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Effect of floristic composition and configuration on plant root mycobiota: a landscape transposition at a small scale

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Summary

- Fungal communities in the root endosphere are heterogeneous at fine scale. The passenger hypothesis assumes that this heterogeneity is driven by host plant distribution. Plant composition and host plant configuration should then influence root fungal assemblages.
- We used a large-scale experimental design of 25 mixtures of grassland plants. We sampled *Brachypodium pinnatum* in each mesocosm, and used amplicon mass-sequencing to analyze the endospheric mycobiota. We used plant distribution maps to assess plant species richness and evenness (heterogeneity of composition); and patch size and the degree of isolation of *B. pinnatum* (heterogeneity of configuration) on fungal community assembly.
- The Glomeromycotina community in *B. pinnatum* roots was not related to either floristic heterogeneity or productivity. For Ascomycota, the composition of OTU (operational taxonomic unit) was driven by plant evenness while OTU richness decreased with plant richness. For Basidiomycota, richness increased with host plant aggregation and connectivity. Plant productivity influenced Ascomycota, inducing a shift in OTU composition and decreasing evenness.
- Plant heterogeneity modified root mycobiota, with potential direct (i.e., host preference) and indirect (i.e., adaptations to abiotic conditions driven by plant occurrence over-time) effects. Plant communities can be envisioned as microlandscapes consisting of a variety of fungal niches.

Introduction

Plant roots are colonized by a wide variety of microorganisms particularly fungi (Coleman-Derr et al., 2016; Lê Van et al., 2017). In plant communities, there is a certain degree of spatial correlation between plant distribution and soil fungal community structure (Chen et al., 2017). Based on the driver and passenger hypotheses, Hart et al. (2001) presented two non-exclusive mechanisms to explain this spatial correlation. The driver paradigm assumes that fungal symbionts regulate plant assemblages. Fungal symbionts indeed play a determinant role in plant nutrition (Marshchner, 1995; Hiruma et al., 2016) and alleviation of environmental stresses (Augé, 2001; Gange et al., 2005; Vannier et al., 2019). Because of these effects, they have a profound impact on the growth and phenotype of their host plant (Vandenkoornhuysse et al., 2015; Berg et al., 2016), in turn influencing competitive interactions in plant communities (Moora & Zobel, 2010). Conversely, the passenger paradigm predicts that plants regulate fungal assemblages. This theory assumes that plants are able to recruit and select particular fungi as partners both because they have barriers to protect their roots from colonization (passive selection) and because they can recognize and attract specific fungi (active recruitment) (Vandenkoornhuysse et al., 2015). In the case of arbuscular mycorrhizal fungi (AMF), even after colonization, host plants are able to sort initial AMF colonizers by selectively rewarding the most cooperative partners (Kiers et al., 2011). However, the ability to detect low quality AMF partners varies among plant species (Kiers & Denison, 2008). Because these mechanisms – recruitment or/and the reward process – result in observed host preference (Vandenkoornhuysse et al., 2003), the plant community is then assumed to provide a variety of fungal niches, driving fungal distribution. Recently, the habitat hypothesis was proposed by Zobel & Opik (2014) as an alternative to the driver and passenger hypotheses. The habitat hypothesis assumes that the apparent interdependence between fungi and host plant communities is due to their independent adaptation to the same abiotic conditions. This hypothesis applies when comparing spatial distribution of plants and fungi along a range of environmental conditions, while in homogeneous environmental conditions, only the driver and passenger hypothesis may apply. Experimental tests of the driver hypothesis are available in the literature. These tests mainly focus on plant interactions following experimental inoculations of fungal assemblages in multispecies mixtures of plants, or on the suppression of fungi with fungicides (Zobel & Opik, 2014). However, rigorous tests of the passenger hypothesis are rare in the literature (Zobel & Opik, 2014). Additionally, the authors usually consider the effect of plant

communities on root fungal distribution is the same as the effect of plant composition or richness only, neglecting the role of plant spatial configuration.

The composition of a plant community is defined as the taxonomic identities of plant species and their relative abundance. Plant composition has been shown to affect the composition of the microbiota (Johnson et al., 2014). Due to the host preference effect, plant species richness should increase the number of available fungal niches, which in turn should increase fungal OTU (operational taxonomic unit) richness. This pattern has been described in several mesocosm experiments, wherein host plant composition was manipulated along a species richness gradient (Burrows & Pflieger, 2002; Hiiesalu et al., 2014), although other similar studies failed to detect a significant relationship (Antoninka et al., 2011). Because different plant species harbor different fungal compositions, the relative abundance of plants, usually measured using evenness indices, may determine the relative availability of specific fungal niches (i.e., based on the availability of preferred hosts).

However, in most plant communities, plant distribution is not random (Law & Dieckmann, 2000). The spatial configuration of a plant community defines the spatial distribution of plant species within that community. In nature, plant species co-occur locally with different degrees of aggregation (Benot et al., 2013). Plant patches within a plant community differ in size and in their degree of isolation but may also differ in the nature and frequency of interface types (i.e., between plant patches of different species). All these parameters are likely to influence the composition of fungal assemblages because they influence the probability of encountering plants of the same or different species. Due to host preference, patch characteristics may affect a fungus' ability to colonize nearby host plants (isolation effect) and the amount of available microhabitat (carrying capacity effect). Therefore, the configuration of a plant community and especially that of a given host plant species should influence the structure of fungal assemblages. For macro-organisms, landscape ecology theory predicts that low patch size and high isolation reduce species richness. A decline in habitat availability and reachability can select for fungal species able to develop and disperse despite such conditions, which likely leads to a potential shift in fungal species composition and a lower evenness index due to the selection of species that are less sensitive to habitat fragmentation (Fahrig et al., 2011). The transposition of these hypotheses to fungal root communities has been made at large landscape scale. Some authors have tested the effect of isolation between host plants (Peay et al., 2010; Vannette et al., 2016), or host plant patch sizes

(Mangan et al., 2004; Grilli et al., 2012), but the transposition of these predictions has only rarely been made at the smaller plant community scale (but see Batten et al., 2006 in the context of invasive plant species).

