropical agricultural soil. Journal of soils and sediments 14: 1179-1188.
Oliver AK, Brown SP, Callaham MA, Jumpponen A (2015) Polymerase matters: non-proofreading enzymes inflate fungal community richness estimates by up to 15%. Fungal Ecology 15: 86-89.
Oria-de-Rueda JA, Hernández-Rodríguez M, Martín-Pinto P, Pando V, Olaizola J (2010) Could artificial reforestations provide as much production and diversity of fungal species as natural forest stands in marginal Mediterranean areas? Forest Ecology and Management 260: 171-180. doi: http://dx.doi.org/10.1016/j.foreco.2010.04.009.
Prescott CE, Grayston SJ (2013) Tree species influence on microbial communities in litter and soil: current knowledge and research needs. Forest Ecology and Management 309: 19-27.
Santalahti M, Sun H, Jumpponen A, Pennanen T, Heinonsalo J (2016) Vertical and seasonal dynamics of fungal communities in boreal Scots pine forest soil. FEMS microbiology ecology 92: fiw170.
Schadt CW, Martin AP, Lipson DA, Schmidt SK (2003) Seasonal dynamics of previously unknown fungal lineages in tundra soils. Science 301: 1359-1361.
Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB, Lesniewski RA, Oakley BB, Parks DH, Robinson CJ (2009) Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Applied and environmental microbiology 75: 7537-7541.
Schmidt DJE, Pouyat R, Szlavecz K, Setälä H, Kotze DJ, Yesilonis I, Cilliers S, Hornung E, Dombos M, Yarwood SA (2017) Urbanization erodes ectomycorrhizal fungal diversity and may cause microbial communities to converge. Nature Ecology & Evolution 1: 0123.
Schnoor TK, Mårtensson L-M, Olsson PA (2011) Soil disturbance alters plant community composition and decreases mycorrhizal carbon allocation in a sandy grassland. Oecologia 167: 809.
Setälä HM, Francini G, Allen JA, Hui N, Jumpponen A, Kotze DJ (2016) Vegetation Type and Age Drive Changes in Soil Properties, Nitrogen, and Carbon Sequestration in Urban Parks under Cold Climate. Frontiers in Ecology and Evolution 4: 93.
Song X, Peng C, Zhou G, Jiang H, Wang W (2014) Chinese Grain for Green Program led to highly increased soil organic carbon levels: A meta-analysis. Scientific reports 4: 4460.
Strickland MS, Callaham Jr MA, Gardiner ES, Stanturf JA, Leff JW, Fierer N, Bradford MA (2017) Response of soil microbial community composition and function to a bottomland forest restoration intensity gradient. Applied Soil Ecology 119: 317-326.

Tedersoo L, Jairus T, Horton BM, Abarenkov K, Suvi T, Saar I, Kõljalg U (2008) Strong host preference of ectomycorrhizal fungi in a Tasmanian wet sclerophyll forest as revealed by DNA barcoding and taxon-specific primers. New Phytologist 180: 479-490.
Tedersoo L, Nilsson RH, Abarenkov K, Jairus T, Sadam A, Saar I, Bahram M, Bechem E, Chuyong G, Kõljalg U (2010) 454 Pyrosequencing and Sanger sequencing of tropical mycorrhizal fungi provide similar results but reveal substantial methodological biases. New Phytologist 188: 291-301.
Twieg BD, Durall DM, Simard SW (2007) Ectomycorrhizal fungal succession in mixed temperate forests. New Phytologist 176: 437-447.
Wedin M, Döring H, Gilenstam G (2004) Saprotrophy and lichenization as options for the same fungal species on different substrata: environmental plasticity and fungal lifestyles in the Stictis–Conotrema complex. New Phytologist 164: 459-465.
Velmala SM, Rajala T, Heinonsalo J, Taylor AF, Pennanen T (2014) Profiling functions of ectomycorrhizal diversity and root structuring in seedlings of Norway spruce (Picea abies) with fast-and slow-growing phenotypes. New Phytologist 201: 610-622.
Verbruggen E, Röling WF, Gamper HA, Kowalchuk GA, Verhoef HA, van der Heijden MG (2010) Positive effects of organic farming on below-ground mutualists: large-scale comparison of mycorrhizal fungal communities in agricultural soils. New Phytologist 186: 968-979.
Vonberg D, Hofmann D, Vanderborght J, Lelickens A, Köppchen S, Pütz T, Burauel P, Vereecken H (2014) Atrazine soil core residue analysis from an agricultural field 21 years after its ban. Journal of environmental quality 43: 1450-1459.
Xiao L, Liu Gb, Zhang Jy, Xue S (2016) Long-term effects of vegetational restoration on soil microbial communities on the Loess Plateau of China. Restoration Ecology 24: 794-804.
Yan Y, Tian J, Fan M, Zhang F, Li X, Christie P, Chen H, Lee J, Kuzyakov Y, Six J (2012) Soil organic carbon and total nitrogen in intensively managed arable soils. Agriculture, Ecosystems & Environment 150: 102-110. doi: http://dx.doi.org/10.1016/j.agee.2012.01.024.
Zak JC (1992) Response of soil fungal communities to disturbance. The fungal community: its organization and role in the ecosystem Dekker, New York: 403-425.

