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**The influence of host-plant connectivity on fungal assemblages in the root microbiota of  
*Brachypodium pinnatum***

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**Running title:**

20 Host-plant connectivity and microbiota

## Abstract

Dispersal limitation may drive the structure of fungal microbiota of plant roots at small spatial  
25 scales. Fungal root microorganisms disperse through the plant rooting systems from hosts to  
hosts. Due to a pronounced host-preference effect, the composition of endophytic root  
microbiota may follow plant distribution. A given plant community may hence include a  
matrix of host-plant species that represent various habitat permeabilities to fungal dispersal in  
the floristic landscape. We experimentally tested the effect of host-plant isolation on  
30 endophytic fungal assemblages (Ascomycota, Basidiomycota, Glomeromycotina) inhabiting  
*Brachypodium pinnatum* roots. We calculated host-plant isolation using Euclidean distance  
(distance-based dispersal limitation) and Resistance distance (functional-based dispersal  
limitation), based on host presences. All fungal groups were more influenced by the resistance  
distance between *B. pinnatum* than by the Euclidean distance. Fungal dispersal was hence  
35 strongly related to the spatial distribution of the host-plants. The fungal groups displayed  
however different responses (in richness, abundance, and composition) to host isolation.  
Additionally, fungal assemblages were more strongly controlled by the degree of connectivity  
between host-plants during the prior year than by current connectivity. This discrepancy may  
be due to changes in plant species coverage in a year and/or to the delay of dispersal response  
40 of fungi. This study is the first to demonstrate how small-scale host-plant distributions  
mediate connectivity in microorganisms. The consequences of plant distributions for the  
permeability of the floristic landscape to fungi dispersal appear to control fungal assemblages,  
but with possibly different mechanisms for the different fungal groups.

## Keywords

45 Plant-fungi interaction, host preference, fungal dispersion, assembly rules, root fungal  
microbiota, micro-landscape

## Introduction

50 Understanding the assembly rules of plant microbiota is a central question of plant biology  
because of the tremendous ecological functions provided by symbiotic microorganisms to  
plants (Davison et al. 2016, Lekberg and Waller 2016). Next generation sequencing  
approaches have demonstrated that the microbiota of plants is composed of a large diversity  
of microorganisms, including bacteria (Lundberg et al. 2012, Bulgarelli et al. 2012), *archaea*

55 (Edwards et al. 2015, Vannier et al. 2018) and fungi (Vandenkoornhuysen et al. 2002,  
Coleman-Derr et al. 2016, Lê Van et al. 2017). An increasing number of studies suggests that  
the identity of the host-plant species (Vandenkoornhuysen et al. 2002, Martinez-Garcia and  
Pugnaire 2011, Yang et al. 2012) and to some extent, its genotype (Bodenhausen et al. 2014,  
Edwards et al. 2015) condition the associated microbiota through the preferential recruitment  
60 of particular fungi. This host-preference effect has been demonstrated in Arbuscular  
Mycorrhizal Fungi (AMF) (Vandenkoornhuysen et al. 2002). It was interpreted as the  
consequence of a plant filtering and a carbon reward process to the AMF best cooperators  
(i.e., providing the highest amounts of phosphorus to the host plant in exchange for carbon)  
(Kiers et al. 2011, Duhamel and Vandenkoornhuysen 2013). Such host-preference has been  
65 overlooked for the other components of the endophyte microbiota. If this host-plant  
preference exists, the plant community at any given location should be regarded as a mosaic  
of habitats with some plant species providing more favorable niches than others for any given  
fungal symbiont. Plant species distributions are then likely to affect the assemblages harbored  
among the roots of individual plants, explaining the observed heterogeneous distribution of  
70 fungi.

Island biogeography theory explains how habitat isolation affects species diversity  
(MacArthur and Wilson 1967). This theory assumes that in fragmented landscapes, local  
populations are linked through the dispersal fluxes of individuals and can hence be structured  
as a network (metapopulation). This theory has been extended to metacommunities (Wilson  
75 1992, Leibold et al. 2004). This conceptual framework assumes that there are four main  
drivers of local community dynamics: patch disturbances, local abiotic and biotic  
environmental conditions on species sorting, dispersal among communities, and stochastic  
extinction and colonization events (Leibold et al. 2004, Chase 2005). When dispersal is the  
main driver (i.e., dispersal rates are high), the species composition of local communities tends  
80 to become more homogeneous. Species richness at the metacommunity scale is also expected  
to be higher because of the lower probability of genetic drift, which counteracts the higher  
probability of stochastic extinctions observed in isolated populations (Hanski 1999). Isolation  
between local communities was at first approximated by a geographic distance. Isolation by  
distance has been transposed and tested recently on microorganisms (i.e. distance-decay  
85 relationships, Bell, 2010; Hanson et al. 2012). But in many species dispersal is not only linked  
with the geographical distance but also driven by landscape connectivity (i.e. the degree to  
which a given landscape facilitates or impedes the movement of organisms among habitat



