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Research

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

Cendrine Mony[1], Philomène Brunellière[1], Nathan Vannier[1,2], Anne-Kristel Bittebiere[3] and Philippe Vandenkoornhuyse[1]

[1]UMR 6553 Ecobio, CNRS – University of Rennes, Avenue du Général Leclerc, 35042, Rennes Cedex, France; [2]Department of Plant Microbe Interactions, Max Planck Institute for Plant Breeding Research, 50829, Cologne, Germany; [3]UMR 5023, LEHNA, CNRS – University of Lyon 1, 43 Boulevard du 11 Novembre 1918, 69622, Villeurbanne Cedex, France

Author for correspondence:
Cendrine Mony
Tel: +33 (0)2 23 23 64 79
Email: Cendrine.mony@univ-rennes1.fr

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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 operational taxonomic units (OTUs) 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 nonexclusive mechanisms to explain this spatial correlation. The driver paradigm assumes that fungal symbionts regulate plant assemblages. Fungal symbionts indeed play a determining role in plant nutrition (Marschner, 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 (Vandenkoornhuyse *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) (Vandenkoornhuyse *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 (Vandenkoornhuyse *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 the 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 focus mainly on plant interactions following experimental inoculations of fungal assemblages in multispecies

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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 operational taxonomic unit (OTU) richness. This pattern has been described in several mesocosm experiments, wherein host plant composition was manipulated along a species richness gradient (Burrows & Pfleger, 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 that are able to develop and disperse despite such conditions, which probably 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 reduced fungal richness in the mycobiota.

Our aim here was to study plant–fungus interactions 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 that has been shown to support a wide range of fungi (Bittebiere *et al.*, 2019), as with 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 (semicontrolled 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, the 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 of 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).

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Materials and 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 × 1.3 × 0.25 m) since May 2009 (Benot *et al.*, 2013). The mesocosms consisted of five types of communities comprising one, two, four, eight or 12 perennial plant species (4–6 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, four 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 refers only 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 m^2) 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 section Analyses of fungal assemblages). The fungal assemblages in the root endosphere were then characterized at the mesocosm scale, that is 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, that is the gamma diversity of *B. pinnatum* mycobiota was assessed at the mesocosm scale.

Characterizing floristic landscapes

Mapping plant distributions We recorded the spatial distributions of plant species in all mesocosms in 2012. All the species were mapped using a square 80 × 80 cm grid positioned at the center of each mesocosm. Each grid consisted of 256 cells, each 5 × 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 (Supporting Information 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 microhabitat available for fungal colonization. Evenness reflects the relative abundance of plant species within the floristic landscape and hence the relative proportion of the different microhabitat types available for colonization. Evenness was measured using Pielou's evenness index, based on plant abundances over the entire grid (Piélou, 1966). Plant abundance was calculated as the total number of

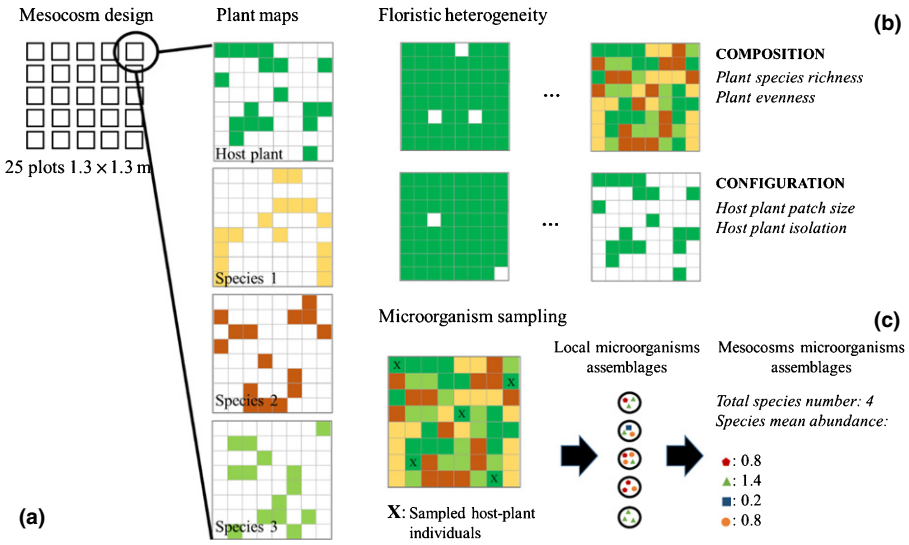


Fig. 1 Presentation of the experimental design. (a) Mesocosm design and plant maps. (b) Floristic landscape heterogeneities with respect to the composition and configuration of plant species. Heterogeneity of composition is determined by plant species richness and evenness. Heterogeneity of configuration 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 the gamma diversity of microorganisms.