Figure and Table Captions

Fig. 1. Soil edaphic conditions (mean ± SE) across land-use type and sampling time. PF represents primary forest, SF secondary forest, and FL farmland (two-way ANOVA results are presented in Table S1).

Fig. 2. Diversity indices of total (upper panels), ectomycorrhizal (ECM; middle panels) and saprotrophic (SAP; lower panels) fungal communities across land-use type and sampling time (GLM results). Note that diversity indices are not comparable between total, ECM and SAP fungi, because the datasets are rarefied differently.

Fig. 3. NMDS plots of total fungal communities (a, d), ECM fungal communities (b, e) and SAP fungal communities (c, f). Statistically significant ($p < 0.05$) soil variables are shown. These analyses

suggest divergent fungal communities ($p < 0.05$) across land-use types (all three communities) and sampling time (total and ECM, but not SAP communities) using the *envfit* analyses in R.

Fig. 4. Relative abundances of fungal phyla (a–e) and functional guilds (f-o) across land-use types and sampling time (GLM results). Statistical differences between land-use types and sampling time are shown in Table S1.

Table 1. Fungal indicator OTUs by land-use type (without farmland) and sampling time.

Supplementary materials

Fig. S1. Fungal population density (qPCR estimated ITS2 copy number per gram of dry soil) across land-use type and sampling time. PF represents primary forest, SF secondary forest and FL farmland.

Table S1. Two-way ANOVA analysis comparing edaphic conditions among land-use type and sampling season. Superscripts in the last three columns indicate where significant differences occur using the Tukey's post hoc test.

Table S2. GLM results of fungal diversity indices, relative abundances of taxa and functional groups by land-use type and sampling season.

Table S3. Fungal indicator OTUs across land-use types (primary forest, second forest and farmland).

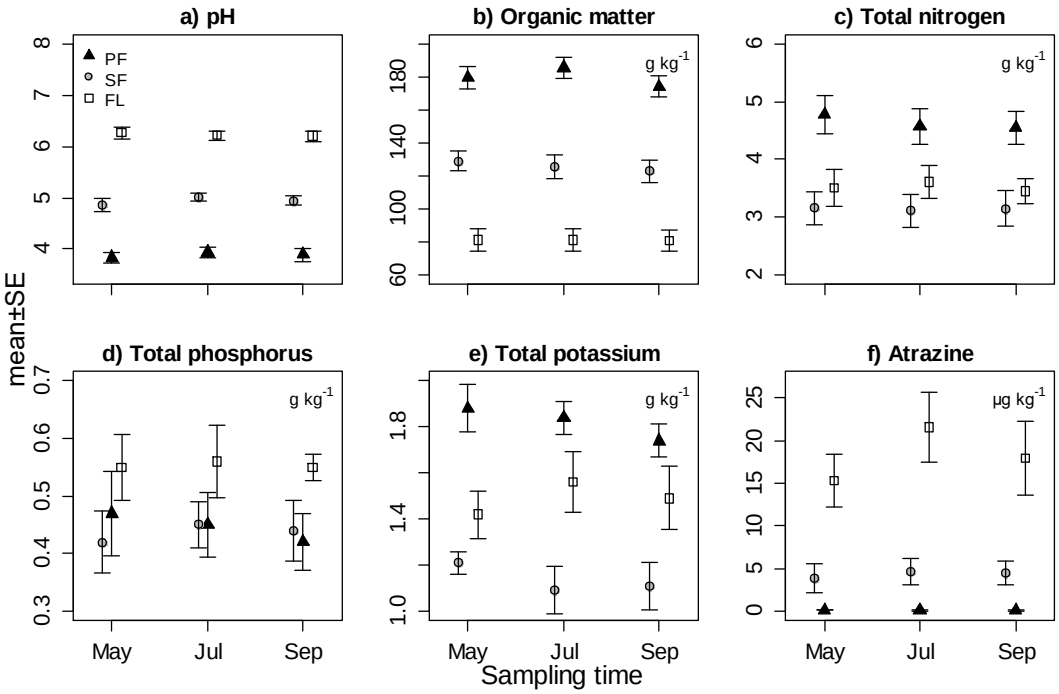

Fig. 1. Soil edaphic conditions (mean ± SE) across land-use type and sampling time. PF represents primary forest, SF secondary forest, and FL farmland (two-way ANOVA results are presented in Table S1).