resources) (Taylor et al. 1993). Connectivity is then dependent on the way landscape elements are distributed over space (structural connectivity) and on how species disperse over these elements (functional connectivity) (Tischendorf & Fahrig, 2000; Calabrese & Fagan, 2004; Taylor et al., 2006). High connectivity increases biodiversity for a large set of macroorganisms (Fletcher et al. 2016). The application of this framework linked to biogeography and landscape ecology to microbial assemblages associated to plant hosts has been mostly developed toward the understanding and the prediction of specific species spread such as pathogens (Thrall et al. 2003, Sullivan et al. 2011). While considering the whole fungal assemblages, the use of island biogeography theory for understanding the fungal microbiota associated with plants has mostly focused on ectomycorrhizal fungi (Peay et al. 2007, Peay et al. 2010) and at large landscape scales, similar to the scales used to study dispersal among macro-organisms.

Recent findings advocated however that fungal dispersal patterns should be examined at small spatial scales because of the high heterogeneity detected at such scales for some groups of fungi (such as AMF) (Bahram et al. 2015). The dispersal of fungi is achieved by dispersing spores and/or by expansion of the hyphal network (Carvalho et al. 2004). However, the relative contribution of these two dispersal types for plant root colonization is difficult to determine (Smith and Read 2008). In some mycorrhizal species, large spore size and belowground spore production restrict propagation distances (Smith and Read 2008). Likewise, hyphal growth is limited in space (Hart and Reader 2002), leading to the assumption that dispersal-limitation likely occurs at small scales for many fungal species. For biotrophic species, fungal species must disperse from one host plant to another. A plant community representing a matrix of different plant species constitutes a variety of potential fungal habitats (floristic landscape) through which fungus species must disperse. It can be imagined that the ability of a fungus species to disperse among plants depends on its host preferences and its dispersal traits, as well as on the root attributes of its host-plants. For a given host-plant in a given floristic landscape, connectivity with individuals of the same species is then likely to increase the rate of fungi dispersal between these host plants.

This study aims to test the effect of host isolation on the composition of root fungal assemblages by focusing on endophytic fungi assemblages of *Brachypodium pinnatum*. We used a mesocosm experiment presenting a large range of *B. pinnatum* spatial configurations in order to test the effect of *B. pinnatum* connectivity degree on the fungal composition associated to roots of the host-plant *B. pinnatum*. We calculated the degree of isolation of the

sampled host-plants with Euclidean distance (i.e. distance-based dispersal limitation) and resistance distance (i.e. functional dispersal limitation (*sensu* Fagan and Calabrese 2006)). We expected that more connected host-plant species within a floristic landscape result in a higher fungal species richness at the metacommunity scale (i.e. group of host-plants), and at the community scale (i.e. individual host-plant) and in closer fungal microbiota compositions between hosts. In contrast, isolated host-plant species within a floristic landscape should result in a lower fungal species richness at the metacommunity and community scales and should harbor different sets of fungal microbiota.

## Methods

### *Sampling host-plants*

We investigated the composition of plant microbiota associated with the focal plant *B. pinnatum*. This *Poaceae* species is a widely-distributed species of temperate grasslands. It displays a clonal plant that forms aggregated patches thanks to its phalanx growth form (*sensu* Lovett-Doust 1981). *B. pinnatum* root-associated mycobiota has been shown to differ depending on the surrounding plant composition and configuration (Bittebiere et al., 2019; Mony et al., 2019). Individual *B. pinnatum* were sampled in 16 outdoor mesocosms (1.30 x 1.30 x 0.25 m) encompassing four different compositions of herbaceous grassland plant species, following an additive design, and with four replicates for each treatment: (1) a *B. pinnatum* monoculture, (2) a two-species mixtures comprised *B. pinnatum* and *Elytrigia repens*, (3) a four-species mixture comprised *B. pinnatum*, *Agrostis tenuis*, *Festuca rubra*, and *Anthemis vulgaris*, and (4) an eight-species mixture comprised of the four species of mixture #3, plus *Elytrigia repens*, *Agrostis stolonifera*, *Holcus mollis*, and *Ranunculus repens*. These nine herbaceous species are widely distributed in grasslands on western France (des Abbayes et al. 1971).

