Root endophytic fungi impact host plant biomass and respond to plant composition at varying spatio-temporal scales

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Title: Root endophytic fungi impact host plant biomass and respond to plant composition at varying spatio-temporal scales

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Abstract

Plant roots are inhabited by a diversity of microorganisms known to be key drivers of plant growth and health. Although the rules governing root microbiota assembly have been investigated and the importance of abiotic determinants highlighted, the consequences of the biotic context of the plant community have often been overlooked. We tested the hypothesis that the abundance of species in a given plant neighborhood could leave a fingerprint on its root-endophytic fungal community, ultimately impacting its biomass. Outdoor experimental mesocosms, comprising a range of floristic compositions and spatially mapped plant distributions, were monitored for 2y. *Medicago truncatula* was used as trap-plant and grown under standardized conditions on soil samples collected in the mesocosms. The root-endophytic fungal community of *M. truncatula* was described by amplicon mass sequencing and *M. truncatula* performance was also assessed. The richness and evenness of *M. truncatula*'s root-endophytic fungal community were explained by the small-scale plant neighborhood of the soil samples. For instance, the occurrence of *Brachypodium pinnatum* in the neighborhood induced higher and lower richness of Sordariomycetes and Glomeromycetes, respectively, whereas *Holcus mollis* decreased the OTU evenness of the entire mycobiota. These changes in fungal clade OTU richness and evenness were related to modifications in the biomass of *M. truncatula*. These results indicate that a given plant endophytic fungal community is determined in part by the neighboring plants. Considering that changes in endophytic fungal community are correlated with the plant biomass, this suggests that plant-plant interactions (i.e. competition, facilitation) impacting plant biomass can be mediated by endophytic fungal community changes.

Keywords

Plant-fungi interactions; *Medicago truncatula*; Fungal communities; Fungal root endophytes; Trap plant experiment; 18S rRNA; Community indices.

Introduction

Soil microorganisms are key-drivers of aboveground ecosystem functioning which determines vegetation composition and dynamics (e.g. van der Heijden et al. 1998, Wagg et al. 2014, Fester et al. 2014) through nutrient cycling and symbioses (e.g. Bardgett and van der Putten 2014). The symbioses between plants and soil microorganisms are widespread in nature and are often important for plant development and growth (Bulgarelli et al. 2013, Vandenkoornhuyse et al. 2015, Hacquard et al. 2016). Among symbiotic microorganisms, mutualistic symbionts and especially arbuscular mycorrhizal (AM) fungi have received much attention since they are arguably the world's most widely distributed root symbionts and among the most important terrestrial symbionts 'that help feed the world' (Marx 2004). However, symbiotic fungi are not limited to mycorrhizal nor mutualistic fungi and a recent study detected over 3000 OTUs in total in the soil, episphere and endosphere of *Agave* species (Agavaceae) with a third of these OTUs (1007 OTUs belonging to nine orders) being found in the endosphere (Colemann-derr et al. 2016).

The rules governing the assembly of this complex endophytic mycobiota remain to be elucidated. Local abiotic conditions are major drivers of plant microbiota composition (Shakya et al. 2013, Schreiter et al. 2014). More recently, authors have demonstrated that plant genotype also affects to a lower extent the composition of root-endophytic fungal communities (Colemann-derr et al. 2016, Vályi et al. 2016). These observed host-genotype effects can be linked to the filtering of surrounding microbes by root exudates (see for review Berendsen et al. 2012) and to the filtering of microbes that colonize the roots by the plant immune system (see for review Jones and Dangl 2006,

Vandenkoornhuyse et al. 2015). In parallel, the ability of plants to preferentially reward AM fungal symbionts according to their level of "cooperativeness" was demonstrated using *Medicago truncatula* (Kiers et al. 2011), suggesting an additional control of the plant over root-endophytic fungi. The combination of all these processes explains a 'host-plant preference' leading plant species to harbor distinct root fungal communities (Vandenkoornhuyse et al. 2002, Duhamel and Vandenkoornhuyse 2013). This filtering effect occurring at the root interface of a plant individual is then likely to influence the local diversity and abundance of fungi in the surrounding soil.

Most studies conducted so far, in a plant community context, focused on the composition of root- endophytic AM fungi communities, neglecting a large diversity of endophytic fungi. The first experimental studies investigating a focal plant root-endophytic fungal community showed that the identity of the plant species in the neighborhood induces drastic changes in the richness, diversity and composition of the AM fungal communities (Johnson et al. 2004, Hausmann and Hawkes 2009). For example, *Vulpia microstachys* grown in monoculture had an AM fungal community richness twice that of the same plant grown in a mixture of two to four plants (Hausmann and Hawkes 2009). This observation remains consistent for AM fungi in more complex mixtures of plants (Hausmann and Hawkes 2009). The influence of neighboring plants on endophytic community assembly of a focal individual was then extended to the temporal dynamics of the fungal assemblages (Hausman and Hawkes 2010). Pre-existing plant species, i.e. established before the introduction of successive species, had a filtering effect on the pool of AM fungi colonizing successive plant species (Hausman and Hawkes 2010). In parallel, large compositional changes in AM fungal communities over seasonal and interannual temporal scales have been observed,

suggesting that temporal dynamics is a key parameter for fungal community assembly (Bennett et al. 2013, Cotton et al. 2015). However, in the above-described experiments, the temporal scale tested was only a few weeks and the plant fingerprint on later root-endophytic fungal communities has never been tested over a longer period of time (Hausmann and Hawkes 2010).

Although the influence of plant community composition on root-endophytic fungal communities has already been described (e.g. Johnson et al. 2004, Hausmann and Hawkes 2010), the spatial scale of this influence is still unclear. In a recent review, Vályi et al. (2016) proposed that the relative influence of environmental conditions, dispersal and host filtering on the AM fungal community is dependent on the spatial scale considered. Previous results indicated that the effect of the host plant (i.e. host filter) is stronger at a local scale (see for review, Vályi et al. 2016). There is, however, very little empirical or experimental support for this hypothesis, and more importantly the distance at which this effect occurs has not been experimentally tested. A single seminal paper demonstrated that the relationship between plant and AM fungal community composition was detectable at the 25 cm^2 point scale but not at the 1 m^2 plot scale (Landis et al. 2005). We can make the parsimonious assumption that the soil reservoir of fungal propagules is likely to be a consequence of the structure and composition of the fungal community in the roots of past host plants (i.e., observed at the previous growth season). Importantly, the effect of a plant on the soil pool of fungi is likely to depend on the environmental conditions such as soil nutrient levels that can determine a fungus' cooperativeness and lifestyle (Hiruma et al. 2016). The effect of the plant community on the soil pool of fungi could also occur indirectly through the effect of the plant on its surrounding environment (habitat hypothesis, Zobel and Opik, 2014). If the fungal species pool

results from the cumulative influences of plants, we should be able to explain and quantify the respective influences of the surrounding plant species on the root-endophytic fungal community composition.

This assumption drives the idea that the plant neighborhood determines the assembly of the fungal pool that is available in the soil for the colonization of a newly established plant. While not all root-endophytic fungi are beneficial (Kogel et al. 2006), many studies have reported benefits for plant nutrition and resistance to abiotic and biotic constraints (see for review Friesen et al. 2011). Well described cases include growth promotion related to phosphate levels provided by *Colletotrichum tofieldiae* (Hiruma et al. 2016), as well as increased tolerance to extreme heat conferred by *Curvularia sp.* (Redman et al. 2002), and increased salinity tolerance conferred by *Fusarium culmorum* (Rodriguez et al. 2008). Changes in the diversity of fungal clades and relative abundance may, therefore, affect the ecological functions provided to the focal plant, thus its ability to cope with environmental conditions, and ultimately impact its fitness components (i.e. growth and reproduction).