Competition among fungal species occurs in plant-fungal interactions, especially depending on the availability of plant roots to colonize. For instance, AMF (obligate biotrophs) are fully dependent on host plants for their carbon supply (Parniske, 2008). The first fungi that colonize are favored (e.g. priority effect) (Werner & Kiers, 2015), which in turn may lead to the dominance of specific fungal species or OTUs as based on DNA sequences. Plant root mass is thus a determinant of competition intensity and hence a driver of fungal assemblages (Cano & Bago, 2005; Engelmoer et al., 2014). In theory, a low root surface area available for colonization can result in rapid saturation of available microhabitats and possibly lead to reduce fungal richness in the mycobiota.

Our aim is to study plant-fungus interaction using the passenger hypothesis, to disentangle the effect of the heterogeneity of plant cover (in composition and configuration) and plant primary productivity (used as a proxy of root mass) on the root fungal endosphere assemblages of *Brachypodium pinnatum*. This focal plant species is a common grassland plant species that has been shown to support a wide range of fungi (Bittebiere et al., in press), like other species of the same genus (Donn et al., 2017). To test the effect of plant heterogeneity and primary productivity, we grew a range of manipulated plant communities in a homogeneous environment (semi-controlled growing conditions in an experimental garden) to represent a wide range of floristic compositions and configurations. We investigated fungi that develop naturally in *B. pinnatum* roots and characterized fungal assemblages (composition, richness and evenness in fungal OTU root endosphere) at the mesocosm scale (i.e., in several samples from each mesocosm) using amplicon sequencing.

By analyzing the structure of fungal assemblages in response to the characteristics of the floristic landscape, we tested the following three predictions:

- (1) Because of the host preference effect, plant community represents a range of different fungal niches, each plant species harboring a particular fungal assemblage. Plant species richness thus enhances fungal OTU richness and plant evenness increases fungal evenness.
- (2) Because of the host preference effect, plant configuration determines the carrying capacity of the host-plant patch, as well as the ability of fungal assemblages to disperse

among the different individual host plants. *B. pinnatum* patch size enhances fungal OTU richness and evenness, whereas *B. pinnatum* isolation decreases these parameters.

(3) Because a higher plant mass represents more roots to colonize and because of the existing competition between fungal microorganisms for available root space, plant productivity promotes fungal OTU richness and modifies the relative abundances of OTU.

Floristic heterogeneity of composition, of configuration and primary productivity – are hypothesized to determine fungal OTU composition. To test these hypotheses, we defined root distribution and availability based on aerial plant coverage; for practical reasons, we assumed that aerial coverage corresponds to root distribution, and estimated root production based on plant aerial biomass. We discuss the limitations of these hypotheses (Hiiesalu et al., 2014).

Methods

Sampling design

In 2012, we analyzed root fungal endosphere assemblages in 25 plant communities already established in an experimental garden at the University of Rennes 1, France. These plant communities have been growing in mesocosms (1.3 m x 1.3 m x 0.25 m) since May 2009 (Benot et al., 2013). The mesocosms consisted of five types of communities comprising 1, 2, 4, 8 or 12 perennial plant species (4 to 6 replicates each), all of which are widely distributed in temperate grasslands in western France (*Agrostis stolonifera*, *Agrostis tenuis*, *Brachypodium pinnatum*, *Centaurea nigra*, *Chamaemelum nobile*, *Dactylis glomerata*, *Elytrigia repens*, *Festuca rubra*, *Holcus lanatus*, *Holcus mollis*, *Lolium perenne* and *Ranunculus repens*). Forty-eight plants were originally planted in each mesocosm using equal proportions of all the species of the mixture (i.e., for the 12-species mixture, 4 plants of each species were planted) in a hexagonal planting pattern. Their spatial positions within the planting pattern were randomized in each mesocosm. The plants were grown on a homogeneous substrate comprising 20% sand, 80% garden soil and a slow release fertilizer. The communities were mown once a year in the fall and all mown biomass was removed. Plant growth only refers to the development of the original plantlets because weeds were removed by hand and flowers were cut off before they went to seed. Each mesocosm (1.69 m²) included at least two patches of *B. pinnatum*.

We analyzed the fungal assemblages of *B. pinnatum* roots in all mesocosms. *B. pinnatum* grows laterally by producing sympodial rhizomes with dense and aggregated ramets (i.e., phalanx form) (Benot et al., 2013). Whenever possible, five individuals of *B. pinnatum* were selected from the corners and center of each mesocosm based on the grid used to map plant distribution (Fig. 1). The composition of the fungal endosphere in the roots of each sample was analyzed by amplicon mass sequencing (see below). The fungal assemblages in the root endosphere were then characterized at the mesocosm scale, i.e. by grouping all samples from one mesocosm (Fig. 1). The effects of floristic heterogeneity and plant productivity were analyzed on the fungal assemblages in the roots of *B. pinnatum* at the population scale, i.e. the gamma diversity of *B. pinnatum* mycobiota was assessed at the mesocosm scale.

Characterizing floristic landscapes

Mapping plant distributions

We recorded spatial distributions of plant species in all mesocosms in 2012. All the species were mapped using a square 80-by-80-cm grid positioned at the center of each mesocosm. Each grid was comprised of 256 cells, each 5 x 5 cm in size. Species were recorded as present or absent in each cell, depending on whether at least one ramet was rooted in the cell. Composition differed between the types of mixture and even within one type of mixture due to the difference in original planting patterns and to the natural dynamics of plant species linked to plant clonal growth and competitive interactions (Table S1).