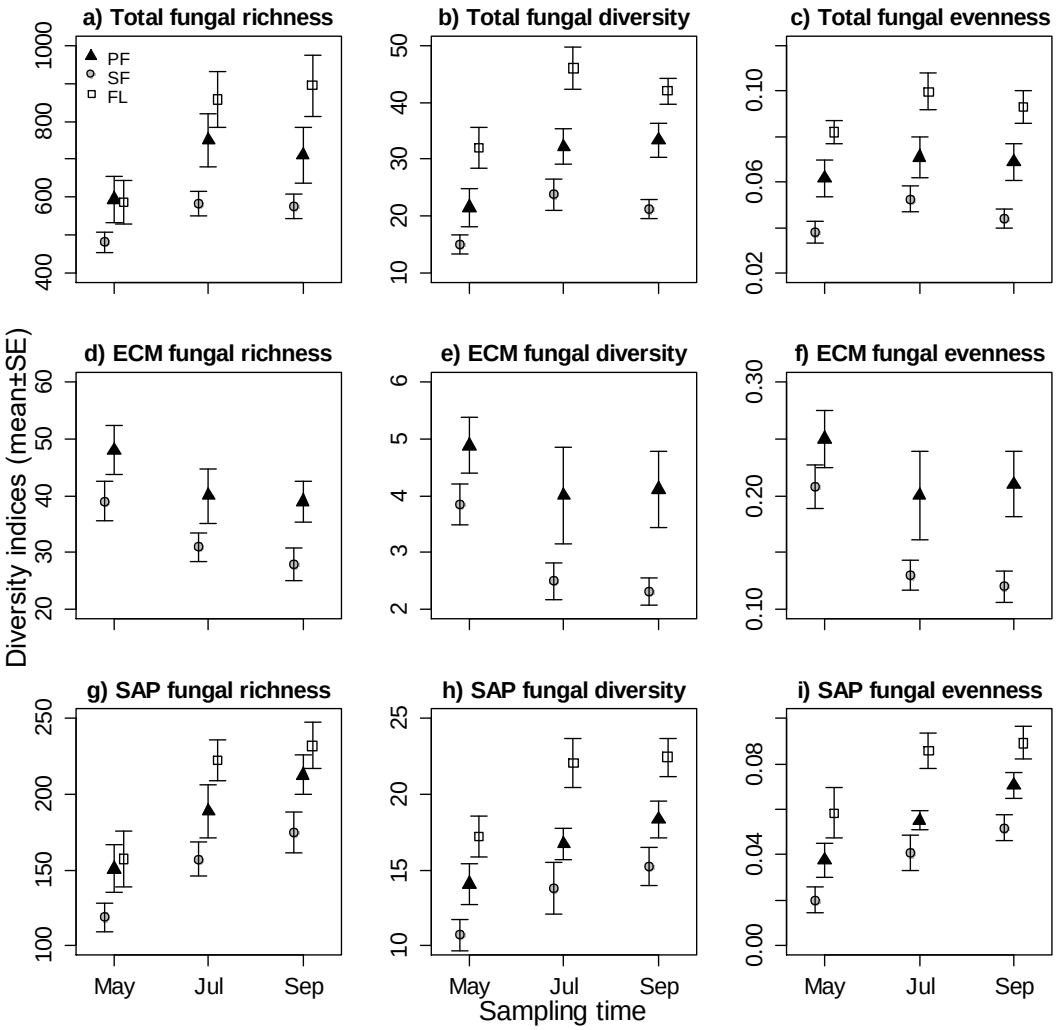

Fig. 2. Diversity indices of total (upper panels), ectomycorrhizal (ECM; middle panels) and saprotrophic (SAP; lower panels) fungal communities across land-use type and sampling time (GLM results). Note that diversity indices are not comparable between total, ECM and SAP fungi, because the datasets are rarefied differently.