Here we tested the influence of the plant neighborhood on the root-endophytic fungal community of a focal plant, including non-mycorrhizal fungi. We used a mesocosm design comprising experimental assemblages of different grassland species with varying levels of plant richness. We sampled soil cores from each mesocosm, and *Medicago truncatula* was grown as a trap plant in pots filled with the soil samples. The trap plant approach allowed us to characterize the fungal community able to colonize plant roots while standardizing the host-plant identity. We mapped the occurrences of individual plants for all species in the mesocosms at the centimetric

scale for two consecutive years. We analyzed the effect of plant neighborhoods on the root-endophytic fungal community of the trap plant *M. truncatula,* and the impact on *M. truncatula* biomass. Additionally, we tested at which spatial and temporal scale the influence of the plant neighborhood was detectable by considering the past and current neighborhood at two diversity scales, the sample scale (i.e., individual soil sample, alpha fungal diversity) and the plot scale (i.e., group of soil samples, gamma fungal diversity).

More specifically, we tested the hypotheses that: (i) the presence and abundance of plant species in the neighborhood leaves a fingerprint on the root fungal community richness and evenness; and (ii) the effect of the plant neighborhood can be detected in the fungal richness and evenness of different fungal clades. As fungal clades have different lifestyles and host preferences, we expect the strength and direction (positive or negative) of the neighborhood effect to depend on the plant species and fungal clade considered. (iii) Because the current plant neighborhood is the consequence of the past one we expect the past neighborhood to exert a stronger effect on the fungal richness and evenness. (iv) Because we expect individual plants to be influential at the scale of their root system, we expect the neighborhood effect to be stronger at small scale (i.e., at the sample, alpha diversity scale) (v) The changes in root fungal communities of *M. truncatula* should ultimately impact the focal plant performance. Because fungal clades have different lifestyles (from pathogenic to beneficial), we expect the richness and evenness of different clades to differentially affect host biomass.

Materials and Methods

Experimental design

To determine whether fungal communities respond to the overlying plant communities depending on their taxonomic identity (i.e. clades), and at which spatio-temporal scales, we used 112 experimental plant communities settled in 1.30 × 1.30 × 0.25 m mesocosms in 2009 and comprising 14 different mixtures of plants (see Benot et al. 2013, Bittebiere et al. 2013 for additional information on the experimental design). This study was conducted in the experimental garden of the University of Rennes 1. Communities were constituted in 2009 from a set of plant species widely distributed in temperate grasslands of Western France (des Abbayes et al. 1971), with one to 12 plant species in mixture with different species compositions (i.e., 1, 2, 3, 4, 8, 12 plant species; the 14 mixtures compositions are described in Table S1) replicated 8 times and maintained since 2009. Forty-eight individuals equally distributed between all co-occurring species within the mesocosm (same number of individuals per species) were initially planted in each mesocosm following a hexagonal pattern with similar densities per species included in the mixture (for example in eight species mixtures, six plantlets per species were initially planted). This hexagonal planting pattern allows balanced interactions between plant individuals (Birch et al. 2007). Plantlets of each species were randomly assigned to a position within the hexagonal pattern (see Bittebiere and Mony 2015 for a visualization of the pattern). The plants were grown on a homogeneous substrate composed of sand (20%) and soil from Western France (80%, collected in March 2009 and previously stored outside for 1 week). The initial chemical composition of the soil was: C/N = 7.2 ±3.2, NO_3^- = 32.5 ±18.6 µg.g^{-1} dry soil, PO_4^{2-} = 28.5 ±16.7 µg.g^{-1} dry soil. The mesocosms were placed on a tarpaulin to isolate the substrate from the ground soil. Weeds were regularly removed

and flowers were cut off to eliminate sexual reproduction in favor of clonal propagation. Juvenile plants that were not connected to other adult plants were considered as developing from seeds instead of emerging from vegetative organs through clonal multiplication. These juvenile plants were then manually removed from the mesocosm. The mesocosms were watered every 2d during the dry season to avoid drought stress. Above-ground vegetation was mown once a year in late September by cutting and removing the aboveground parts at 10 cm from soil surface allowing to limit litter accumulation. Plant community dynamics was, therefore, dependent on the plant clonal growth only. The present plant neighborhood, therefore, resulted from the past one.

Plant neighborhood characterization

The plant species spatial distributions in the mesocosms changed over time due to the ongoing community dynamics. To take these dynamics into account, occurrences of plant individuals were, therefore mapped in all mesocosms after 2y and 3y of experimental community cultivation (i.e. early spring 2011 and 2012), using an 80 × 80 cm squared lattice centered on the mesocosm (Fig. 1, called plot from here on). The use of this 80 × 80 cm lattice aimed at avoiding an edge effect within mesocosms. We recorded presence/absence data in 5 × 5 cm cells of the lattice based on above-ground coverage (i.e. 256 cells in total per lattice). A plant species was considered as present when at least one individual rooted within the cell, with each individual belonging to a single cell. GIS (ArcGIS ver. 9.3., ESRI) was used to calculate the number of cells colonized by each plant species (i.e. their abundances) at each spatial scale tested.

To test our hypotheses (i) at the plot scale (gamma scale), we calculated plant species abundances within the entire plot as the total number of cells occupied over the square-lattice; (ii) at the sample scale (alpha scale), we analyzed plant species abundances at five spatial scales around the central sampling point, ranging from 5 to 25 cm (i.e. radius of 5, 10, 15, 20 and 25 cm from the center of the plot; Fig. 1) (see Bittebiere and Mony 2015 for details on the method). These spatial scales were chosen because a previous paper detected a relationship between plants and AM fungi at a 25 cm^2 scale (Landis et al. 2005) and because competitive interactions between plants happen at such neighborhood scales (Bittebiere and Mony 2015). We considered two temporal scales by performing these calculations in 2011 (past plant neighborhood) and 2012 (present plant neighborhood).

Analysis of fungal root endophytic assemblages through a trap plant bioassay

We sampled five soil cores per plot within the 80×80 cm lattice in 2012 (the four corners and the center of the lattice) (i.e. 560 soil samples in total). This design enabled the fungal community to be captured both at the sample (sample in the center of the plot) and the plot scales (set of the five sampling points within the plot). These soil samples were used as substrates for the cultivation of *Medicago truncatula* individuals, which are trap plants. Using a trap plant bioassay enabled us to focus on fungal species that were able to colonize the trap plants. The advantage of the trap plant approach is thus to avoid the bias of detecting traces of resident DNA in the soil by focusing on living fungi. Additionally, *M. truncatula* has been regularly used as a model trap plant in the literature, and this species displays a very low host preference (Cook 1999). *M. truncatula* was

transplanted as seedlings on the sampled soil cores, after being germinated in sterile conditions. *M. truncatula* individuals were cultivated for 7 weeks under controlled conditions (constant temperature and water availability) with a 12 h day/light cycle, and nutrients were provided with a watering solution (see Table S2 for watering solution composition). To evaluate *M. truncatula* performance, root and shoot samples from each individual were weighed at the end of the experiment after harvesting. Plant total fresh mass was used as a proxy of performance.

DNA extraction and amplicon preparation

Medicago truncatula root samples were carefully washed with detergent (Triton 100X, 1% V/V), thoroughly rinsed in sterile distilled water, and ground to powder using a pestle and mortar under liquid nitrogen. Then, total DNA was extracted using the DNeasy plant kit (Qiagen, Courtaboeuf, France) according to the manufacturer recommendations. A 480 bp fragment of the fungi SSU rRNA was specifically amplified by PCR using NS22/SSU0817 primers (Lê Van et al. 2017) with PuReTaq Ready-to-go PCR beads (GE Healthcare, Buc, France). All the PCRs were done using fusion primers containing sequencing adapters and multiplex identifiers in addition to PCR primer (more details about amplifications in Lê Van et al. 2017). For each of the 560 samples, true technical amplicon replicates were performed (i.e. two independent PCRs for each extracted DNA sample). Amplicons were purified using AMPure XP – PCR kit (Agencourt/Beckman-Coulter, Paris, France). Purified amplicons were then quantified with Quant-iT Picogreen ds DNA assay (Invitrogen, Villebon sur Yvette, France). An equimolecular amount of each amplicon was pooled to prepare the sequencing library. Traces of concatemerized primers were removed with LabchipXT

(Caliper Life Sciences, Runcorn, United Kingdom) before emPCR and sequencing on a GS FLX+ instrument (Roche, Boulogne-Billancourt, France), following the manufacturer instructions.