Using landscape indices to characterize floristic heterogeneity

From the species distribution maps, we analyzed floristic cover as a landscape within which plant patches define specific landscape elements. Following methods used to describe landscape heterogeneity, two categories of indices were calculated and used to characterize plant composition and configuration (see review by Fahrig *et al.*, 2011) (Fig. 1; Table 1).

Plant heterogeneity of composition was assessed through two indices: plant species richness and evenness. Plant species richness was defined as the number of different host plant species present in the mesocosm representing the types of micro habitat available for fungal colonization. Evenness reflects the relative abundance of plant species within the floristic

landscape and hence the relative proportion of the different micro habitat types available for colonization. Evenness was measured using Pielou's evenness index, based on plant abundances over the entire grid (Pielou, 1966). Plant abundance was calculated as the total number of cells occupied by a given plant species within a grid. Both indices were calculated using the vegan package of R software (Oksanen et al., 2013).

Plant heterogeneity of configuration was assessed relative to the spatial distribution of the host plant, *B. pinnatum*. We selected two indices, patch aggregation and patch isolation. Patch aggregation characterizes patch size. A low aggregation value reflects a low patch carrying capacity for fungi assemblages, but also implies a stronger edge effect, i.e. the potential of neighboring plants to influence fungi of the host-plant in the patch. Patch aggregation was calculated using the percentage of like adjacencies (PLADJ), which is computed as the sum of the diagonal elements of the adjacency matrix divided by the total number of adjacencies. PLADJ is high when *B. pinnatum* is aggregated. When patches of *B. pinnatum* are highly fragmented into small patches, PLADJ is low, i.e. they contain fewer like adjacencies. Degree of patch isolation was measured as the mean Euclidean nearest neighbor distance between patches of *B. pinnatum*. Nearest neighbor distance is defined using simple Euclidean geometry as the shortest straight line distance between the cell center of the focal patch and its two nearest neighbors. We calculated PLADJ and ENN indices using the presence-absence of *B. pinnatum* with FRAGSTAT software v4 (McGarigal et al., 2012).

Characterizing plant production

In 2012, we harvested the aboveground vegetation in each mesocosm at the end of summer, as had been done in prior years. Biomass was defined as total fresh mass of aboveground cover and weighed. We used a subsample to calculate water content (to convert fresh biomass into dry biomass). The subsample was weighed before and after drying at 65°C for 72 h. Productivity was calculated by converting fresh biomass into dry biomass per unit surface area (g.m⁻²).

Analyses of fungal assemblages

In 2012, samples of *B. pinnatum* roots belonging to single plants were collected in each mesocosm (Fig. 1). The roots were cleaned in a 5‰ Triton X100 solution and thoroughly rinsed with tap water, then with sterile, distilled water. Roots were reduced to a powder under liquid nitrogen using a sterile mortar and pestle. Total DNA was extracted from a 100 mg sub-sample using the DNEasy plant mini kit (Qiagen) following the manufacturer's recommendations. A specific 550 bp of the fungal SSU rRNA gene fragment, including the V4 and V5 regions, was amplified from ~50 ng of extracted DNA using the primers SSU0817 (5'-TTA GCA TGG AAT AAT RRA ATA GGA-3') and -NS22B (5'-AAT TAA GCA GAC AAA TCA CT-3') (Borneman & Hartin, 2000; Lê Van et al., 2017). The PCR reactions, performed with Illustra™ PuReTaq Ready-to-go (GE Healthcare®), contained 0.2 µM of primers in a final volume of 25 µL. The cycling regime was identical to methods used by Lê Van et al. (2017). On 5', the two primers contained the Illumina® tails necessary to perform a second PCR and the amplicon identification necessary for multiplexing. Each amplicon was then purified (Agencourt® AMPure™) using a Bravo Automated Liquid Handling Platform (Agilent®), then quantified (Quant-iT™ PicoGreen™ dsDNA Assay Kit) and normalized to the same concentrations before pair-end sequencing (Illumina®, Miseq). Both the sequencing library and mass sequencing were performed at the Human and Environmental Genomics platform ([https://geh.univ-rennes1.fr/?\[en\]](https://geh.univ-rennes1.fr/?[en])). The sequences were deposited in the European Nucleotides Archive ENA (<http://www.ebi.ac.uk/ena>) and are accessible under accession number PRJEB27968.

Sequence data were analyzed using the FROGS pipeline (Escudié et al., 2017). In FROGS, sequence clustering was performed using SWARM (Mahé et al., 2014). This clustering process enables grouping of sequences with no threshold. To limit the risks of obtaining artificial OTUs, we only kept identical sequences we observed in at least five samples. OTUs were assigned to taxa by comparing them to the Silva 18S database release 132 (Quast et al., 2013). Data were organized in a contingency table and the number of sequences per sample was normalized (i.e. 19,224) using the VEGAN package in R. This normalized database was used for all statistical analyses.

Mycobiota assemblages of the root endosphere were studied in (1) the entire assemblage (hereafter “All fungi”) and (2) in each group, focusing on Ascomycota, Basidiomycota and Glomeromycotina. Fungal OTU richness and evenness were calculated based on the fungal composition pooled over all samples of the mesocosm (i.e., total OTU pool with their mean abundances; Fig. 1). OTU richness was calculated as the total number of OTU present. Evenness

was measured using Pielou's evenness index based on the mean abundance of OTU at the mesocosm scale (Pielou, 1966). Both OTU richness and evenness were calculated using the vegan package in R.

Statistical analysis

Prior to analyses, we center-reduced all independent variables to enable comparison between regression coefficients within models. To be sure landscape variables were not redundant, we analyzed the Pearson correlations between all pairs of variables. Most correlations were lower than 0.7 (Dormann et al., 2013, Table S2). Because our experimental design might include a sampling effect due to the occurrence of different plant community mixtures (see preceding section), we checked that the heterogeneity and productivity values were not strongly related to the mixture types used (Fig. S1). Except for plant richness, values strongly overlapped among the mixture types.