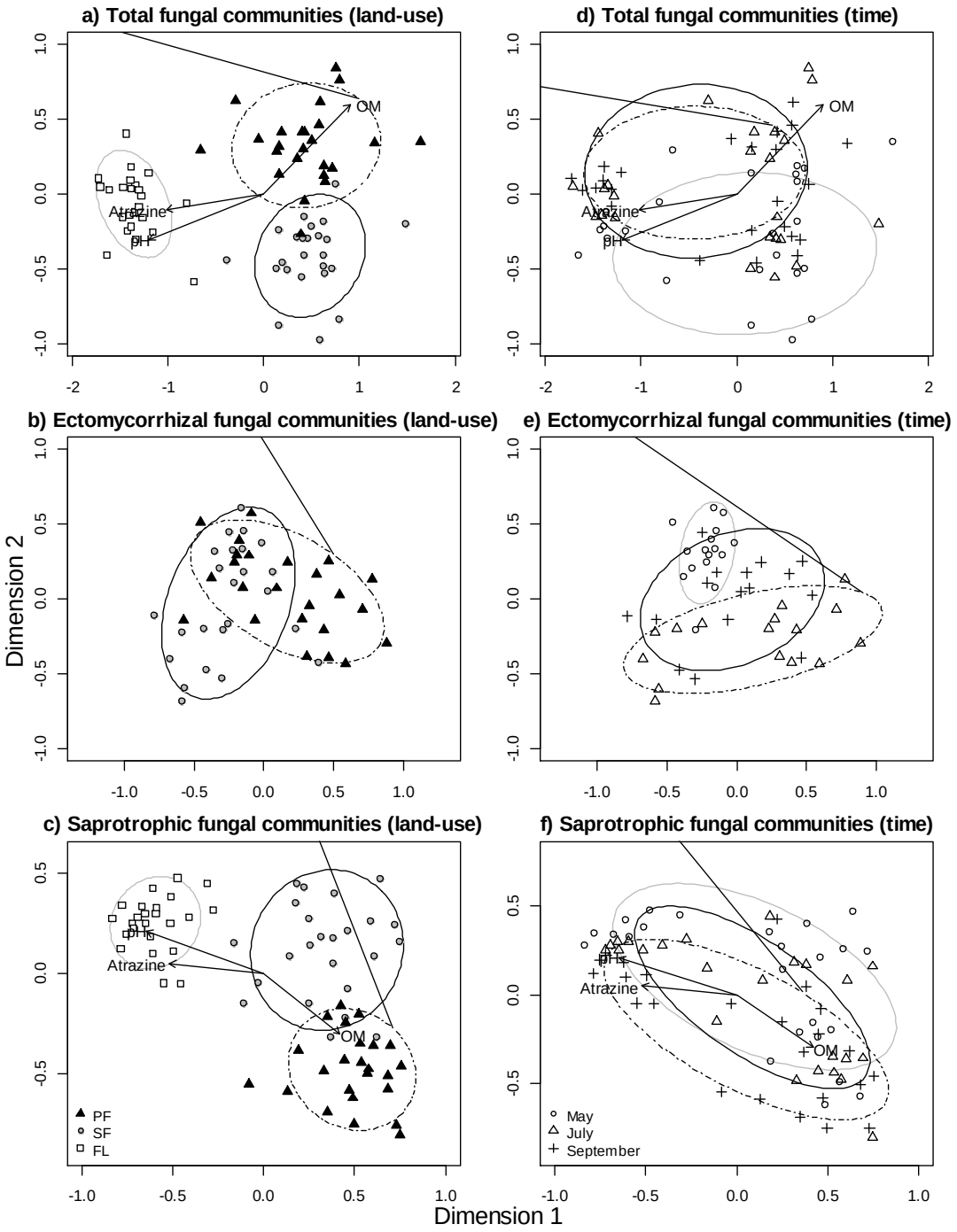

Fig. 3. NMDS plots of total fungal communities (a, d), ECM fungal communities (b, e) and SAP fungal communities (c, f). Statistically significant ($p < 0.05$) soil variables are shown. These analyses suggest divergent fungal communities ($p < 0.05$) across land-use types (all three communities) and sampling time (total and ECM, but not SAP communities) using the *envfit* analyses in R.