Data trimming and contingency matrix preparation

Trimming, filtering, clustering, OTU identification and taxonomic assignments were performed as described elsewhere (e.g. Ben Maamar et al. 2015, Lê Van et al. 2017). To summarize the strategy, short sequences (<200 bp), sequences with homopolymers (>8 nucleotides) or ambiguous nucleotides, sequences containing errors in the multiplex identifier or primer, were deleted from the dataset. Chimeric sequences were detected using UCHIME (Edgar et al., 2011) and were deleted. After these steps, and from the two replicates, only sequences displaying 100% identity were kept (sequences are available under the accession number PRJEB22724, European Nucleotide Archive). The remaining sequences were grouped into OTUs using DNAclust (Ghodsi et al. 2011) with a 97% sequence identity threshold, and a contingency matrix was built. We removed 154 samples with less than 1000 reads and the remaining samples were normalized to the lower read count of the remaining samples, 1351 sequences. The sequencing depth (i.e. number of sequences per sample to describe the community) was checked from rarefaction curves computed using the function "rarefaction" in the package vegan (version 2.2-1) (Oksanen et al. 2015) in R (version 3.3.0) (R Core Team, 2013). The sequencing depth was high enough to describe the fungal communities in detail (Supplementary Fig. 1).

OTUs affiliation and clades selection

A total of 3471 fungal OTUs for the 406 samples were obtained. A large proportion of these OTUs were rare (i.e. >70% of the OTUs represented by less than 25 reads). To avoid over representation of rare OTUs and limit chimeric OTUs contaminants in the dataset (e.g. Mysara et al. 2017), OTUs occurring in less than 1% of the samples were removed. The resulting dataset contained 2057 fungal OTUs. All the statistical analyses were performed at three taxonomic levels: (i) all fungi, (ii) within the three most abundant phyla (i.e. Ascomycota, Basidiomycota, Glomeromycota), and (iii) within the most abundant class in each phylum (i.e. Sordariomycetes, Glomeromycetes, and Agaricomycetes) (i.e. seven datasets in total). Phyla and classes were thus selected according to their respective dominance in the entire assemblage and within the phyla. The Ascomycota, the Glomeromycota, and the Basidiomycota contained 1587 OTUs (77.2% of the total richness), 308 OTUs (15% of the total richness) and 100 OTUs (4.86% of the total richness), respectively. In each of these three fungal phyla, Sordariomycetes (186 OTUs), Glomeromycetes (80 OTUs) and Agaricomycetes (86 OTUs) were the dominant classes in terms of OTU richness. Together with the entire fungal community (comprising all phyla) these three phyla and three classes constituted the seven taxonomic clades that were used to calculate diversity indices.

Diversity indices calculation

We based our analyses on indices describing fungal community richness, diversity and evenness. We calculated the relative abundances of OTUs as the proportion of reads attributed to the OTU in a sample, and then calculated the indices for different clades following a down scale approach (from the entire assemblage to the phyla and classes). We calculated the indices at the plot scale (i.e., the

five samples from each plot pooled) and at the sample scale (i.e., based on the sample from the center of the plot only) for the seven fungal datasets (see above). For both scales (alpha and gamma diversity), we calculated the OTU richness (S) giving the number of co-occurring species, and Pielou's evenness indice (J) that informs on competitive equilibrium in the community. The evenness was calculated on OTU relative abundances computed from the normalized number of reads per OTU. We used Pielou's evenness because strong correlations (i.e. > 90%) between Shannon diversity, Simpson diversity, and Pielou's evenness were found. The Shannon and Simpson indices were thus discarded before further analyses. No strong correlations (i.e. > 90%) were found between the richness and evenness regardless of the taxonomic level analyzed. Indices were calculated using the VEGAN package (Oksanen et al. 2013) in R (R Core Team, 2015).

Statistical analyses

To determine whether the fungal community structure was influenced by the past and present plant neighborhoods at the sample and plot scales, we used multiple regression analyses with plant-species abundances as explanatory variables in linear models with the function "lm" in R (3.3.0). These analyses were performed on the seven fungal datasets (from the entire assemblage to the three phyla and the three classes).

At the plot scale, we tested the influence of past and present plant composition on the fungal OTU richness using the total abundances of the plant species over the lattice, for each date (see above section *Plant neighborhood characterization*). We thus constructed two models (past and present) for each taxonomic level analyzed (i.e. 14 models in total), which were optimized using a

backward stepwise selection procedure of the explanatory variables based on the Akaike's Information Criterion (AIC, Burnham and Anderson 2002).

At the sample scale, we tested the influence of past and present plant neighborhood*s* on the fungal pool at the sample scale (i.e. corresponding to the soil sample from the plot center). We tested the effect of the plant abundances for each date at five spatial scales (see above section *Plant neighborhood characterization*) on the fungal OTU richness and evenness. This enabled us to determine the spatio-temporal scale of response of clades from the local fungal pool to the plant neighborhood. One model was developed for each date and neighborhood size. We, therefore, constructed a total of ten models per index (two indices in total) and per taxonomic level analyzed (i.e. 140 models in total), and each model was optimized using a backward stepwise selection procedure of the explanatory variables based on the AIC (Burnham and Anderson 2002).

For both the plot and the sample scale we then used the information-theoretic model comparison approach based on AIC and compared for each index, all the optimized models through second-order AIC corrected for small sample sizes (AICc) (Burnham and Anderson 2002). In our analyses, we considered models with smaller AICc values and with a substantial level of empirical support (i.e., a difference of AICc > 2 with other models) as the most probable (Burnham and Anderson 2002). This procedure thus enabled us to compute and compare multiple models to determine the best model according to the AICc criteria.

To determine the impact of fungal clade OTU richness and evenness on the trap plant performance, we used linear models with the OTU richness and evenness as explanatory variables and *M. truncatula* biomass as the dependent variable. This was done at the sample scale (central

point of the plot) and at the three taxonomic levels analyzed (all fungi, phyla, classes). Models for phyla and classes where constructed as follows:

biomass~richness phylum 1 + richness phylum 2 + richness phylum3

Each model was optimized using a backward stepwise selection procedure of the explanatory variables based on the AIC (Burnham and Anderson 2002). Only phyla and classes that significantly participated in the construction of the best model were kept in the model and were thus presented in the results section.

For all models, data were log or root-square transformed when necessary to satisfy the assumption of a normal distribution of the residuals. The model coefficients and the proportion of index variation that was accounted for by the regression (R^2) were calculated. The significance of each explanatory variable was tested with the function "Anova" in R. All the statistical analyses were performed using the packages "car" (Fox and Weisberg 2011) and "AICcmodavg" (Mazerolle 2012) in R (3.3.0) (R Core Team 2013).