To analyze the effects of floristic variables on fungal OTU richness and evenness, we used multiple regression analyses with productivity, plant species richness, plant equitability, *B. pinnatum* patch aggregation and degree of *B. pinnatum* patch isolation as explanatory variables. When necessary, the dependent variables were log-transformed to ensure normal distribution of model residuals. A stepwise selection procedure based on Akaike's information criterion (AIC) was used to select the optimal set of explanatory variables. We calculated the regression coefficients and the proportion of variation of the explained variables accounted for by the R^2 coefficient of determination. The significance of each explanatory variable in the model and of the global model was assessed using Type II ANOVA.

To confirm that the effects observed were not an artifact of *B. pinnatum* abundance, we checked that the optimized models displayed a higher R^2 than the model with only *B. pinnatum* abundance, indicated as the number of occupied cells in the grid, as an explanatory variable (see Table S3). In all cases, the models were better explained by the variables used in the optimized model than only by host plant abundance. Differences between the two models were higher when fungal OTU evenness was the dependent variable.

Canonical correspondence analyses (CCA) were used to investigate whether the fungal OTU composition depended on landscape variables. Separate analyses were performed for "All

fungi”, Ascomycota, Basidiomycota and Glomeromycotina. We coupled the matrix of OTU composition under the constraint of the environmental variables matrix (composition, configuration and productivity variables). Fungal OTU composition of the mesocosm was assessed using presence-absence data for OTUs. The significance of the independent landscape variables in the CCA structure was tested using ANOVA like permutation tests. These analyses were performed using the vegan package. All statistical tests were performed using R 2.15.3 software (R Foundation for Statistical Computing, Vienna, Austria).

Results

The 25 mesocosms encompassed a wide range of floristic compositions and configurations (Table 1).

Characteristics of fungal assemblages

Sequencing depth was sufficient to describe the mycobiota in *B. pinnatum* roots in detail (Fig. S2). The mycobiota was dominated by the three groups Ascomycota, Basidiomycota and Glomeromycotina (Fig. 2) that accounted for 66.4%, 26.9% and 4.6% of the sequences respectively. The dataset contained a total of 1 172 OTUs. Basidiomycota accounted for 305 OTUs, of which 276 belonged to the class Agaricomycetes. Ascomycota was the most diverse group, with a total of 718 OTUs. The dominant classes in term of OTU richness were Sordariomycetes (253 OTUs), Dothideomycetes (158 OTUs) and Leotiomycetes (151 OTUs). Within the Glomeromycotina group, we found 62 OTUs of which 54 were Glomerales (91 560 sequences), seven were Diversisporales (20 sequences) and one was Paraglomerales (4 sequences). Fungal OTU richness varied from 111 to 232, depending on the specific mesocosm examined (Table 2). Ascomycota, Basidiomycota and Glomeromycotina represented an average of 61% +/- 5%, 24% +/- 5% and 5% +/- 1% of the fungal OTUs, respectively.

*Impact of floristic composition and configuration on fungal OTU richness and evenness associated with *B. pinnatum* roots*

The structure of the fungal community in *B. pinnatum* roots depended significantly on landscape composition and/or configuration in four of the eight statistical models tested (Table 2). The OTU richness of Glomeromycotina was not affected by the floristic landscape structure, nor was the OTU evenness of 'All fungi', Basidiomycota or Glomeromycotina. The OTU richness of Ascomycota depended on plant species richness, whereas the OTU richness of Basidiomycota depended only on configuration with a significant effect of the degree of aggregation and isolation of *B. pinnatum*. Therefore, the OTU richness of Ascomycota was the highest in monocultures of *B. pinnatum* (host plant) and the lowest in species rich communities. The OTU richness of Basidiomycota was significantly lower in *B. pinnatum* growing in mesocosms in which *B. pinnatum* patches were small and isolated than when the patches were large and located close to one another. In the 'All fungi' assemblages, both plant species richness and the degree of *B. pinnatum* aggregation influenced OTU richness. In addition, higher plant evenness was associated with lower OTU evenness of Ascomycota, whereas the other groups were not significantly linked to any floristic landscape variable.

Plant productivity varied from 324 to 1 612 g/m² (mean = 701 g/m²) with a standard deviation of 327 g/m². Productivity had a significant effect on two of the eight models we tested. Productivity reduced evenness in both the 'All fungi' assemblage and in the Ascomycota group. The effect of productivity on the OTU evenness of Ascomycota was so strong that it was even detectable in the 'All fungi' model. However, plant productivity did not influence OTU richness in any of the fungal groups we examined.

Impact of floristic composition and configuration on fungal assemblage composition associated with B. pinnatum roots

'All fungi' and the OTU of *Ascomycota* composition were driven by the floristic landscape structure with a significant explained deviance above 0.20 (Table 3). The OTU composition of *Ascomycota* depended on plant evenness and productivity, whereas the composition of the 'All fungi' assemblages depended on plant evenness and the degree of isolation of *B. pinnatum*. The non-significance of the other tests, especially for Glomeromycotina, despite the proportion of variance explained, may be linked with the limited number of sequences assigned to this group.