The mesocosms were established in 2009 in outdoor experimental gardens (regular watering but no control of light, rainfall, insect herbivory) three years before we sampled the fungi associated with *B. pinnatum* roots. We assumed that this 3 year duration after establishment of the mesocosm guarantees the fact that the host-preference effect is the main driver of host-plant recruitment of fungi over priority effect. Each mesocosm treatment consisted of a mixture of 48 plantlets grown in a hexagonal pattern. The density of the 48 plants was equally distributed among species in the mixtures (e.g. for the four-species

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155 mixtures, 12 plantlets of each per species were planted in each mesocosm). Plantlets were randomly assigned to each position within the hexagonal pattern (Appendix S1: Fig. S1). Inflorescences were periodically cut to suppress seed production and all new seedlings were removed manually. This ensured that the plant cover is the result of the initially planted individuals that developed through clonal multiplication without any individuals coming from other mesocosms. In absence of existing knowledge, we assumed that the stress induced to the plant by the cut has a minor effect on fungi composition and, if it exists, this potential effect is similar across sampled plots. Mesocosms were mown each year in fall and biomass was removed. Mesocosms were watered during the dry season to avoid drought stress. Plants colonized bare soil in contrasting distribution patterns, even within the same type of mixture (treatment) due to random clonal propagation of individuals. Therefore, the 16 mesocosms (plots) varied widely in their spatial distribution of *B. pinnatum*, meaning that plots encompassed a wide range of *B. pinnatum* isolation intensities (Appendix S1: Fig. S2). Mesocosms varied widely in plant species compositions and richness, allowing us to test our expectations across a large range of plant communities. Within our experimental plots, five *B. pinnatum* individuals, one located near each of the four corners of the plot at a minimum distance of 20cm to the border, and one at the center of the plot (Appendix S1: Fig. S2) were sampled. Due to the patchiness of *B. pinnatum* in some plots, three individuals in four of the plots and four individuals in two of the plots were sampled.

#### *Measuring current and past connectivity between host-plants*

175 Two metrics were used to assess the connectivity between pairs of focal plants: Euclidean distance and resistance distance (Appendix S1: Fig. S3). Euclidean distance corresponds to the degree of isolation (by geographic distance alone) between pairs of plants, which models degree of connectivity due to distance-isolation. Euclidean distance is independent of the presence of the focal plants (*B. pinnatum*) or other plant species. Resistance distance corresponds to the degree of isolation due to landscape resistance to dispersal. It is based on circuit theory (McRae et al. 2008). It assumes that a landscape consists of a mosaic of patches, each with a different resistance to dispersal. Less resistant areas are assumed more permeable to dispersal, whereas areas that are more resistant inhibit dispersal (less permeable). In the present study, we assumed that the dispersal of fungi associated with *B. pinnatum* is preferentially achieved along paths inhabited by other *B. pinnatum* plants than along paths of

185 other species. This distance is considered as a functional-based connectivity index as it takes into account the functional response of the microbiota to the spatial distribution of host-plants.

To calculate Euclidean and resistance distances, *B. pinnatum* sampled host plants were assimilated as a graph where the sampled plants represented nodes. The distances for each pair of nodes were calculated using the software ArcGis for the Euclidean distances and the  
190 software Circuitscape for the resistance distances (McRae et al. 2008). The resistance distance for a given pair of nodes was calculated based on the cumulated resistance for all possible paths between the two nodes (plants) relatively to the probability that the path would be used for dispersal. Resistance distances were defined based on *B. pinnatum* distribution maps because we defined low resistant areas as patches where *B. pinnatum* occurred and high  
195 resistant areas as areas where the host-plant was absent. Resistance distances were calculated for each pair of nodes based on the presence of *B. pinnatum* in an 80 x 80-cm grid of 256 cells, each cell being of 5 x 5 cm in size. This grid was positioned at the center of each plot. A plant was considered to be present in a grid-cell if at least one shoot was rooted within it. To take into account the plant-plant competition and resulting plant distribution changes, plant  
200 communities were mapped in 2011, one year before we began sampling them (i.e., the 'past' map) and again in 2012 (i.e., the 'current' map), the same year that we sampled *B. pinnatum* individuals. Assessing resistance distances for each of these dates is necessary for taking care of any potential lag-time response of microbiota to host plant isolation,