Results

Medicago truncatula's root endophytic fungal community

The 2057 fungal OTUs found in the *M. truncatula* root endosphere belonged to five phyla (i.e. Zygomycota, Chytridiomycota, Glomeromycota, Ascomycota, and Basidiomycota), with Zygomycota and Chytridiomycota accounting together for less than 3% of the total number of OTUs (Fig. 2). The root-endophytic fungal community ranged from 82 to 265 OTUs per sample with an average of 138±28 and 163±25 OTUS per sample and plot respectively, comparably to

previous studies (e.g. 133 fungal OTUs per sample in Lê Van et al. 2017). The root-endophytic fungal community was dominated by Ascomycota OTUs which represented more than 80% of the reads and ~77% of the total fungal richness. The 1587 Ascomycota OTUs were distributed in nine classes, with the Sordariomycetes being the class with the most OTUs (~11%) (Fig. 2). The rest of the Ascomycota OTUs were not classified at the level of the class. Among the 308 Glomeromycota OTUs, only 25% belonged to the class Glomeromycetes whereas the other OTUs were not affiliated at the class level while most of the 101 Basidiomycota OTUs where affiliated to the class Agaricomycetes (Fig. 2). Principal coordinates analysis (PCoA) on Bray-Curtis dissimilarities between samples indicated that there was no clear clustering of the samples based on the fungal community composition (Fig. 3A). This was also the case for dissimilarities at the plot scale (Fig. 3C). However, the distribution of fungal OTUs in the samples (Fig. 3B and Supplementary Fig. 2A) and plots (Fig. 3D, and Supplementary Fig. 2B) interestingly pointed out that Glomeromycota OTUs were mainly present in samples from which Ascomycota OTUs were absent even if this was less clear at the plot scale.

Root-endophytic fungal community response to plant richness

We first analyzed the effect of the plant mixture richness on the composition of the root-endophytic fungal community of the trap plant. PCoA ordinations on Bray-Curtis dissimilarity indicated that that there was no clustering per richness mixture at both the sample and the plot scale (Fig. 3A, C). This result was confirmed by a PCoA analysis constrained by the richness of the plant mixture, indicating that the richness only explained 1.53% and 5.31% of the variance in composition of the

fungal community at the sample and plot scales respectively (Supplementary Fig. 3). These results indicated that the overall plant community richness was a poor predictor of the composition of the fungal community colonizing *Medicago truncatula*'s roots.

Root-endophytic fungal community richness response to the plant neighborhood

At the plot scale – To investigate the effect of the plant composition on the richness of the fungal community at the scale of the entire plot (i.e. gamma diversity), we produced linear models at the plot scale (i.e. the five sampling points from the same plot pooled together) with the plant species abundances as explanatory variables (Table 1). The plot richness of the entire fungal community was significantly determined by the present plant composition. However, the proportion of the variation in fungal richness explained by the model was low ($p=0.04$; $R^2=0.04$). In addition, the fungal richness within the Ascomycota and Basidiomycota was not determined by the plant composition and only 5% of the variance in Glomeromycota richness could be attributed to the present plant composition ($P=0.04$; $R^2=0.05$) (Table 1). When considering the past plant composition, only Basidiomycota richness was weakly determined by the plant composition ($P=0.03$; $R^2=0.04$).

At the sample scale – We used the same linear model approach at the sample scale (i.e. center of the plot) with plant species abundances as explanatory variables. The richness of the fungal community was significantly determined by the plant neighborhood for all clades tested (Table 2). In comparison with the models produced at the plot scale, a larger proportion of the variance in fungal

community richness was explained at this sample scale. At the level of the entire fungal community, the richness only increased significantly with the abundance of *Agrostis tenuis* in the neighborhood, whereas the abundance of the other plants had no significant effect on the fungal community richness ($P<0.05$, $0.07\geq R^2\geq 0.1$). Nevertheless, *A. tenuis* was one of the rarest species in the experiment and the effect detected could be due to this rarity.

Considering the fungal phyla and classes separately, Ascomycota richness increased with the abundance of *A. tenuis* and *Festuca rubra* ($P<0.05$; $0.04\geq R^2\geq 0.12$) whereas Glomeromycota richness increased with *Brachypodium pinnatum* and *Dactylis glomerata*, and decreased with *Elytrigia repens* ($P<0.05$; $0.06\geq R^2\geq 0.09$). The effects (positive or negative) of plant species at the phylum level were not necessarily the same at the class level. The presence of *D. glomerata* in the plant neighborhood for example, significantly increased the richness of the phylum *Basidiomycota* ($P<0.05$, $0.06\geq R^2\geq 0.09$) but decreased the richness of the class Agaricomycetes ($P<0.05$, $0.07\geq R^2\geq 0.1$). In contrast, several species had a consistent effect between taxonomic levels (Table 2). *A. tenuis* for example, significantly increased the richness of the entire fungal community and at the phylum levels for both Ascomycota and Basidiomycota, and at the class level for Sordariomycetes.

Fungal evenness at the sample scale

The linear models analysis revealed that the evenness of the entire fungal community, of the Ascomycota, Basidiomycota, Glomeromycota, Sordariomycetes, Glomeromycetes, and Agaricomycetes were all significantly determined by the plant neighborhood (Table 2). At the level

of the entire fungal community, the evenness only decreased significantly with the presence of *Holcus mollis* in the neighborhood and not with other species abundance ($P<0.05$; $0.04 \geq R^2 \geq 0.07$).

Considering the fungal phyla and classes separately, we detected stronger effects of the plant-species than for entire fungal community (Table 2). For example, the evenness of the phylum Ascomycota significantly increased with *B. pinnatum* but decreased with *H. mollis* abundance ($P<0.01$; $0.11 \geq R^2 \geq 0.14$), whereas Glomeromycota evenness increased with *F. rubra* abundance ($P<0.01$; $R^2=0.09$). Similarly to the results obtained for the fungal richness, several plant species had the same effect on the evenness for every fungal clade whereas others had different effects between clades. In general, the variance explained by the models increased with the fungal taxonomic levels. For example, 14% and 24% of the variance in evenness were explained for the phylum Ascomycota and the class Sordariomycetes respectively.

Effect of temporal and spatial scales on the link between plant neighborhood and fungal community

We determined the spatio-temporal scale of response of the root-endophytic fungal community to the plant neighborhood by producing linear models with the past and present distributions of the plant species at five neighborhood sizes (5 to 25 cm). These analyses were performed at the scale of the sample (center of the plot) and the models with the lowest AICc criteria were selected (see Material and Methods section).

The selected models indicated that the fungal community richness and evenness equally responded to present and past plant neighborhoods (i.e. the models were significant and the AICc criteria were not different; Table 2). Furthermore, the species explaining the variations in richness

and evenness of the fungal community were the same at both temporal scales (i.e. past and present). Only the evenness of Glomeromycota, Basidiomycota, and Sordariomycetes, and the richness of Glomeromycetes, responded to a single temporal scale.

The entire fungal community richness and evenness indifferently responded to the plant neighborhood at the five neighborhood scales analyzed (i.e. 5, 10, 15, 20 and 25cm around the sampling point) (Table 2). Only the evenness of Glomeromycota responded to a single specific neighborhood size (i.e. 25 cm), whereas the other phyla and classes responded to at least two of the five neighborhood scales for both richness and evenness.

Effects of fungal and plant communities on the trap plant biomass

We first tested whether the biomass of the trap plant *Medicago truncatula* was affected by the plant mixture richness in the mesocosms. No significant difference of the trap plant biomass was detected between all the pairs of treatments (pairwise Wilcoxon rank sum test, $p>0.05$, supplementary Fig. 4). To go further we then tested the effect of the richness and evenness of the root-endophytic fungal community on the trap plant biomass. The biomass of the trap plant *Medicago truncatula* was not affected by the OTU richness of its entire root-endophytic fungal community (Table 3). However, the biomass of the trap plant increased significantly with the richness of the Basidiomycota and Glomeromycota phyla ($P<0.01$ and $P<0.05$ respectively) (Table 3) but not with the phylum Ascomycota (Table 3). The combined effects of Basidiomycota and Glomeromycota OTU richness explained ~12% of the variations in plant biomass ($P<0.05$; $R^2=0.12$). The results were conserved at the class level for Glomeromycetes and Agaricomycetes richness but not for Sordariomycetes richness ($P<0.01$; $R^2=0.13$; Table 3). The biomass of the trap

plant also increased with the evenness of its entire fungal endophytic community ($P<0.01$; $R^2=0.07$; Table 3). However, the biomass increased only with the evenness of the phylum Ascomycota but not with the Basidiomycota and Glomeromycota phyla ($P<0.01$, $R^2=0.1$; Table 3), and this result was conserved at the class level.