Discussion

*The complexity of mycobiota on the roots of *B. pinnatum**

The microbiome composition varied widely among individual plants, with only ~3% of OTUs shared by all plants, and this may be an underestimation related to the description of the rare biosphere. This high variability is likely due to variation in the floristic landscapes where individual plants grow. Indeed, other possible factors were standardized. Genotypic differences between plants were restricted because plant fragments were sampled in a single population. Donn et al. (2017) also reported for *Brachypodium distachyon*, a closely related species to our focal host plant, that only a small fraction of the observed variation in AM fungal community composition was due to differences among plant genotypes. Soil conditions were also standardized at the beginning of our experiment. However, we cannot exclude the possibility that plants induced changes in the soil characteristics over the three years of plant development in the different manipulated communities. The effects of plants on soil conditions are likely to have indirect effects on microbial nutrient cycling thereby influencing plant root mycobiota. Due to these potential indirect effects, our results do not strictly demonstrate the passenger paradigm (Hart et al., 2001) but rather show a broader impact of floristic landscapes on root mycobiota, including direct and indirect effects of plants.

Floristic heterogeneity determines fungal assemblages

As expected, the heterogeneity of floristic composition or configuration or both impacted the composition, richness and evenness of root fungal assemblages. Nevertheless, a possible sampling effect even if weak (see methods section for more details), is likely as the mesocosms originally contained five different types of mixture, that were used to create the gradient of floristic heterogeneity and productivity.

Plant cover determined both the presence and relative abundance of OTUs within the mycobiota of *B. pinnatum* roots. We cannot exclude the possibility that a partial limitation of this result might be due to our estimation of root distribution and production based on aboveground cover. Indeed, Hiiesalu et al., (2014) demonstrated 50% underestimation of plant species richness when aboveground plant distribution was used relative to belowground distribution at a small scale of 0.01m², possibly due to plant clonality (occurrence of belowground clonal organs) and

dormant belowground organs. This underestimation led the authors to infer stronger relationships in AMF-plant richness and productivity using belowground than aboveground plant cover, suggesting that we may have underestimated the strength of some relationships in the present study.

Beyond the effects already reported for plant richness and evenness on microbiota in previous studies on AMF, (Burrows & Pflieger, 2002; Landis et al., 2004), our study demonstrated that the spatial configuration of host plants also affects the structure of mycobiota assemblages. The spatial configuration of plants may explain the strong small-scale spatial heterogeneity often reported for fungi (Bahram et al., 2015). The effect of floristic heterogeneity and productivity we demonstrated in our study is likely, at least partly due, to the relative abundance of *B. pinnatum*, which impacts both the composition and the configuration of the floristic cover. In two of the three fungal groups we examined, OTU richness and to a lesser extent OTU evenness were both influenced by the abundance of the host plant. However, the abundance of *B. pinnatum* appeared to play a less prominent role in determining the characteristics of fungal assemblages than did floristic heterogeneity. The preferential associations between plants and fungal communities (i.e., host preference effect), which is already known for Glomeromycotina (Vandenkoornhuysen et al., 2003; Gollote et al., 2004; Martinez-Garcia & Pugnaire, 2011; Torrecillas et al., 2012) was a strong assumption in the present work, as it was assumed to be the mechanism underlying the floristic heterogeneity effect. Interestingly, in the mesocosm experiment we conducted, we did not detect any such effect in Glomeromycotina. The absence of relationship may be due to a lower statistical power because of the low limited number of sequences assigned to this group; or to a weaker host preference effect in this group than in other plant-microbe associations. To validate the relationships we found, further sampling campaigns are necessary to account for temporal dynamics in OTU composition.

Contrasted responses of fungal groups to floristic heterogeneity

Fungal groups differed in their responses to floristic landscape structure: Ascomycota assemblages associated with *B. pinnatum* roots were mostly driven by the heterogeneity of composition, whereas Basidiomycota assemblage richness was driven by the host plant configuration in the floristic landscape (Tab. 2; Fig. 3).

Ascomycota assemblages were richer in species-poor plant communities than in species-rich plant communities. This may suggest that the abundance of the host plant is a dominant driver of both coexistence and of competition between fungal OTUs. Because plant species richness is also closely related to changes in the types of plant mixture, we cannot exclude a possible alternative explanation due to the composition of plant species. Richer plant assemblages, which would include species that are not present in the other mixture types, may include species that are less mycotrophic or that secrete allelopathic molecules that prevent plants from being colonized by fungi; and possibly explaining the lowest richness of Ascomycota in species rich plant cover. To enable deeper interpretations of the results presented here, future work would need to analyze the root colonizing microorganisms of both the target plant and its neighbors to disentangle the effect of the floristic variables from the effect of plant identities. Changes in fungal composition associated with *B. pinnatum* also indicated that the relative balance of plant abundances influences the composition of the fungal assemblages associated with plant roots, probably by acting on the fungal pool available for *B. pinnatum* recruitment.

Basidiomycota assemblages were richer in plant communities in which *B. pinnatum* patches were aggregated and close to one another. Such habitat size effects on biodiversity is a classical paradigm in macro-ecology. Large habitat patches have been demonstrated to have a higher carrying capacity than smaller ones (MacArthur & Wilson 1967; Fahrig et al., 2011), and to offer more micro habitats (Honnay et al., 2005). In this particular case, large patches of *B. pinnatum* may provide roots of different ages and morphologies that provide more available fungal niches locally. Additionally, the high sensitivity of Basidiomycota assemblages to patch isolation suggests dispersal limitation of microorganisms when considering the assemblages at the host population level. Isolation indeed leads to the thinning out of the assemblage by selecting resistant species able to disperse in fragmented habitats. In our experimental system, vegetative multiplication through hyphae may have been favoured over sexual reproduction. We suggest that hyphal growth leads to short dispersal distances, thereby explaining the dispersal limitation.