Euclidean and resistance distances were then computed for both maps. Because *B.*  
205 *pinnatum* has a very superficial and narrow rooting system, we assumed that the grid cells adequately reflected root distributions at a 5-cm spatial resolution. Each grid-cell of the resistance maps represented a cost for dispersal (1 = low cost), which depended on the presence of *B. pinnatum* in the cell. Patches where *B. pinnatum* occurs had a low cost of 1. When *B. pinnatum* was absent in a given grid cell, the cell was either occupied by another  
210 plant species or by bare soil (infrequent). Where *B. pinnatum* was absent, we determined a specific cost of dispersal, which depended on the relative effort required for a fungal species to disperse through such a grid cell relative to cells that harbored *B. pinnatum*. In order to test the sensitivity of the index to the cost attributed to the absence data (cells without *B. pinnatum*), we tested several relative dispersal costs: 10, 30, 50, 70, and 100. For example, a  
215 dispersal cost of 10 for a cell meant that in the grid-cell, fungi might be 10 times less likely to disperse through it than through a grid-cell occupied by *B. pinnatum*. Resistance distances were then calculated for each pair of the sampled host-plants. We determined the correlation

between resistance distances calculated with the various magnitudes of costs we tested for grid-cells without *B. pinnatum*. Pearson coefficients between resistance distances obtained with costs of 10, 30, 50, 70 and 100 attributed to cells without *B. pinnatum* were all  $>0.99$  ( $p<0.001$ ) both for 2011 and for 2012. This suggests that dispersal paths simulated were independent on the ratio of dispersal costs in absence or presence of *B. pinnatum* in the range 10-100. Therefore, the following dispersal costs were assigned to cells: 1 (for cells with *B. pinnatum*) and 50 (to cells without *B. pinnatum*).

We calculated two metrics to represent the degree of isolation for sampled host-plants based on resistance distances. One metric was the mean of pairwise resistance distances for a given host-plant with all other host-plants (4 pairs) and the other was the mean of the pairwise resistance distances relative to the nearest host-plants, with the number of pairs examined dependent on the location of the sampled plants in the plot (i.e. based on three pairs if the sampled plant was located in a plot corner and based on four pairs if the plant was located at the center of the plot). Because both metrics were highly correlated with one another (Pearson coefficient,  $R=0.99$ ;  $p<0.001$  for 2012,  $R=0.98$ ;  $p<0.001$  for 2011), we presented only the results for the mean pairwise resistance distances based on the nearest host-plants. Hereafter, these pairwise comparisons represent the “sample scale.” The degree of isolation of *B. pinnatum* plants was also calculated at the plot scale. This was determined by the mean resistance distance between all pairs of sampled individuals (10 pairs). This characterized the average connectivity of the plant community (hereafter, these means represent the “plot scale”). The degree of isolation at the sample and plot scales was not calculated based on Euclidean distance because all samples and plots displayed the same values (all samples nearly equidistant in the plot).

#### *Assessing root fungal assemblages*

We collected ~ half of the rooting system of a *B. pinnatum* individual plant in each of the sampled mesocosms. Roots of each sample were cleaned in a 1‰ solution of Triton X100, and thoroughly rinsed with tap water then sterile distilled water. For a given sampled plant, ~80 mg out of at least ~1g of fresh mass were subsampled. Healthy root fragments were selected for being representative of the different parts of the rooting system. After this cleaning step, only root-adhering epiphytic and endophytic microorganisms were still present with the roots (e.g. Lê Van et al. 2017). Cleaned roots of each sample were ground to powder

250 under liquid nitrogen using a pestle and mortar. Total DNA was extracted from the 80mg sub-  
sample using the DNeasy plant kit (Qiagen) according to the manufacturer's  
recommendations. We amplified a specific 550 bp of the fungal SSU rRNA gene fragment,  
including the V4 and V5 regions, using the primers SSU0817 and -NS22B following  
255 procedure outlined by Borneman and Hartin (2000) and Lê Van et al. (2017). The two primers  
were modified on their 5' end with the addition of Illumina® tails. The PCRs performed with  
Illustra™ PuReTaq Ready-to-go (GE Healthcare®) contained 0.2 μM of primer within a final  
volume of 25 μL following the procedure of Lê Van et al. (2017). This first PCR was  
followed by a second PCR to tag the amplicons. Amplicons were purified in Agencourt®  
(AMPure™) solution using a Bravo Automated Liquid Handling Platform (Agilent®).  
260 Purified amplicons were then quantified with a Quant-iT™ PicoGreen™ dsDNA Assay Kit  
and normalized to the same concentrations before a second PCR to conduct multiplexing  
tagging, which was performed with a Smartchip Wafergen/Takara instrument. The pooled  
PCR products were purified and quantified by qPCR before pair-end sequencing (Illumina®,  
Miseq). Both the sequencing library and mass sequencing were performed using the Human  
265 and Environmental Genomics platform ([https://geh.univ-rennes1.fr/?\[en\]](https://geh.univ-rennes1.fr/?[en])).