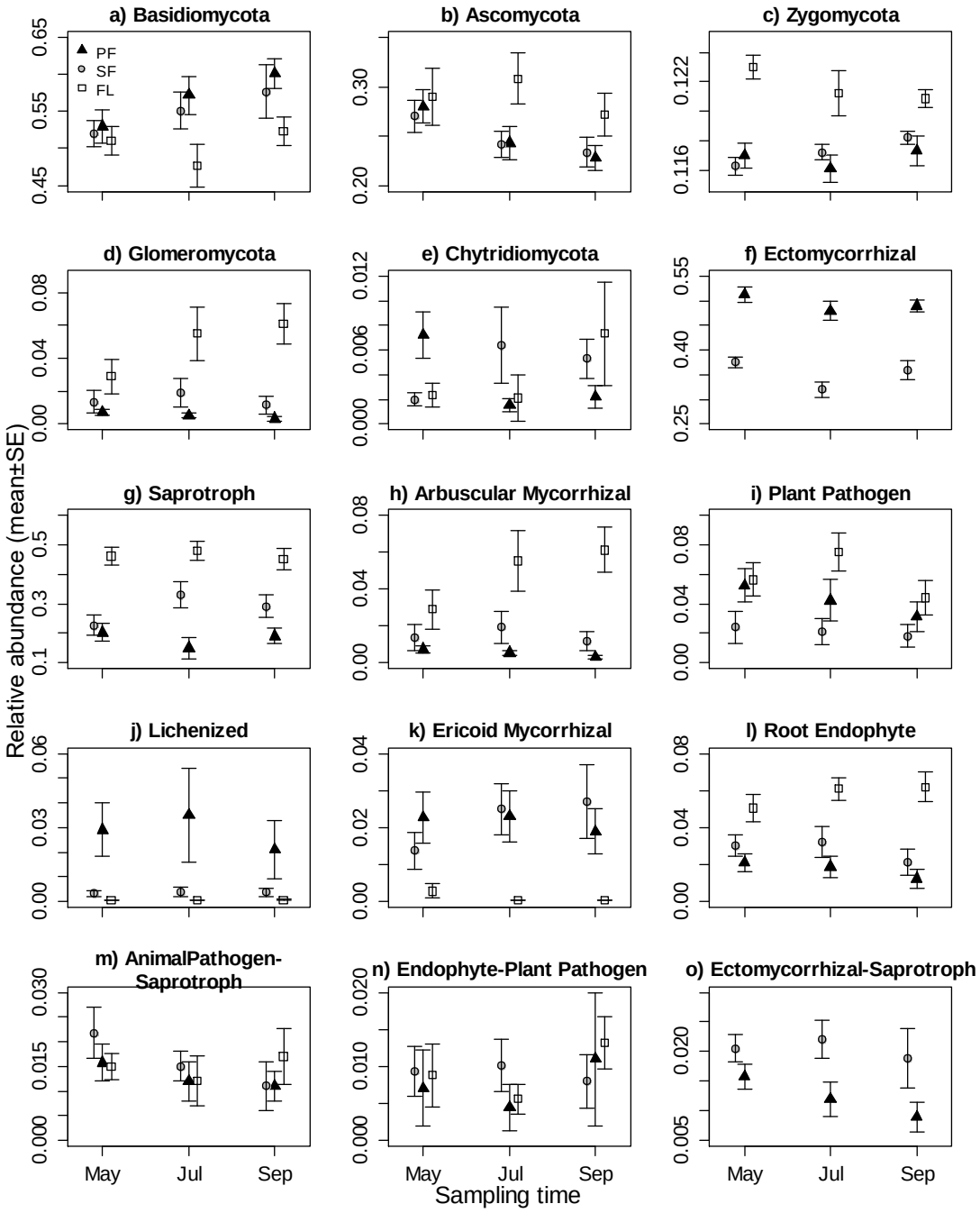

Fig. 4. Relative abundances of fungal phyla (a–e) and functional guilds (f-o) across land-use types and sampling time (GLM results). Statistical differences between land-use types and sampling time are shown in Table S1.

Table 1. Fungal indicator OTUs by land-use type (without farmland) and sampling time.

	OTU	stat	p-value	Classification	Trophic mode
Land-use type					
Primary forest	Otu0033	0.780	0.021	*Trametes versicolor*	SAP
	Otu0052	0.741	0.016	*Sepedonium laevigatum*	PAT
	Otu0224	0.662	0.021	*Amphinema sp.*	ECM
Secondary forest	Otu0213	0.583	0.001	*Schizophyllum commune*	SAP
	Otu0331	0.711	0.001	*Schizopora paradoxa*	SAP
Sampling time					
May	Otu0625	0.866	0.001	*Bjerkandera fumosa*	SAP
	Otu0130	0.751	0.006	*Trechispora sp.*	SAP
	Otu0114	0.624	0.025	*Phlebia sp.*	SAP
	Otu0736	0.584	0.004	*Phlebia sp.*	SAP
	Otu0517	0.629	0.001	*Elaphocordyceps sp*	PAT
July	Otu0048	0.650	0.033	*Eutypa sp.*	SAP
	Otu0092	0.769	0.001	*Hypocrea sp.*	SAP
September	Otu0433	0.872	0.001	*Lecanicillium fusisporum*	SAP
	Otu0271	0.543	0.001	*Lophodermium piceae*	PAT

ECM represents ectomycorrhizal fungi; SAP saprotrophic fungi; END root endophyte fungi; PAT pathotrophic fungi.