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Table 1 List of the floristic landscape variables and potential and observed ranges of variation.

Variable	Formula	Potential variation	Observed variation in mesocosms, mean (SD)/min–max	
Heterogeneity of composition				Landscape 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				Landscape 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 *B. pinnatum* g_k = number of adjacencies between a cell of *B. pinnatum* and a cell f a patch type k (involves focal and non-focal patch types)	Varies from 0 (fragmented patches) to 1 (aggregated patches)	78.8 (14.1)/33.3–92.3	
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 (113 cm 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	

SD, standard deviation.

cells occupied by a given plant species within a grid. Both indices were calculated using the VEGAN package with the R software (Oksanen *et al.*, 2018).

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, namely 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, that is they contain fewer like adjacencies. Degree of patch isolation was measured as the mean Euclidean nearest neighbor (ENN) 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 v.4 (McGarigal *et al.*, 2012).

Characterizing plant production

In 2012, we harvested the above-ground vegetation in each mesocosm at the end of summer, as had been done in previous years. Biomass was defined as total fresh mass of above-ground 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-X 100 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 subsample using the DNEasy plant mini kit (Qiagen) following the manufacturer's recommendations. A specific 550 bp of the fungal small subunit (SSU) rRNA gene fragment, including the V4 and V5 regions, was amplified from *c.* 50 ng of extracted DNA using the primers SSU0817 (5′-TTAGCATGGAATAATRRAATAGGA-3′) and -NS22B (5′-AATTAAGCAGACAAATCACT-3′) (Borneman & Hartin, 2000; Lê Van *et al.*, 2017). PCRs, performed with Illustra PuReTaq Ready-to-go (GE Healthcare, Chicago, IL, USA), 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, Santa Clara, CA, USA), then quantified

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(Quant-iT PicoGreen dsDNA Assay Kit) and normalized to the same concentrations before pair-end sequencing (Miseq; Illumina, San Diego, CA, USA). Both the sequencing library and mass sequencing were performed at the Human and Environmental Genomics platform (https://geh.univ-rennes1.fr/?[en]). The sequences were deposited in the European Nucleotides Archive (http://www.ebi.ac.uk/ena) and are accessible under accession no. 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 the entire assemblage (hereafter 'All fungi'), and 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 OTUs present. Evenness was measured using Pielou's evenness index based on the mean abundance of OTUs at the mesocosm scale (Piélou, 1966). Both OTU richness and evenness were calculated using the VEGAN package in R.

Statistical analysis

Before analyses, we center-reduced all independent variables to enable comparison between regression coefficients within models. To ensure 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 the 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 a 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 (CCAs) 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), which accounted for 66.4, 26.9 and 4.6% of the sequences respectively. The dataset contained a total of 1172 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 terms 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 (four 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 $61 \pm 5\%$, $24 \pm 5\%$ and $5 \pm 1\%$ 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