Discussion

Local plant neighborhood as a driver of fungal community

The use of a trap plant bioassay allowed us to specifically investigate the fraction of soil fungi that is active and able to colonize the trap plant roots. In agreement with our expectations (hyp.1), we demonstrated that the presence and abundance of specific plant species in the neighborhood can be linked to the richness and evenness of the fungal community colonizing the roots of a focal plant. Interestingly, the distribution of the plant species in the neighborhood was a better predictor of the root-endophytic fungal community of the trap plant than the richness of the plant mixture (Fig 2, 3 and Supplementary Fig 4; Tables 1 and 2). This suggests that more than the overall plant community richness, it is the identity of the plants in the neighborhood that sculpts the root-endophytic fungal community of a given plant. Additionally, the fact that the influence of the plant neighborhood could be detected for all the fungal clades investigated (hyp II) suggests that this effect is not specific to a fungal group in particular. This result resonates with the Driver and Passenger hypotheses that aim at addressing whether AM fungi or plants communities drive the other (Zobel and Opik, 2014). In this aspect, all the fungal clades at least partly responded to the past plant landscape indicating that fungi are driven by the plant communities. However, and as

explained by Zobel and Opik (2014), the observed effect could also be an indirect consequence of plant-driven environmental changes (i.e plants acting as source and sink of nutrients) thus fitting the habitat hypothesis. However, and as predicted in our hypothesis (hyp. II), the fungal clades responded to the presence of key plant species that were different between clades. This result leans toward a specific response of a given fungus to a given host-plant, which suggests that the fungi are either driven by a given nutrient or compound made available by the plant, or have a preferential association (i.e. host preference) towards this plant species. Our experiments were, however, conducted at a small (centimetric) scale and such effects might not be perceptible at larger scales, and may thus be irrelevant for plant-fungi co-variation at larger scales.

When looking at the spatial scale of the neighborhood influence we found that the plant distribution at the sample scale (i.e. alpha diversity) better explained the richness of *M. truncatula* endophytic fungi than at the plot scale (hyp. III, Tables 1 and 2). This result suggests that the biotic interactions structuring the fungal endophytic communities (i.e., host preference, host filtering) mostly act at the scale of a few centimeters (Hazard et al. 2013, Vályi et al. 2016). Such heterogeneity of fungal richness and evenness within a particular host-plant species is a recurrent observation (e.g. Schlaeppi et al. 2014, Lê Van et al. 2017) and has been linked to plant recruitment from the soil "reservoir" (Vandenkoornhuyse et al. 2015). For example, root-associated fungi in agave species are mainly recruited from the surrounding soil (Coleman-Derr et al. 2016). The importance of abiotic factors, notably soil properties, as determinants of the soil microbial pool composition (Shakya et al. 2013, Schreiter et al. 2014, Coleman-Derr et al. 2016) has been repeatedly demonstrated and is

considered as the main source of variation of the plant microbiota (Vandenkoornhuyse et al. 2015). We highlighted here that the plant neighborhood at a centimeter scale (i.e. 5 cm to 25 cm around the sampling point) also determines, in part, the fungal soil pool available for plant recruitment introducing the role of the local plant community context (i.e. the plant neighborhood) as a structuring factor.

The fungal community also results from past plant neighborhoods

In agreement with our expectations (hyp. 3), we showed that the past plant neighborhood determined, at least in part, the root-endophytic fungal community richness and evenness of the trap plant. This was confirmed at the phyla and classes scales, although a low percentage of variance was explained for all clades tested. This suggests that past plants can leave a significant "footprint" on the richness and evenness of root-endophytic fungi colonizing a focal plant. The observed persistence of the plant composition effect over the years could be due to the short period investigated in the present study (two consecutive years) because fungal spores and propagules can survive in soil for more than a year and the existence of a "spore bank" has been proposed (McGee et al, 1997, Nguyen et al., 2012). Interestingly, Nguyen et al. (2012) also proposed, although this has not been experimentally validated, that spore longevity might not be a phylogenetically conserved trait within Basidiomycota or Ascomycota. A study involving a longer period of plant community mapping (i.e. >2y), would allow determination of the temporal limits of this potential soil fungal bank "memory" and whether some fungal clades with high spore longevity are more affected by the past plant community.

The structure of the root endospheric fungal community impacts M. truncatula biomass

In agreement with our expectations (hyp.4) the performance of *Medicago truncatula* was affected by the richness and evenness of the community of fungal endophytes. The observed relationship between Glomeromycota richness and plant performance has already been demonstrated for AM fungi (van der Heijden et al. 1998, Klironomos et al. 2000, Hiiesalu et al. 2014), and is likely due to their beneficial effects on nutrient uptakes. The increase in AM fungal diversity has experimentally been shown to result in more efficient exploitation of available resources such as soil phosphorus (van der Heijden et al. 1998), and to decrease plant pathogens (van der Putten et al. 2009). To our knowledge, however, the positive effect of fungal species richness on plant performance has never been demonstrated with the phylum Basidiomycota or the class Agaricomycetes. Little is known about the functions of the endospheric Agaricomycetes in grass plants and the role of this clade on plant growth has still to be clarified. Even if these clades have been described to a limited extent, Ascomycota is a phylum known to be composed of much diversified organisms performing various functions for host plants. In this context, an even community could also represent a higher diversity of organisms able to colonize the plant and provide functions that can help the plant to adjust to environmental conditions (Vannier et al. 2015). Our results are, however, limited by the fact that we used DNA-based approaches and thus have no information on the activity and functions of the endophytic communities. Additionally, it is important to note that we investigated the root-endophytic fungal compartment only and that fungi colonizing the shoots of the plant like Epichloae, which have been described to colonize the plant species present in this study (Márquez

et al. 2010, Schardl et al. 2012, Tadych et al. 2014), could also alter the plant biomass and/or mediate the observed effect on the host biomass. More generally, other compartments of the plant microbiota, such as bacteria that exert a strong control on fungal communities and buffer their effects on plant biomass (Duran, et al. 2018), could also be involved in the observed effect.

Plant-plant interactions mediated by fungi affect plant performance

Knowing that the endophytic fungi richness and evenness were determined by the abundance of specific plants in the neighborhood and that these changes affected plant performance (biomass productivity), this study suggests the existence of plant-plant interactions mediated by the root-endophytic fungal communities. The trap plant approach allowed us to control for the effects of plant-plant competition to detect biomass changes only linked to soil microorganisms. It is thus important to notice that these effects could be counterbalanced by plant-plant interactions in nature. Our results nevertheless indicate that the shifts in fungal community richness and evenness can have positive or negative effects on the trap plant biomass, suggesting that the plant neighborhood can have either a facilitative or a competitive effect on the trap plant through the root fungal community. Studies that have investigated the shift between plant-plant facilitation and competition suggested that this shift is linked to environmental stress or disturbance intensity and is spatially heterogeneous (O'Brien et al. 2017). Importantly, previous studies have indicated that plant-plant facilitation (i.e. a beneficial effect of a particular plant presence) may be linked to the composition of their AM fungal communities (Montesinos-Navarro et al. 2012). Montesinos-Navarro et al.