Glomeromycotina assemblages associated with *B. pinnatum* roots were surprisingly independent of the floristic composition and configuration patterns of the host plants. This may be due to the small number of sequences detected (see preceding section), or to weak host preference in the plant communities we used. There is empirical evidence that the plant host species acts both on hyphal growth and sporulation (Bever et al., 1996; Eom et al., 2000). Hawmann & Hawes (2010) additionally demonstrated that neighboring plant composition may strongly affect the

composition of AMFs associated with a focal host plant. However, existing evidence for this host plant-AMF interaction has only been reported in particular cases, such as exotic plant species invading a native community (Mummey et al., 2005; Mummey & Rillig, 2006; Lekberg et al., 2013) or when phylogenetically diverse plant composition was experimentally manipulated (Burrows & Pfleger, 2002). In both situations, the composition of their root endospheric mycobiota may differ considerably among the plant species. In the present study, 75% of the plant species growing in our experimental mesocosm mixtures belonged to the same taxonomic group (*Poaceae* species) and dominated the plant cover. In such cases, plant landscape heterogeneity may have little effect on Glomeromycotina composition.

Plant productivity affects mycobiota composition and evenness in Ascomycota

Contrary to our expectations, productivity did not result in higher richness in the fungal assemblage. This suggests that there is no direct relationship between plant productivity and the quantity of roots available for fungal colonization. It would be possible to investigate this relationship by only examining the productivity of the host *B. pinnatum*, due to the strong host preference effect we detected for Ascomycota and Basidiomycota. However, we did not collect these data and were thus not able to test this relationship. The composition and evenness of Ascomycota assemblages were influenced by plant productivity. Because of the sampling design, i.e., we had established and maintained the experimental plant communities for three years before sampling the fungal community, the direction of the causal effect is difficult to assess. Differences in productivity may result from characteristics of associated fungal assemblages (not the reverse) (van der Heijden et al., 1998; Vogelsang et al., 2006; Maherali & Klironomos, 2007). The overwhelming dominance of some Ascomycota found in the more productive plots suggests that this particular mycobiota stimulates plant productivity. Because the Ascomycota pool contained a wide diversity of fungal symbionts, ranging from pathogens to mutualists, the dominance of highly mutualist species in the fungal community would contribute to the ecosystem productivity by mediating plant-plant interactions.

A micro landscape approach: plant spatial arrangements may influence microbiota assemblages

A debate arose regarding the effect of plant composition on fungal assemblages. Our work provides additional information on the relationship between the assembly of the root endospheric mycobiota and plant composition. Besides influencing plant composition, our data suggest that fine scale spatial arrangements of host plants influence the structure of the mycobiota associated with its roots. A plant community can be seen as a micro landscape for fungal assemblages and its composition and configuration appear to modify the assemblages. Plant influence on fungal assemblages possibly includes direct host preference effects, already shown to be a driver of AM fungal assemblages, which we here extend to the whole fungal endosphere assemblage. Plant influences could also include indirect effects on soil composition and structure (Cornellissen et al., 2014). These two candidate mechanisms cannot be easily disentangled. It may consequently be difficult to separate the effect hypothesized in the passenger paradigm from the effect hypothesized in the habitat paradigm in this particular medium term garden experiment, where individual plants impact their own local microhabitat. This study is the first to explore the role of plant spatial heterogeneity on plant-microbiota assembly rules by transposing methods of landscape ecology to a much finer scale grain, thus identifying possible new avenues for understanding plant-microbiota assembly rules. These results provide additional insights into other ways to manipulate root microbiota based on the spatial arrangements of plant species.

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Authors' contributions

CM and PV conceived the project and all the authors contributed to the methodology of the study. PB, NV, A-KB and CM collected the data. PB, NV and PV did the sequence analyses. CM

statistically analyzed the data and wrote the manuscript. All authors contributed critically to the interpretations of results and to the drafts and gave their final approval for publication.

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Supporting Information

Fig. S1 Value distribution of floristic variables (Heterogeneity of composition, heterogeneity of configuration, productivity) for each mixture type (M1 to M5, composition of the mixtures described in Fig. 1).

Fig. S2 Rarefaction curves for all samples.

Table S1 Range of plant abundance for each mixture type: minimum and maximum of number of cells occupied above 256, expressed as a percentage.

Table S2 Spearman correlation between landscape characteristics.

Table S3 Statistics of the linear models explaining fungal OTU richness and evenness, based on *B. pinnatum* abundance.

Figure captions

Fig. 1: Presentation of the experimental design. a. Mesocosm design and plant maps; b. Floristic landscape heterogeneities with respect to the composition and configuration of plant species. Heterogeneity of composition is determined by plant species richness and evenness. Heterogeneity of configuration is determined by patch size and the distance between patches. We only considered the spatial arrangement of the focal host plant, *Brachypodium pinnatum* (adapted from Fahrig et al., 2011); c. Measurement of gamma diversity of microorganisms.

Fig. 2: The mycobiota of *Brachypodium pinnatum* roots. Number of sequences (left panels) and operational taxonomic unit (OTU) richness at the group level (top panels, 'All fungi'), and in the Basidiomycota, Ascomycota and Glomeromycotina groups for all samples combined.

Fig. 3: Effects of floristic landscape heterogeneity and productivity on fungal assemblages. Heterogeneity is based on plant composition (plant richness and evenness), host plant spatial configuration (degree of aggregation and isolation of *Brachypodium pinnatum*). Fungal assemblage characteristics comprised the richness and evenness of operational taxonomic unit (OTU). Fungal groups in which significant effects were detected include "All fungi" (green dots), Ascomycota (orange dots), Basidiomycota (blue dots). Only significant effects are presented (see Table 2 for complete statistical results). Each graph provides partial residuals, i.e., the effects of a given independent variable when all other independent variables are statistically fixed.