Supplementary materials

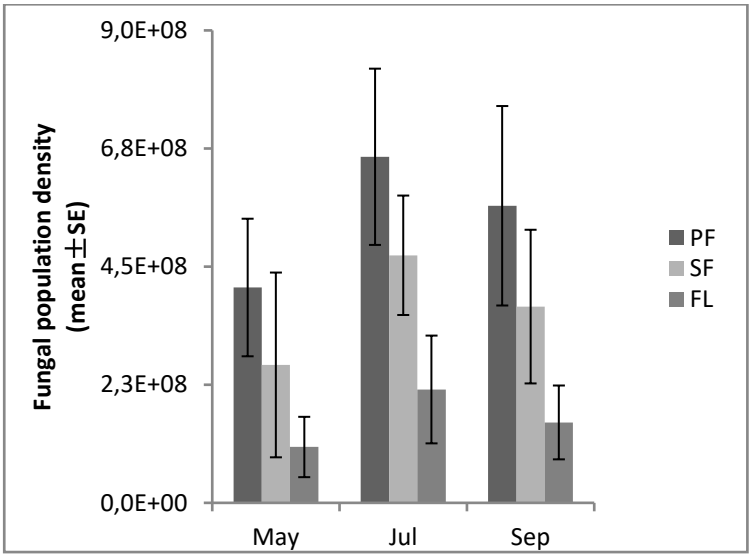

Fig. S1 Fungal population density (qPCR estimated ITS2 copy number per gram of dry soil) across land-use type and sampling time. PF represents primary forest, SF secondary forest and FL farmland.

Table S1. Two-way ANOVA analysis comparing edaphic conditions among land-use type and sampling season. Superscripts in the last three columns indicate where significant differences occur using the Tukey's post hoc test.

Edaphic conditions	**Factors**	df	n	F	p	**Tukey's *post hoc* test**		
pH*	Land-use type	2	72	15.636	<0.001	PF[a]	SF[b]	FL[c]
	Sampling time	2	72	0.653	0.564			
Organic matter*	Land-use type	2	72	9.36	<0.001	PF[a]	SF[b]	FL[c]
	Sampling time	2	72	1.179	0.399			
Total nitrogen	Land-use type	2	72	6.77	0.005	PF[a]	SF[b]	FL[b]
	Sampling time	2	72	2.237	0.228			
Total phosphorus*	Land-use type	2	72	4.726	0.013	PF[a]	SF[a]	FL[b]
	Sampling time	2	72	2.973	0.193			
Total potassium*	Land-use type	2	72	5.011	0.009	PF[a]	SF[b]	FL[c]
	Sampling time	2	72	3.295	0.106			
Atrazine#	Land-use type	2	72	40.263	<0.001	PF[a]	SF[b]	FL[c]
	Sampling time	2	72	3.221	0.153			

To approximate normality, the response variables were log transformed (*) or square root transformed (#). PF represents primary forests; SF secondary forests; FL farmland.

Table S2. GLM results of fungal diversity indices, relative abundances of taxa and functional groups by land-use type and sampling season.