(2012) argued that stronger facilitation occurs between pairs of plant species with different associated AM fungi. This phenomenon underlies a potential mechanism which increases AM fungal diversity in the shared rhizosphere and promotes complementarity between the beneficial effects of each AM fungus (van der Heijden et al. 1998, Wagg et al. 2011). We herein provided experimental evidence supporting this assumption by showing that the richness of Glomeromycota in the roots of the trap plant increased with the abundance of specific plants in the mesocosm neighborhood, which ultimately increased *M. trunculata* biomass. The idea that particular plant species can increase the abundance of some fungal groups has already been suggested: for instance, spore abundance in salt marsh was determined by the proximity of mycotrophic hosts (Carvalho et al. 2003), whereas the presence of the grass *Anthoxantum odoratum* increased the abundance of AM fungi in the soil regardless of the plant mixtures (De Deyn 2011). Our results also indicate that changes in plant fungal communities can be detrimental as several plant species had a negative effect on fungal richness and/or equitability. We thus propose that changes in the fungal communities in the surrounding soil of a plant is a mechanism that can contribute to the shift from plant-plant facilitation to competition. More importantly, we herein propose that the potential links between plant-plant facilitation and endophytic fungi are not restricted to AM fungi but could be extended to other fungal clades such as Ascomycota and Basidiomycota. We thus encourage future studies to consider the entire fungal community and to consider the feedbacks between plant and fungal communities that can affect ecosystem properties such as productivity (Cadotte et al. 2008).

Conclusions

We highlighted herein that the abundance of key plant species in the neighborhood of *Medicago truncatula* can contribute to sculpt the evenness and richness of its root-endophytic fungal community. These findings indicate that the assembly of *M. truncatula* root-endophytic fungal community could also be driven in part by the fingerprint that past plants leave on the soil fungal community. This neighborhood effect was detectable and stronger in the fungal community richness and evenness at a very fine scale (below 25 cm). The influence of the plant neighborhood on the root-endophytic fungal community could be an ecological force driving root-associated fungal complexity and heterogeneity with consequences for the focal plant biomass.

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Availability of data and materials

All sequence data generated in this study have been deposited in the European Nucleotide Archive under the accession number PRJEB22724.

Plant abundances in the mesocosms are also available as supplementary files.

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Figures legends

Figure 1. Sampling protocol. Plant neighborhoods were determined by mapping the abundances of the different plant species with a 0.8 × 0.8m lattice, in the past (1y before sampling, 2011) and present (the same year than sampling, 2012). Five soil cores were sampled within each plot (1.3 × 1.3m) and an individual of *M. truncatula* was grown on each soil sample as a trap plant.

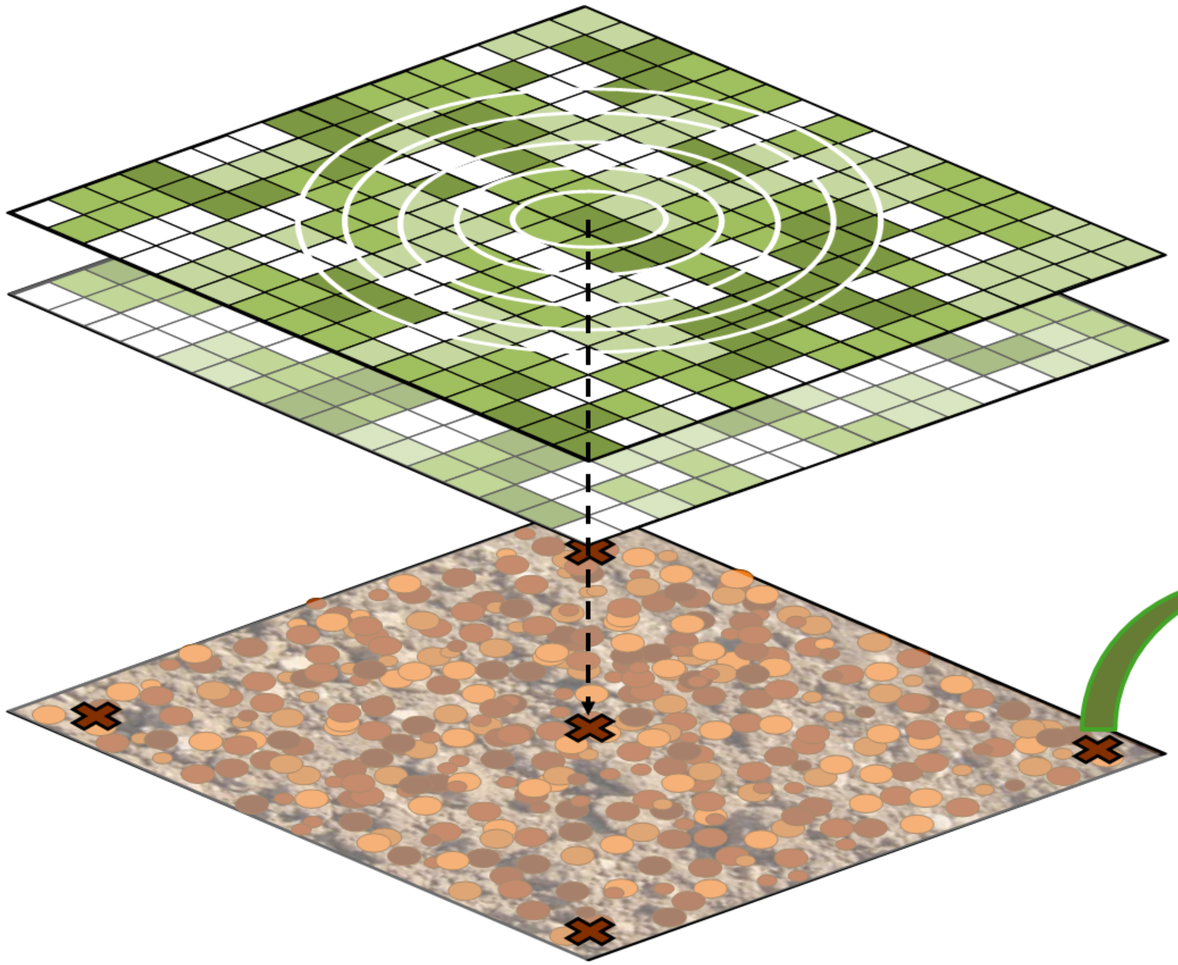
Figure 2. (A) Relative sequence abundance and total OTU richness of the different phyla within the entire fungal community. Relative abundance and OTU richness of classes within the three major phyla (B) Ascomycota, (C) Basidiomycota and (D) Glomeromycota. Unclassified represent OTUs affiliated to sequences without classification and Undefined represent OTUs affiliated to unknown organisms at this taxonomic level.

Figure 3. Dissimilarity between root samples fungal communities of the trap plant *M. truncatula* and distribution of fungal OTUs across samples. (A) PCoA on bray-curtis distances between samples calculated on normalized OTU tables at the sample scale (alpha diversity). (B) Distribution of fungal OTUs on the PCoA ordination of samples dissimilarity at the sample scale. (C) PCoA on bray-curtis distances between plots calculated on normalized OTU tables (aggregated abundances of all samples in a plot) at the plot scale (gamma diversity). (D) Distribution of fungal OTUs on the PCoA ordination of plot dissimilarity at the plot scale (gamma diversity). The richness level of the plant mixture in the mesocosm from which the soil of the trap plant was harvested is indicated in color on the samples' ordination. OTU affiliation at the phylum are indicated in colors in the OTU distribution ordination.