The FROGS pipeline was used to analyze amplicon sequences (Escudié et al. 2017).  
The clustering within FROGS was performed using SWARM (Mahé et al. 2014) a new  
generation method allowing to avoid the use of a threshold. To limit the risks of obtaining  
artificial OTUs (Operational Taxonomic Units), we only retained identical sequences  
270 observed in at least five different samples. OTUs were affiliated to taxa by comparing the data  
to the Silva 18S database, release 132 (Quast et al. 2013). We built a contingency matrix from  
the data. The number of sequences was normalized at the same size for all the samples by  
rarefying to the smallest number of sequences observed (i.e. 19,224 sequences for each  
sample) using the VEGAN package in R (function 'rarefy'; Oksanen et al., 2018; Rarefaction  
275 plot provided in Appendix S1: Fig. S4).

From this normalized matrix, richness and evenness of OTUs were calculated for the  
whole fungi assemblage we obtained from the roots of the sampled plants collected from each  
plot. We additionally analyzed separately the different fungal groups because the effects  
detected at the scale of the entire fungal community might be driven by the most dominant  
280 phylum. This allows also to focus on interesting taxonomic groups such as the sub-phylum  
Glomeromycotina which was previously proven of major importance for plant growth (Van  
der Heijden et al., 1998). Richness at the plot scale was calculated as the total number of  
OTUs for all sampled host plants in a plot, and at the sample scale as the number of OTUs

285 that were associated with roots of the sampled plant. Evenness was calculated only at the  
sample scale using Pielou evenness index. Dissimilarity indices were also calculated for each  
*B. pinnatum* pair based on Bray-Curtis dissimilarity index using the VEGAN package  
(function ‘vegdist’). This dissimilarity index for fungal assemblages was used as a proxy to  
determine the extent of dispersal between pairs of *B. pinnatum*. They are then interpreted as  
follows: the higher the dissimilarity index, the more dissimilar the assemblages are in  
290 composition.

### *Statistical analyses*

295 Dissimilarity in fungal community composition was described through a PCoA on bray-curtis  
distances between samples computed on the normalized OTUs table for both the plot scale  
(Appendix S1: Fig. S5) and the sample scale (Appendix S1: Fig. S6) (package “vegan” and  
“phyloseq”; McMurdie & Holmes, 2013). We used then a linear model to analyze the effect  
of host-plant isolation in 2011 and in 2012 on fungal OTU richness at the plot scale. Host-  
plant isolation corresponded to the mean of pairwise resistance distances over all possible  
pairs of host plants in a given plot. To analyze the effect of a given host-plant’s isolation on  
300 the richness of its fungi assemblage and its evenness at the sample scale, we applied linear  
mixed models using the mean pairwise resistance distance for a given host plant to its nearest  
sampled host plants (resistance distance for 2011 or 2012) as the independent variable and  
fungal species richness (or evenness) as the dependent variable. OTUs richness was log-  
transformed to ensure normal distributions. Plots were integrated in the model as a random  
305 factor. We did not test for the effect of Euclidean distance on OTU richness and evenness  
because all samples had similar distance to the nearest neighbor (sample scale) nor mean  
distance between sampled plants (plot scale) due to the standardized sampling design  
(sampling in the four corners and in the center). The effect of Euclidean distance was tested  
though on the similarity index

310 The effect of host-plant isolation on fungi similarity between pairs of sampled  
individuals were analyzed using linear mixed models. The independent variable was isolation  
distance for pairs of sampled individuals with three different distances tested (Euclidean  
distance, Resistance distance calculated for 2011 and Resistance distance calculated for  
2012). The dependent variable was the dissimilarity between the fungi assemblages measured  
315 using the Bray-Curtis index for a total of 3 models per fungal group. In these models, the  
sampled individuals of each pair were considered as random factors nested into the plot factor

(i.e. three random factors with two nested into one). This structure enabled us to manage the partial dependencies in the data.

The independence and normality of the residuals for all tested models were examined graphically. The significance of each model was tested using an ANOVA based on the Type II sums of squares and calculated the proportion of variance explained by the fixed effect (marginal  $R^2$ ). The proportion of variance explained by random effect(s) (conditional  $R^2$ ) (*sensu* Nakagawa and Schielzeth 2013) was calculated for mixed models. We used the following software packages for statistical analyses: ‘nmlr’ (Pinheiro et al., 2018), ‘lme4’ (Bates et al. 2015), ‘MuMIn’ (Barton 2016), and ‘car’ (Fox & Weisberg 2011) in R.3.0.4 (R Core Team).