													Interaction			
		Intercept	**PF**	**FL**	**July**	**Sep**	**pH**	**Organic matter**	**Nitrogen content**	**Phosphorus content**	**Potassium content**	**Atrazine**	**PF x Jul**	**FL x Jul**	**PF x Sep**	**FL x Sep**
Diversity indices																
Total fungal richness	Coefficient	550.750	138.167	233.766	175.833	172.833	238.421						-70.638	40.221	45.269	53.880
	SE	30.876	68.503	71.667	58.988	62.784	32.025						19.342	13.663	15.275	11.856
	p-value	0.000	**0.008**	**<0.001**	**0.015**	**0.009**	**0.006**						**0.039**	0.436	0.275	0.863
Total fungal diversity*	Coefficient	1.331	0.161	0.300	0.173	0.149			-0.954			0.426				
	SE	0.303	0.201	0.508	0.207	0.063			0.245			0.210				
	p-value	0.000	**0.017**	**<0.001**	**0.025**	0.135			**0.002**			**0.009**				
Total fungal evenness*	Coefficient	-1.279	0.176	0.310	0.090	0.055			-0.725			0.533				
	SE	0.321	0.075	0.170	0.122	0.008			0.139			0.174				
	p-value	0.000	0.011	**<0.001**	**0.043**	0.095			**0.001**			**0.016**				
ECM fungal richness	Coefficient	38.083	9.667		-7.126	-9.158										
	SE	2.950	4.187		3.640	3.225										
	p-value	0.000	**0.010**		**0.036**	**0.028**										
ECM fungal diversity	Coefficient	3.626	1.445		-1.108	-1.148		-2.533								
	SE	0.303	0.673		0.585	0.460		1.311								
	p-value	0.000	**0.007**		**0.029**	**0.011**		**0.026**								
ECM fungal evenness	Coefficient	0.191	0.068		-0.064	-0.064		-0.110								
	SE	0.015	0.031		0.026	0.021		0.036								
	p-value	0.000	**0.010**		**0.013**	**0.008**		**0.015**								
SAP fungal richness	Coefficient	146.187	33.720	53.541	47.080	64.101	0.374						35.428	-10.850	18.771	-16.311
	SE	11.171	15.505	15.543	14.029	13.895	0.065						7.550	3.212	5.215	4.223
	p-value	0.000	**0.035**	**0.006**	**0.018**	**0.006**	**0.019**						**0.022**	0.630	0.153	0.198
SAP fungal diversity*	Coefficient	1.134	0.092	0.191	0.097	0.125	0.478					0.335				
	SE	0.312	0.032	0.063	0.021	0.008	0.133					0.082				
	p-value	0.000	**0.042**	**0.015**	0.088	**0.041**	**0.004**					**0.019**				
SAP fungal evenness*	Coefficient	-1.419	0.160	0.315	0.195	0.261	0.021					0.223				

	SE	0.464	0.058	0.047	0.066	0.062	0.005				0.139				
	p-value	0.000	**0.037**	**0.009**	0.062	**0.025**	**0.007**				**0.006**				
Relative abundance of fungal phyla															
Basidiomycota	Coefficient	0.535	0.018	-0.046	0.013	0.047	-0.237	0.159							
	SE	0.027	0.023	0.022	0.026	0.025	0.087	0.052							
	p-value	0.000	0.275	**0.008**	0.145	0.098	**0.015**	**0.009**							
Ascomycota*	Coefficient	-0.578	0.003	0.067	-0.025	-0.059	0.139	-0.235							
	SE	0.015	0.016	0.026	0.019	0.016	0.066	0.034							
	p-value	0.000	0.684	**0.033**	0.845	0.769	**0.028**	**0.014**							
Zygomycota	Coefficient	0.118	-0.002	0.016	-0.001	0.000					0.052	0.053	-0.021	-0.061	-0.043
	SE	0.001	0.001	0.001	0.001	0.001					0.013	0.012	0.007	0.015	0.011
	p-value	0.000	0.234	**0.003**	0.564	0.248					**0.029**	**0.043**	0.299	**0.036**	0.651
Chytridiomycota	Coefficient	0.004	-0.001	-0.001	-0.001	0.001									
	SE	0.002	0.001	0.002	0.002	0.002									
	p-value	0.000	0.254	0.436	0.772	0.663									
Glomeromycota*	Coefficient	-1.396	-0.419	1.228	0.135	0.156				0.265					
	SE	0.007	0.001	0.040	0.019	0.015				0.112					
	p-value	0.000	0.884	**<0.001**	**0.021**	**0.013**				**0.013**					
Relative abundance of fungal functional groups															
Ectomycorrhizal*	Coefficient	-0.488	0.148	-1.854	-0.043	-0.020		0.312	-0.312						
	SE	0.016	0.016	0.003	0.014	0.011		0.005	0.008						
	p-value	0.000	**0.003**	**<0.001**	**0.029**	0.228		**0.021**	**0.003**						
Saprotroph*	Coefficient	-0.538	-0.195	0.215	0.033	0.020		-0.512			0.412				
	SE	0.039	0.031	0.033	0.038	0.034		0.034			0.034				
	p-value	0.000	**0.032**	**0.011**	0.237	0.358		**0.011**			**0.016**				
Arbuscular Mycorrhizal*	Coefficient	-1.396	-0.419	1.228	0.135	0.156				0.265					
	SE	0.007	0.001	0.040	0.019	0.015				0.112					
	p-value	0.000	0.884	**<0.001**	**0.021**	**0.013**				**0.013**					