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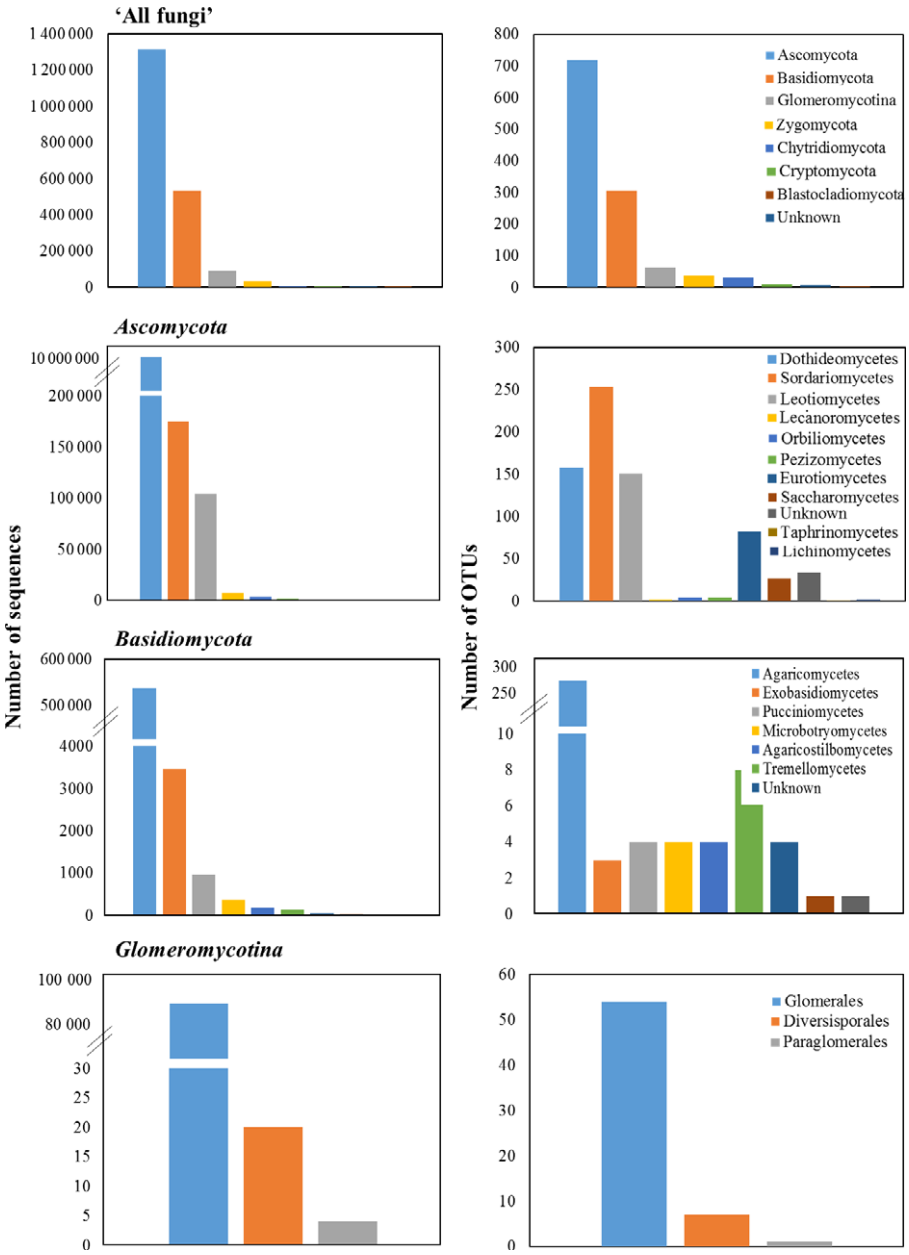


Fig. 2 The mycobiota of *Brachypodium pinnatum* roots. Number of sequences (left panels) and operational taxonomic unit (OTU) richness at the group level (top panels, 'All fungi'), and in the Basidiomycota, Ascomycota and Glomeromycotina groups for all samples combined.

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 1612 $g\,m^{-2}$ (mean = 701 $g\,m^{-2}$) with a standard deviation of 327 $g\,m^{-2}$. 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.

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

				Plant composition				*Brachypodium pinnatum* configuration						
				Richness		Evenness		Aggregation		Isolation		Plant productivity		
	Mean (SD)	Range	Inter	Est	*F*	Est	*F*	Est	*F*	Est	*F*	Est	*F*	Adj-R^2
Richness														
All fungi	184 (37)	111–232	184.0	**−14.5**	**5.4***			**16.0**	**6.5***					**0.42****
Ascomycota	111 (24)	63–150	111.4	**−10.6**	**6.3***									**0.35****
Basidiomycota	45 (14)	20–67	44.7					**6.2**	**6.3***	**−7.8**	**10.4****	−3.0	1.75	**0.60*****
Glomeromycotina	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*****
Ascomycota	0.34 (0.06)	0.20–0.44	0.34			**−0.02**	**7.2***					**−0.06**	**40.7*****	**0.63*****
Basidiomycota	0.26 (0.08)	0.12–0.43	0.27											0.11ns
Glomeromycotina	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 type: 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 are shown.

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	*Brachypodium pinnatum* aggregation	*Brachypodium pinnatum* isolation	Plant productivity
All fungi	**0.22***	0.98 ns	**1.19***	1.02ns	**1.18 ***	1.09ns
Ascomycota	**0.23***	1.01ns	**1.21****	1.06ns	1.14ns	**1.18***
Basidiomycota	0.22ns					
Glomeromycotina	0.24ns					

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. ns, not significant; *, $P<0.05$; **, $P<0.01$. Significant values are in bold type.

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

'All fungi' and the OTUs 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 nonsignificance of the other tests, especially for Glomeromycotina, despite the proportion of variance explained, may be linked to 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 *c.* 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 probably 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 AMF 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 3 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

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likely as the mesocosms originally contained five different types of mixture, which were used to create the gradient of floristic heterogeneity and productivity.