## Results

### *Fungi assemblage characteristics*

*B. pinnatum* roots were colonized by 1006 fungal OTUs described by 1,345,680 sequences (Fig. 1). The root fungal microbiota of the entire fungi assemblage were dominated by Ascomycota (67.1% of the sequences and 61.8% of OTU richness). Basidiomycota constituted 25.9% of the sequences and 25.0% of OTUs, whereas Glomeromycotina constituted 5.1% of the sequences and 5.4% OTU richness. The dominant classes of the Ascomycota were Dothideomycetes (78.0% of Ascomycota sequences and 22.5% of OTU richness; orders: Jahnulales, Pleosporales). Sordariomycetes constituted 12.5% of Ascomycota sequences and 35.6% of OTU richness (orders: Boliniales, Hypocreales, Magnaporthales, Microascales, Ophiostomatales, Sordariales, Xylariales), whereas Leotiomyces constituted 8.7% of Ascomycota sequences and 20.4% of OTU richness (orders: Erysiphales, Helotiales, Medeolariales, Thelebolales). Other classes were lower than 1% of total sequences. The dominant class of the Basidiomycota was the Agaricomycetes (99.0% of Basidiomycota sequences and 90.1% of OTU richness; orders: Agaricales, Geastrales, Trechisporales, Gomphales). The dominant class of the Glomeromycotina was Glomerales, which represented 99.99% of the sequences and 87.0% of OTU richness. The composition in fungal OTUs varied among assemblages both at the plot (Appendix S1: Fig. S5) and sample scales (Appendix S1: Fig. S6).



### *Effect of host isolation on fungal assemblages assessed at sample and plot scales*

Indices used to characterize the fungal microbiota in *B. pinnatum* roots are provided in Tables  
350 1 & 2. OTU richness and evenness for “all fungi”, Asco-, Basidio-, and Glomeromycotina  
varied widely between samples, both at sample and at plot scales. We did not test the effect of  
Euclidean distance on fungal richness and evenness as all samples and plots displayed similar  
values of isolation due to the sampling design (see method section). This means that the  
strong variability of the OTUs richness and evenness observed is not related to changes in  
355 geographic distance. The results testing the effect of the resistance distance on fungal richness  
and evenness are presented below.

At the plot scale (Table 1), only one statistical model of the eight we produced  
was significant ( $R^2 = 0.20$ ;  $p=0.047$ ). For “all fungi”, Ascomycota and Glomeromycotina, we  
detected no significant relationship between degree of isolation of *B. pinnatum* and OTU  
360 richness of fungal microbiota inhabiting its roots. For Basidiomycota, we detected a  
significant effect of host isolation on OTU richness only for 2011. Host isolation decreased  
Basidiomycota richness, as indicated by the negative Estimate (‘Est’) value of -0.1.

At the sample scale (Table 2), five of 16 models were statistically significant with  
marginal  $R^2$  ranging from 0.06 to 0.13. In Ascomycota and Basidiomycota, we did not detect  
365 a significant effect of OTU richness on resistance distance in 2011 or 2012. In contrast,  
Glomeromycotina OTU richness increased with mean resistance distance in both the 2011 and  
2012 samples, as indicated by the positive Estimate (‘Est’) values of 0.009 and 0.02. We  
found no effect of mean resistance distance of 2012 on evenness for Asco-, Basiodio-, or  
Glomeromycotina, nor was mean resistance distance of 2011 related to evenness in  
370 Ascomycota. The effect of mean resistance distance of 2011 on evenness differed between  
fungi assemblages: higher values of evenness for “all fungi” and Basiodiomycota assemblages  
occurred in more isolated *B. pinnatum* plants (positive ‘Est’ of 0.001 and 0.003 respectively in  
Table 2), whereas low values of evenness for Glomeromycotina assemblages occurred in  
more isolated *B. pinnatum* plants (negative ‘Est’ of -0.004 in Table 2).

### *Effect of host-isolation on the similarity of fungal assemblages between B. pinnatum plants*

Euclidean distance between sampled *B. pinnatum* plants was not correlated with resistance  
distance (Pearson coefficients:  $R= 0.04$ ;  $p>0.05$  for resistance distance in 2011;  $R= -0.07$ ;  
 $p>0.05$ ). Resistance distance in 2012 was significantly correlated with resistance distance in  
380 2011, although not very strongly ( $R = 0.43$ ;  $p<0.001$ ).

For “All fungi” and Ascomycota colonizing *B. pinnatum* roots, the Bray-Curtis dissimilarity index declined with degree of host-plant isolation with Euclidean and resistance distance in 2011 (Table 2). Assemblages were more similar when host plants were isolated than when they were more connected to other *B. pinnatum* (i.e. lower resistance distance) (negative ‘Est’ of -0.002 and -0.003 respectively). We did not detect a significant relationship between the Bray-Curtis index and Euclidean distance or resistance distances for Basidiomycota nor Glomeromycotina.