Table 1: List of the floristic landscape variables and potential and observed ranges of variation. SD (Standard Deviation)

Variable	Formula	Potential Variation	Observed variation in mesocosms Mean (SD) / Min-Max
Heterogeneity of composition			
Plant species richness (Richness)	$Richness = S$ S: Number of different species	Varies from 0 to 12	3.8 (2.9) / 1-10
Plant Evenness (Equi) (Pielou, 1966)	$Equi = \frac{-\sum_{i=0}^S \log(p_i)}{\log(S)}$	Varies from 0 (dominance of one host-plant species) to 1 (equal distribution of all host-plant species)	0.62 (0.38) / 0-1
Heterogeneity of configuration			
Proportion of Like Adjacencies (PLADJ) (Gardner & O'Neill, 1991)	$PLADJ = \frac{g}{\sum_{k=1}^S g_k} \times 100$ g = number of adjacencies between two patches of <i>B. pinnatum</i> g _k = number of adjacencies between	Varies from 0 (fragmented patches) to 1 (aggregated patches)	78.8 (14.1) / 33.3 – 92.3

a cell of *B. pinnatum* and a cell of a patch type k (involves focal and non-focal patch types)

Mean Euclidean Nearest Neighbor (ENN-MN)
(McGarigal & Marks, 1995)

$$ENN - MN = \frac{\sum_{i=1}^n d_i}{n}$$

n = number of patches
d_i: distance (m) from *B. pinnatum* patch i to nearest neighboring *B. pinnatum* patch, based on patch edge-to-edge distance, computed from cell center to cell center

Varies from 0 to 113 cm (113cm is the diagonal of the grid if only two patches are present and located at the most extreme corners of the grid)

7.0 (6.7) / 0-20.6

Table 2: Summary of the best models explaining fungal operational taxonomic unit (OTU) richness and evenness.

	Mean (SD)	Range	Plant composition				<i>B. pinnatum</i> configuration				Plant productivity		Adj-R ²	
			Richness		Evenness		Aggregation		Isolation		Est	F		
			Inter	Est	F	Est	F	Est	F	Est				F
Richness														
All fungi	184 (37)	111-232	184.0	-14.5	5.4*			16.0	6.5*					0.42**
<i>Ascomycota</i>	111 (24)	63-150	111.4	-10.6	6.3*									0.35**
<i>Basidiomycota</i>	45 (14)	20-67	44.7					6.2	6.3*	-7.8	10.4**	-3.0	1.75	0.60***
<i>Glomeromycotina</i>	9 (4)	3-16	8.92	-1.22	2.12	1.25	2.20							0.03ns
Evenness														
All fungi	0.41 (0.04)	0.29-0.50	0.41					-0.01	2.5			-0.02	9.7**	0.52***
<i>Ascomycota</i>	0.34 (0.06)	0.20-0.44	0.34			-0.02	7.2*					-0.06	40.7***	0.63***
<i>Basidiomycota</i>	0.26 (0.08)	0.12-0.43	0.27											0.11ns
<i>Glomeromycotina</i>	0.28 (0.10)	0.10-0.56	0.28											0.16ns

Models are based on variables of floristic composition (plant richness and plant evenness), configuration, (patch aggregation and degree of isolation of *Brachypodium pinnatum*), and plant productivity. Model parameters provided are the selected variables derived from the Akaike procedure with

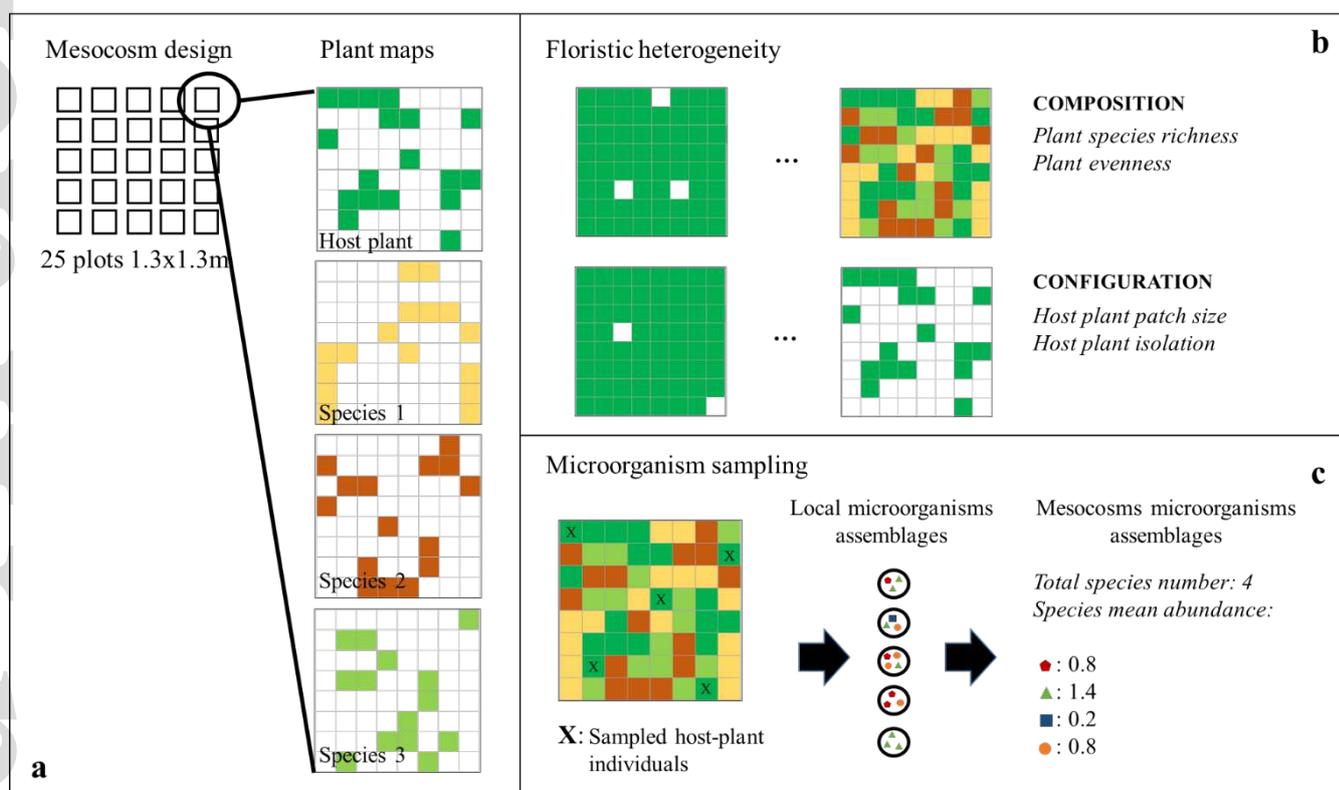
their estimated coefficient (Est.) and significance (F-test) values. Significant models are in bold: ns (not significant), * ($P < 0.05$), ** ($P < 0.01$), *** ($P < 0.001$), adj-R, (adjusted- R^2). Mean, standard deviation (SD), and the minimum and the maximum values.