Plant Pathogen*	Coefficient	-1.486	0.298	0.443	0.018	-0.154									
	SE	0.009	0.012	0.012	0.012	0.010									
	p-value	0.000	0.086	0.001	0.337	0.632									
Lichenized	Coefficient	0.004	0.027	-0.002	0.011	0.006	0.021					0.011	-0.015	0.028	0.016
	SE	0.013	0.000	0.001	0.010	0.011	0.004					0.003	0.006	0.009	0.004
	p-value	0.000	**<0.001**	0.229	0.633	0.350	**0.015**					0.651	0.226	**0.015**	0.432
Ericoid Mycorrhizal*	Coefficient	-1.756	-0.007	-1.260	0.089	0.071		1.126							
	SE	0.007	0.007	0.001	0.005	0.005		0.008							
	p-value	0.000	0.276	**<0.001**	0.317	0.535		**0.041**							
Root Endophyte*	Coefficient	-1.511	-0.204	0.319	0.042	-0.027									
	SE	0.007	0.005	0.007	0.007	0.007									
	p-value	0.000	0.101	**0.004**	0.343	0.221									
Animal Pathogen-Saprotroph	Coefficient	0.017	0.018	-0.046	-0.004	-0.005	0.011			-0.847		-0.008	0.017	-0.006	0.004
	SE	0.004	0.004	0.004	0.004	0.005	0.001			0.024		0.002	0.003	0.001	0.001
	p-value	0.000	0.355	0.622	0.245	0.169	**0.023**			**0.005**		0.254	**0.015**	0.433	0.362
Endophyte-Plant Pathogen*	Coefficient	-2.056	-0.088	0.002	-0.099	0.106									
	SE	0.004	0.006	0.003	0.003	0.005									
	p-value	0.000	0.237	0.566	0.741	0.855									
Ectomycorrhizal-Saprotroph	Coefficient	0.016	-0.102	0.181	-0.001	-0.003		-0.011			0.014				
	SE	0.004	0.003	0.000	0.002	0.003		0.001			0.005				
	p-value	0.000	**0.013**	**<0.001**	0.122	0.083		**0.037**			**0.016**				

Intercept refer to SF and May. To approximate normality, the response variables are log transformed (*).

Table S3. Fungal indicator OTUs across land-use types (primary forest, second forest and farmland).

Land-use type	OTU	stat	p-value	Classification	Trophic mode
Primary forest	Otu000224	0.561	0.011	*Amphinema sp.*	ECM
Secondary forest	None				
Farmland	Otu000102	0.997	0.001	*Rhizophagus albidum*	AM
	Otu000059	0.999	0.001	*Rhizophagus mosseae*	AM
	Otu000142	0.999	0.001	*Rhizophagus mosseae*	AM
	Otu000162	0.934	0.001	*Acremonium rutilum*	PAT
	Otu000168	0.974	0.001	*Acremonium rutilum*	PAT
	Otu000148	0.903	0.001	*Microdochium bolleyi*	PAT
	Otu000176	0.963	0.001	*Nectria ramulariae*	PAT
	Otu000086	0.999	0.001	*Acremonium strictum*	PAT
	Otu000018	0.999	0.001	*Curvularia lunata*	PAT
	Otu000035	0.887	0.001	*Curvularia pallescens*	PAT
	Otu000071	0.998	0.001	*Marasmiellus sp.*	PAT
	Otu000112	0.999	0.001	*Mortierella humilis*	SAP
	Otu000072	0.946	0.001	*Mortierella sp*	SAP
	Otu000075	0.993	0.001	*Mortierella sp*	SAP
	Otu000087	0.935	0.001	*Mortierella sp*	SAP
	Otu000139	0.989	0.001	*Podospora glutinans*	SAP
	Otu000156	0.978	0.001	*Pseudaleuria sp*	SAP
	Otu000178	0.922	0.001	*Pseudaleuria sp*	SAP
	Otu000184	0.706	0.001	*Pseudaleuria sp*	SAP
	Otu000141	0.998	0.001	*Pseudeurotium hygrophilum*	SAP
	Otu000066	0.996	0.001	*Schizothecium carpinicola*	SAP
	Otu000100	0.999	0.001	*Schizothecium carpinicola*	SAP
	Otu000120	0.999	0.001	*Schizothecium carpinicola*	SAP
	Otu000151	0.997	0.001	*Schizothecium carpinicola*	SAP
	Otu000137	0.888	0.001	*Tetracladium sp*	SAP
	Otu000145	0.916	0.001	*Tetracladium sp*	SAP

ECM represents ectomycorrhizal fungi, SAP saprotrophic fungi, END root endophyte fungi, PAT pathotrophic fungi, AM arbuscular mycorrhizal fungi and ERM ericoid mycorrhizal fungi.