## Discussion

We demonstrated the effect of *B. pinnatum* isolation on fungal microbiota assemblages. This effect is robust for the relationships between isolation and the richness and evenness of the assemblages both at the plot and sample scales. For dissimilarity indices however, significances of signals were low. This can be due to the limitation of the Bray-Curtis indices to fully and quantitatively represent dispersal fluxes of fungi species between local communities. These indices can also reflect possible other mechanisms than dispersal such as environmental similarities. Despite homogeneous environmental conditions of soil, water or light of the mesocosms, we cannot exclude that there is no heterogeneity at very fine grain, possibly due to the effect of plants themselves over the duration of experiment. Slight nutrient or water depletion in the soil below more competitive plant individuals is likely to occur, as well as slight increase in herbivore pressure around more palatable plant individuals. This fine-scale heterogeneity reflects real conditions of grassland communities despite their possible environmental homogeneity at the field scale. Discussion is made below within these limitations.

### *Host-plant connectivity affects plant fungal microbiota assemblages*

As expected, we demonstrated the effect of the degree of host-plant isolation on OTUs composition, richness, and evenness (Tables 1, 2 & 3). Differences in plant fungal microbiota assemblages depended on the degree of host-plant (*B. pinnatum*) isolation, which varied widely and were independent of plant species richness and plant composition. Interestingly, the effect of the degree of plant isolation on plant fungal microbiota differed among fungal groups.

For “all fungi” and Ascomycota, OTU assemblages between host plants were more similar in isolated populations as measured by resistance distance, whereas they differed in relative abundance among highly-connected populations (Table 3). This suggests that when  
415 there is no limitation to dispersal, OTUs relative abundances in fungal assemblages of the focal plant species (*B. pinnatum* in this study) are essentially regulated by other parameters acting at local scale. We can speculate that they are due to slight changes in environmental conditions (abiotic or biotic) or fungal traits that should drive assemblages in the local patches (species sorting paradigm, sensu Leibold et al. 2004).

420 In Basidiomycota, the degree of isolation as measured as resistance distance, was related to OTU richness at the plot scale (i.e. the entire assemblage recorded over the sampled individuals of our plots) (Table 1), suggesting that Basidiomycota are more impacted by the degree of isolation of their host plants (habitat fragmentation at the “metacommunity” scale) than Ascomycota or Glomeromycotina are. In these later two groups, we can speculate that  
425 their higher resistance may be due to traits that promote efficient dispersal ability between isolated host plants. For instance, large spores in Glomeromycotina have been shown to be more effective than small spores in initiating root colonization (Daniels et al. 1981), whereas rapid hyphal growth and particular hyphal architectures are known to promote root colonization at small spatial scale (Hart and Reader 2002).

430 Our study showed that the response of Glomeromycotina to host-plant isolation (measured as OTU richness) is higher and more equitable in roots of more isolated host plants than in more connected ones (Table 2). They tend also to be more similar while connected though this result needs to be supported by further experimentations as it is only a statistical tendency. Isolated host plants are surrounded by other plant species. These neighboring  
435 species may display different AMF assemblages and thus enrich the local soil with them, from which AMFs are recruited by *B. pinnatum*. Fungal assemblages of neighboring plants have indeed been shown to impact the AMF root composition of plants located within a few centimeters from them (Shi et al. 2014; Bittebiere et al. 2019).

#### 440 **Effect of host-plant connectivity is not necessarily linked to their geographic distance**

“All fungi”, Ascomycota, Basidiomycota, and Glomeromycotina appeared to be more dependent on resistance distance than on Euclidean distance between host-plants (Tables 1, 2 & 3) indicating that the isolation-by-distance connectivity model is not adequate for

445 endophytic fungi. We indeed only recorded for Ascomycota group a tendency to depend on  
Euclidean distance while no effect was detected for the other groups. The Ascomycota group  
includes a morphologically diverse group of species, including single-celled yeasts,  
filamentous fungi, and even more complex fungi, thus representing a wide range of dispersal  
mechanisms and patterns (Bennett and Turgeon 2017). Additionally, within the endophytic  
450 assemblages, some species are not obligatory biotrophs and so their ability to disperse may be  
independent of whether a suitable host is present. If we could conduct a similar study based  
on more precise taxonomic subdivisions, it might be possible to obtain more insight into  
Ascomycota dispersal ecology and their response to the connectivity models.