Table 3: The effect of the plant landscape variables and plant productivity on the mycobiota inhabiting *Brachypodium pinnatum* roots.

	Explained deviance	Plant species richness	Plant species evenness	<i>B. pinnatum</i> aggregation	<i>B. pinnatum</i> isolation	Plant productivity
All fungi	0.22*	0.98 ns	1.19 *	1.02 ns	1.18 *	1.09 ns
Ascomycota	0.23*	1.01 ns	1.21 **	1.06 ns	1.14 ns	1.18*
Basidiomycota	0.22 ns					
Glomeromycotina	0.24 ns					

Percentages of explained deviance and significances were calculated using ANOVA-like permutation tests with canonical correspondence analysis (CCA). Values are given for the 'All fungi', Ascomycota, Basidiomycota, and Glomeromycotina groups when the explained deviance is significant. Abbreviations: ns (not significant), * ($P < 0.05$), and ** ($P < 0.01$). Significant values are in bold.

Figure 1



1 **Figure 2**

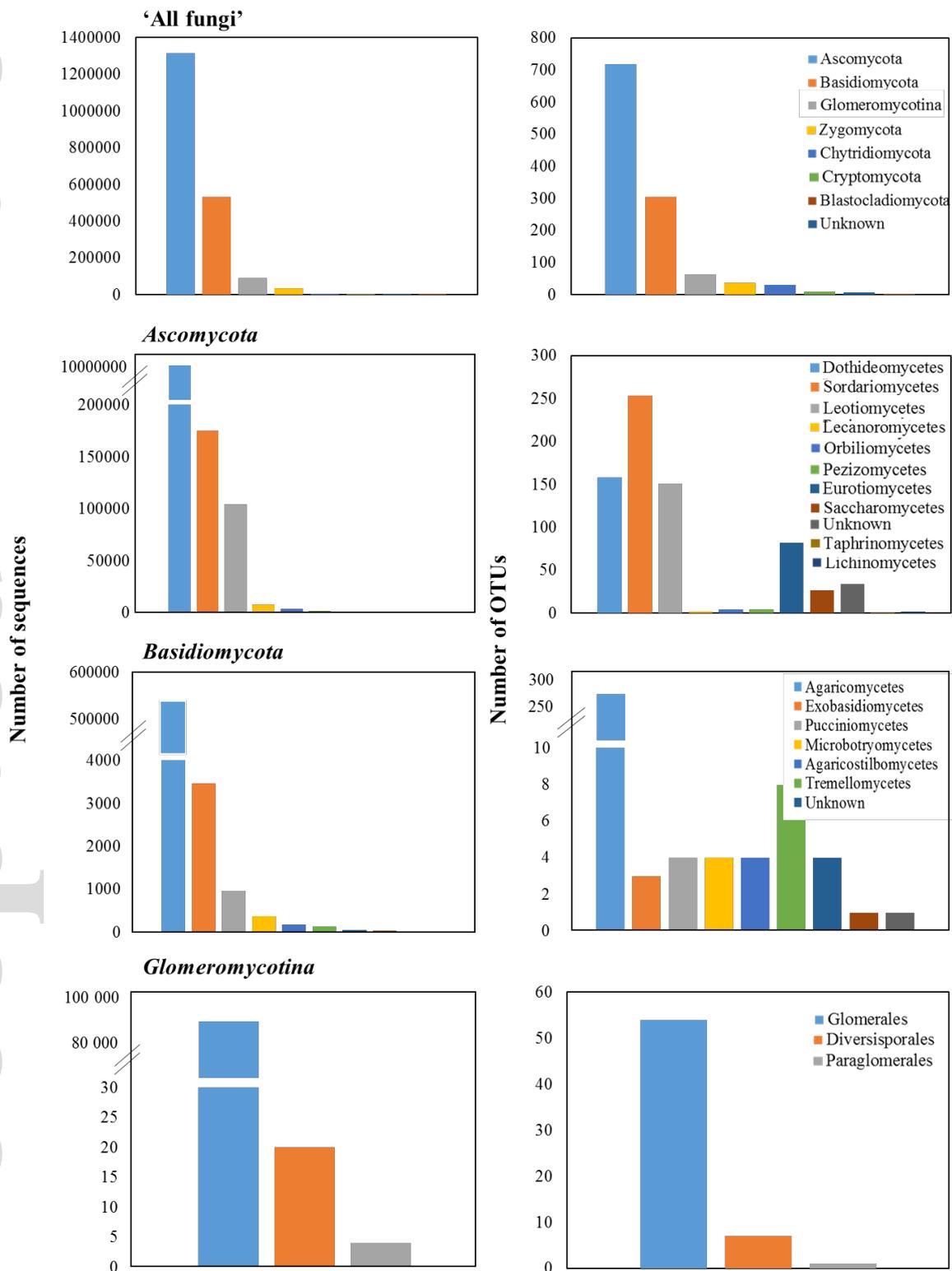


Figure 3