Present

Past

Plant neighborhood



Soil sampling

***M. Truncatula* trap culture**

Plant species 1

Plant species 2

Plant species 3

Plant species 4

Soil sampling point

Fungi

(A)

Relative abundance

OTUs richness

Zygomycota
Glomeromycota
Chytridiomycota
Basidiomycota
Ascomycota

All fungi

(B)

Relative abundance

OTUs richness

undefined
unclassified
Taphrinomycetes
Sordariomycetes
Saccharomycetes
Pezizomycetes
Orbiliomycetes
Lecanoromycetes
Laboulbeniomycetes
Eurotiomycetes
Dothideomycetes

Ascomycota

(C)

Relative abundance

OTUs richness

undefined
unclassified
Exobasidiomycetes
Agaricostilbomycetes
Agaricomycetes

Basidiomycota

(D)

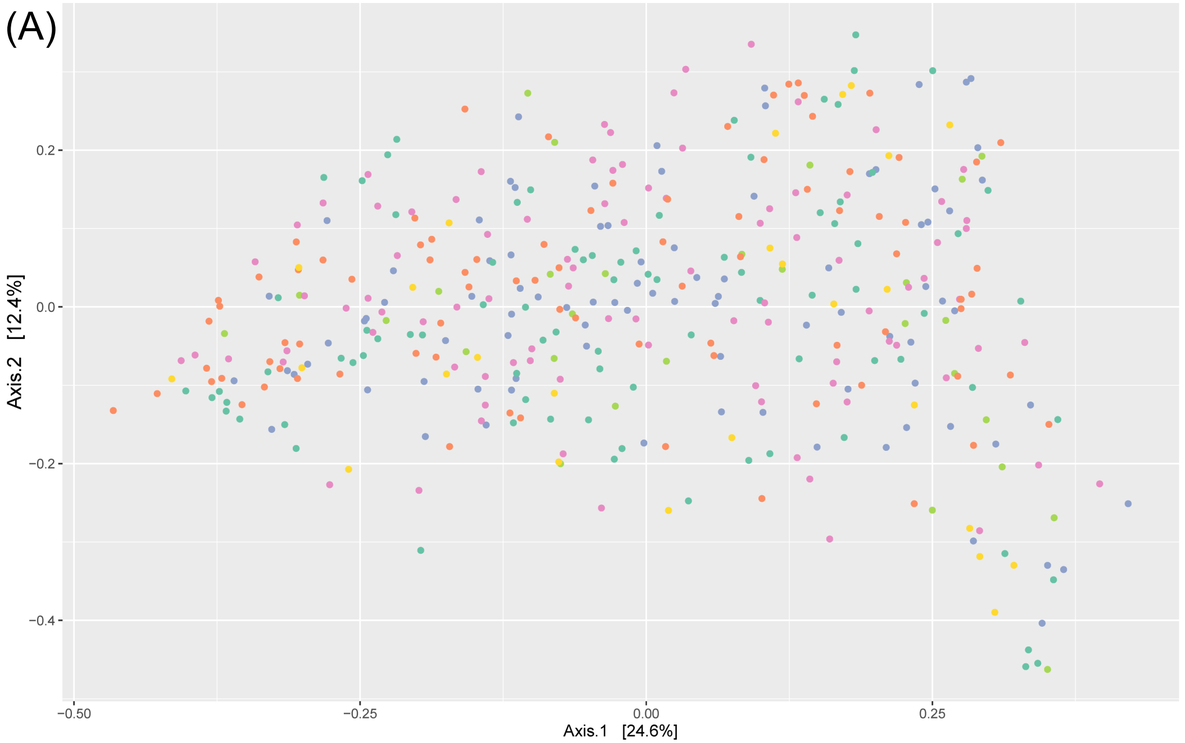
Relative abundance

OTUs richness

unclassified
Glomeromycetes

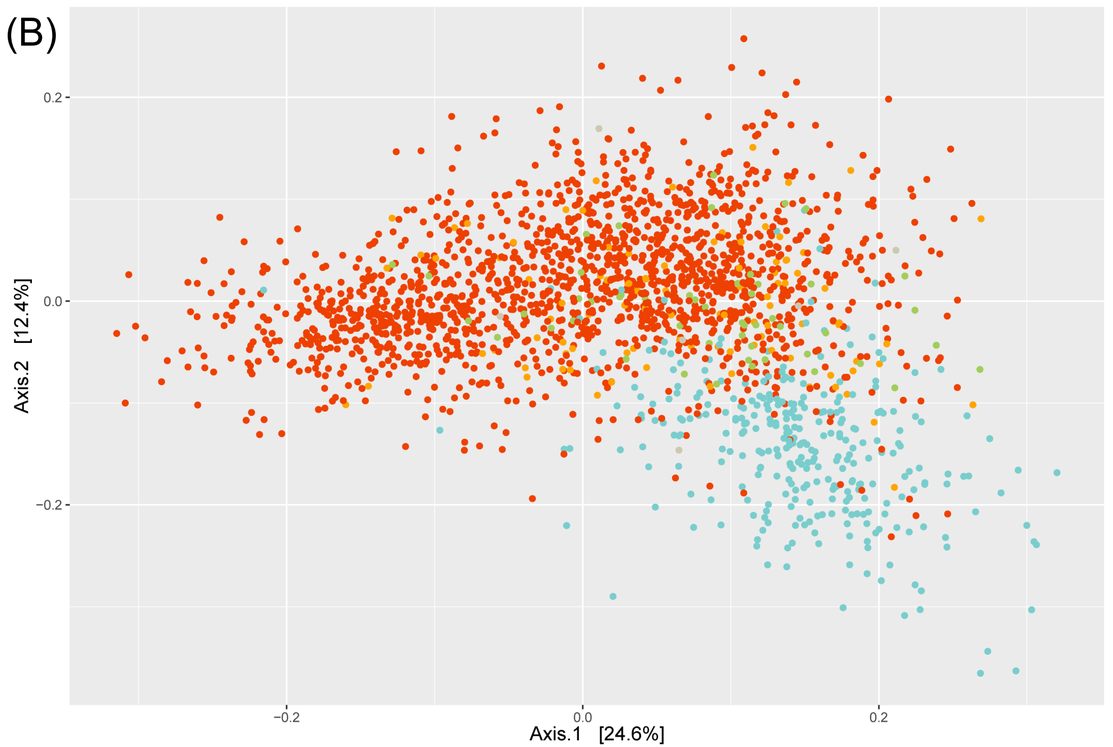
Glomeromycota

(A)



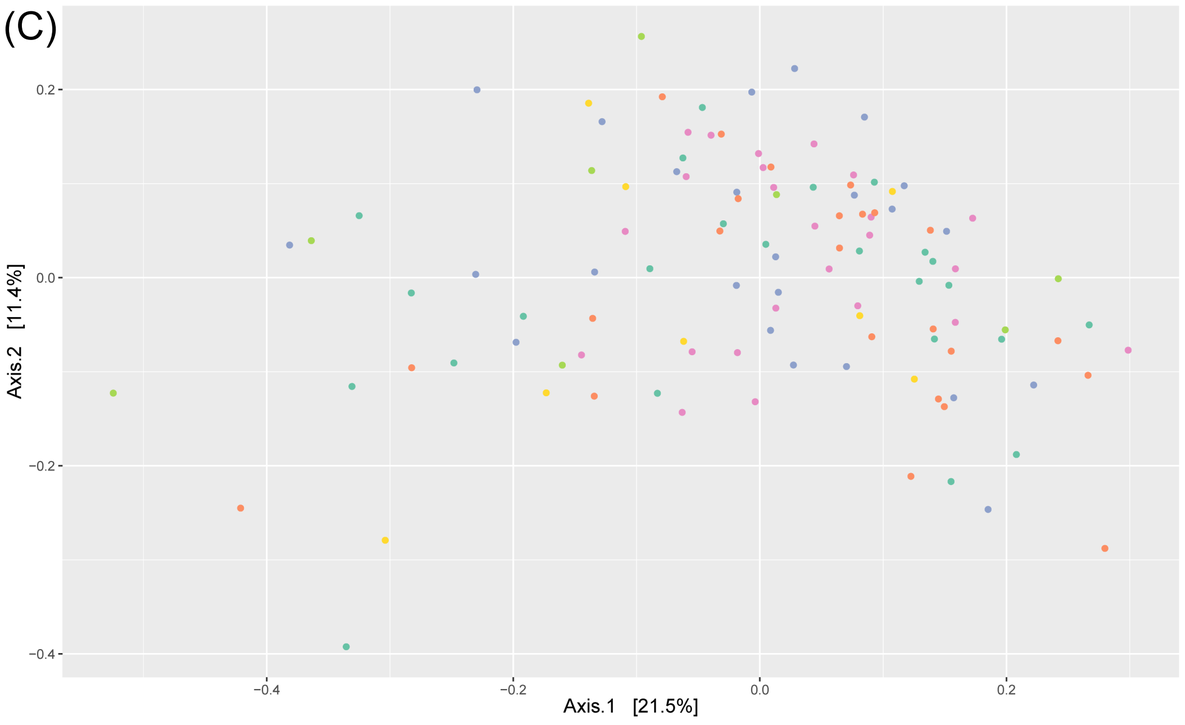
Richness
1
2
3
4
8
12

(B)