Plant cover determined both the presence and the 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 above-ground cover. Indeed, Hiiesalu *et al.* (2014) demonstrated 50% underestimation of plant species richness when above-ground plant distribution was used relative to below-ground distribution at a small scale of 0.01 m^2, possibly due to plant clonality (occurrence of below-ground clonal organs) and dormant below-ground organs. This underestimation led the authors to infer stronger relationships in AMF–plant richness and productivity using below-ground than above-ground 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 & Pfleger, 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 to be, at least in part, 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 (Vandenkoornhuyse *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 (Table 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 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; this may possibly explain 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 microhabitats (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 that are able to disperse in fragmented habitats. In our experimental system, vegetative multiplication through hyphae may have been favored 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 the 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 on sporulation (Bever *et al.*, 1996; Eom *et al.*, 2000). Hausmann & Hawkes (2010) additionally demonstrated that neighboring plant composition may strongly affect the composition of AMF 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

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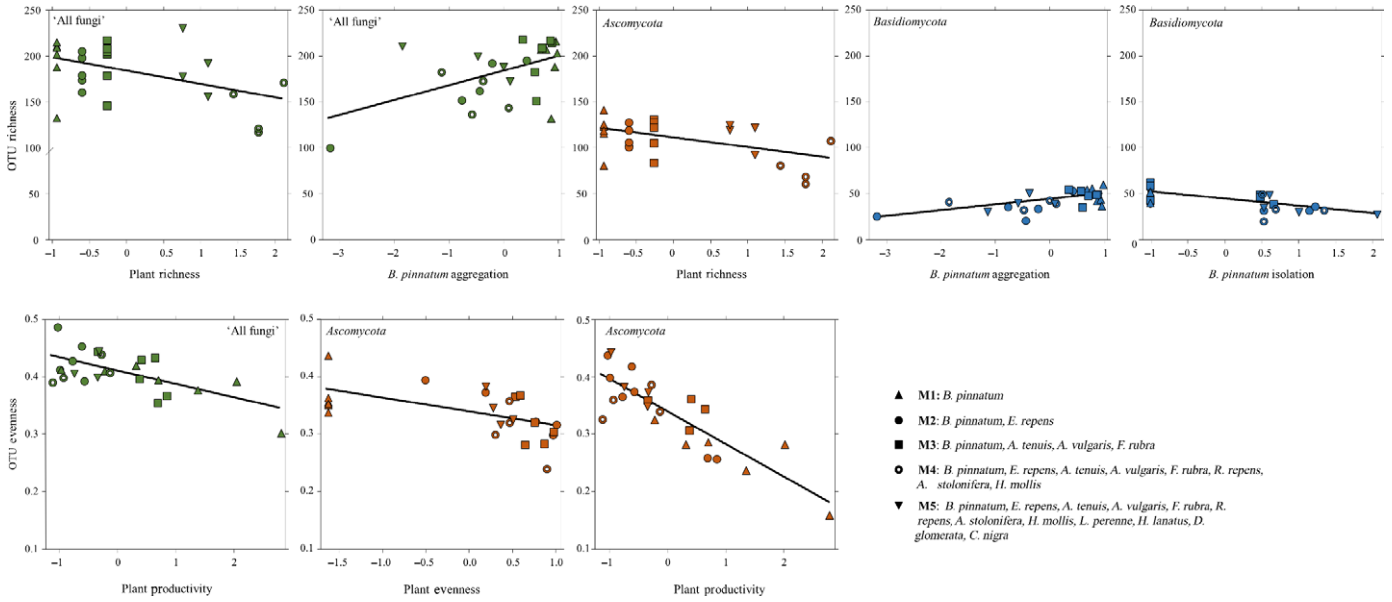


Fig. 3 Effects of floristic landscape heterogeneity and productivity on fungal assemblages. Heterogeneity is based on plant composition (plant richness and evenness), host plant spatial configuration (degree of aggregation and isolation of *Brachypodium pinnatum*). Fungal assemblage characteristics comprised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

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 3 yr 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 microlandscape approach: plant spatial arrangements may influence microbiota assemblages

Debate has arisen 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. Our data suggest that, besides influencing plant composition, 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 microlandscape 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 AMF 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, thus identifying possible new avenues for

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

ORCID

Anne-Kristel Bittebiere https://orcid.org/0000-0002-9882-968X
Cendrine Mony https://orcid.org/0000-0002-0061-6521
Philippe Vandenkoornhuyse https://orcid.org/0000-0003-3029-4647
Nathan Vannier https://orcid.org/0000-0002-5459-2051

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

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.

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