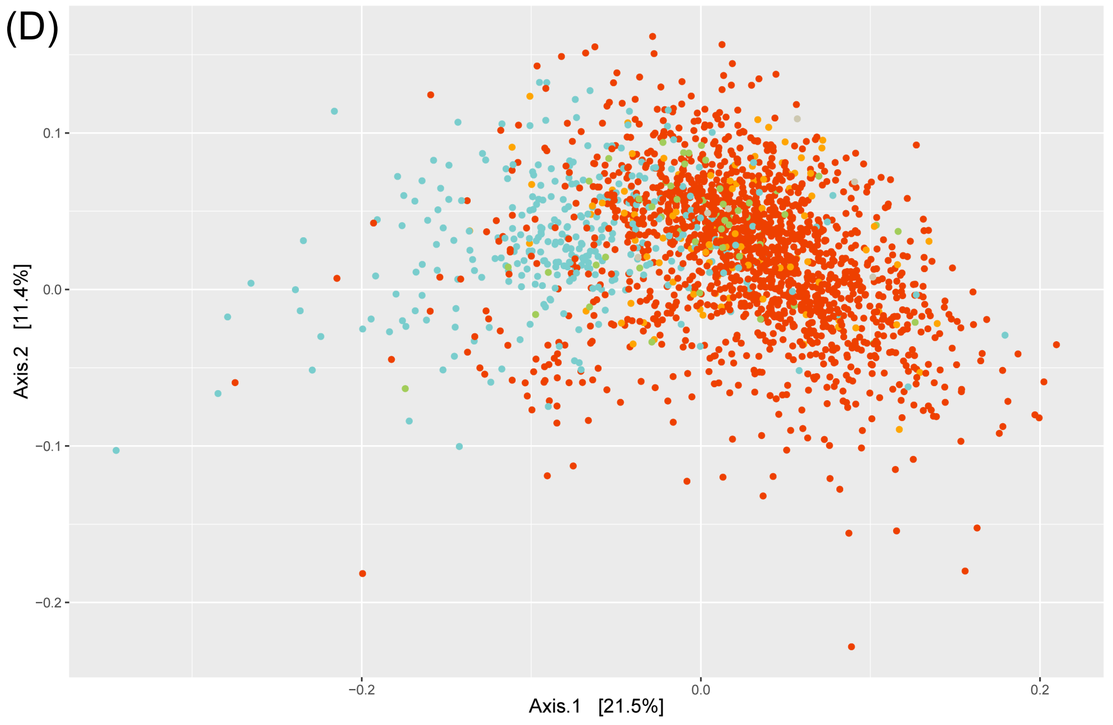
Phyla
Ascomycota
Basidiomycota
Chytridiomycota
Glomeromycota
Zygomycota

(C)



Richness
1
2
3
4
8
12

(D)



Phyla
Ascomycota
Basidiomycota
Chytridiomycota
Glomeromycota
Zygomycota

Table 1. Responses of each fungal clade OTU richness at the plot scale to the past and present plant compositions (results of linear models). P-values and adjusted R^2 are presented. Only species contributing significantly to model building are showed as well as their effect on the fungal richness (+ increasing the richness; – decreasing the richness).

	Present			Past		
Clades	P-value	R^2	Significant plant species	P-value	R^2	Significant plant species
All Fungi	**0.04**	0.04	Asto (-)	0.11	0.01	-
Ascomycota	0.07	0.03	Hmol (-)	0.09	0.02	-
Glomeromycota	**0.04**	0.05	Cnig (-)	0.08	0.02	-
Basidiomycota	0.12	0.03	Bpin (+)	**0.03**	0.04	Hlan (-)
Agaricomycetes	**0.02**	0.04	Hlan (-)	**0.02**	0.04	Hlan (-)
Sordiariomycetes	**0.03**	0.07	Erep (+) Dglo (+) Lper (+)	**0.01**	0.08	Aten (+) Cnig (+) Lper (+)
Glomeromycetes	**0.004**	0.08	Hmol (-) Cnig (+)	**0.01**	0.06	Cnig (-)

Table 2. Responses of each fungal clade OTU richness and evenness at the sample scale to the past and present plant compositions (results from linear models). P-values and the range of adjusted R^2 of best models are presented. Significant time and spatial scales of neighborhood are also indicated. Only species significantly contributing to the model building are showed as well as their effect on the fungal richness and evenness (+ increasing; – decreasing). *, P<0.05; **, P<0.01; ***, P<0.001.

Clades	Time scale of response	Spatial scale of response (radius)	R^2	Significant plant species
All Fungi				
Richness	Present/Past	5 to 20cm	0.07- 0.1 (*)	Aten (+)
Evenness	Present/Past	10 to 25cm	0.04 – 0.07 (*)	Hmol (-)
Ascomycota				
Richness	Present/Past	5 to 25cm	0.04 – 0.12 (*)	Aten (+) Frub (+)
Evenness	Present/Past	5 to 20cm	0.11 – 0.14 (**)	Hmol (-) Bpin (+)
Glomeromycota				
Richness	Present/Past	5 to 25cm	0.06 – 0.09 (*)	Erep (-) Bpin (+) Dglo (-)
Evenness	Present	25cm	0.09 (**)	Frub (+)
Basidiomycota				
Richness	Present/Past	5 to 25cm	0.06 – 0.09 (*)	Dglo (-) Asto (-) Aten (+) Hlan (-) Cnig (-)
Evenness	Present	15 and 25cm	0.07 (*)	Asto (-)
Sordariomycetes				
Richness	Present/Past	20 and 25cm	0.1 – 0.11 (*)	Erep (-) Bpin (-) Frub (-) Aten (+)
Evenness	Past	10 and 25cm	0.22 – 0.24 (***)	Bpin (+) Hmol (-) Frub (+) Aten (+)
Agaricomycetes				
Richness	Present/Past	5 to 20 cm	0.07 – 0.1 (*)	Dglo (+) Asto (-) Hlan (-)
Evenness	Present/Past	5 to 20 cm	0.08 – 0.15 (**)	Dglo (-) Asto (-) Hmol (+) Anob (-)
Glomeromycetes				
Richness	Past	5 to 25cm	0.08 – 0.1 (**)	Bpin (+)
Evenness	Present/Past	5 to 25cm	0.07 – 0.12 (*)	Bpin (+) Asto (+) Aten (+) Hlan (+) Lper (+)

Table 3. Results of linear models testing the effect of each fungal clade OTU richness and evenness on the biomass of the trap plant *M. truncatula*. ANOVA P-values, F-values and adjusted R^2 of the best models are presented. Only fungal clades significantly contributing to the best model building are presented.

Clades		Richness			Evenness		
		P-value	F-value	R^2	P-value	F-value	R^2
	All Fungi	0.24	1.42	0.005	**0.007**	**7.67**	**0.08**
Phyla	Ascomycota	---	---		**0.003**	**9.57**	
	Basidiomycota	**0.01**	**2.56**	**0.12**	---	---	**0.1**
	Glomeromycota	**0.02**	**2.38**		---	---	
Classes	Sordariomycetes	---	---		**0.007**	**7.62**	
	Agaricomycetes	**0.01**	**6.83**	**0.14**	0.057	3.71	**0.1**
	Glomeromycetes	**0.04**	**4.34**		---	---	