Our results suggest then that fungal dispersion is more strongly related to the spatial  
distribution of host-plants at the plot scale than by geographic distance; supporting existing  
455 empirical results. For example, Carvalho et al. (2003) found that spore abundance in soil is  
related to the proximity of mycotrophic hosts. Similarly, Shi et al. (2014) demonstrated that  
the composition of Glomeromycotina species in the roots of an invasive plant species and in  
its neighboring plants depended on the density of the invasive species in the patch and hence  
its higher probability of being in closer proximity to other individuals of the same species.  
460 When resistance distance is low, individuals of host plants tend to be aggregated, thus  
providing a higher probability of plant rooting systems being in contact with one another.  
Transfers of fungi among aggregated host plants of a given species means that they are more  
likely to harbor more similar fungi microbiota (i.e. via direct contacts between rooting  
systems or hyphal growth) (McGee et al. 1997, Smith and Read 2008). Propagules produced  
465 by fungi are also expected to form similar symbiotic reservoirs.

Resistance distance rather than geographic distance has proved to be a better predictor  
of the effects of patch isolation on macro-organisms (McRae and Beier 2007). In our study,  
we were able to use a similar approach to gain insight into dispersal potentials among  
microorganisms but we used a simple binary distribution of resistance costs based on the only  
470 presence/absence of the host-plant *B. pinnatum*. Refinements to our approach might be  
possible by imposing different permeability degrees (i.e. resistance costs) for each plant  
species comprising the plant community. Endophytic fungal assemblages are preferentially  
associated with specific plant species but some other plant species might constitute alternative  
hosts (Gollotte et al. 2004). Fixing permeability degrees needs to better describe fungal  
475 microbiota over a large number of plant species in order to analyze the similarities and  
divergence of fungal composition between host species. Studies suggest that AMF community

is determined by the taxonomic family of the plant species (Scheublin et al. 2003, König et al. 2010) or the plant life form (Yang et al. 2012, Lopez-Garcia et al. 2014). These attributes could be used to predict similarity of microbiota and ultimately calibrate the resistance costs.

480 In addition, permeability is assumed here to be similar for all fungi while the cost of dispersal is likely to differ between fast- and slow-growing fungi and between those that can use vectors or have a high specificity and those that are not transported by vectors or have a low specificity. This work paves the way for studies looking at studies on connectivity effect based on more refined interactions between fungi and host-plant isolation and extended to the  
485 entire endophyte microbiota.

*Past B. pinnatum connectivity drives plant fungal microbiota assemblages more than present connectivity*

For “all fungi” and the Asco- Basidio-, and Glomeromycotina groups, characteristics of  
490 fungal assemblages were equally or better explained by degree of connectivity in 2011 than degree of connectivity in 2012 (the year we sampled *B. pinnatum* microbiota). This discrepancy may be partly due to changes in plant species coverages that occurred between 2011 and 2012 (Fig. 2; 0.5 to 3.3 fold increase in cover respectively in *B. pinnatum* monoculture and the eight-species mixture). This led to an overall decline in the degree of  
495 host-plant isolation in plots in 2012 (isolation assessed with *B. pinnatum* presence/absence data).

An alternative explanation of the delay of response of the plant fungal assemblages to changes in vegetation distribution might be due to the time necessary for dispersing in these microorganisms. Many root fungi produce propagules to disperse, which in some Ascomycota  
500 and Basidiomycota may be produced either aboveground and/or in soils. However, Glomeromycotina only produce propagules belowground, likely leading to a low probability of long-distance dispersal events. A large proportion of fungi do not sporulate at all or sporulate infrequently (Clapp et al. 1995). In these types, a large part of dispersal is achieved by hyphal growth and infections by root contacts between plants (Carvalho et al. 2004). Thus,  
505 vegetative multiplication in these types of fungi occurs then within a few centimeters around a given rooting system (Warner and Mosse 1980) leading to a slow dispersal from hosts to hosts. Lastly the timing of fungi dispersal may also depend on host plant phenology (Johnson-Green et al. 1995) and especially on the growth and architecture of host roots (Bundrett and

Kendrick 1990). Additional studies, with controlled experimental designs that manipulate  
510 various configurations of host plants may help to deepen our understanding of dispersal  
mechanisms among root-associated fungi.

*The concept of connectivity for microorganisms: toward a better understanding of fungi  
assembly rules*

515 The importance of connectivity in regulating dispersal has been a central ecological question  
for understanding assembly rules in macro-organisms. Our study is the very first to apply  
these approaches to better understand dispersal at a small scales (centimeters) and obtain  
insight into how plant microbiota communities assemble. We demonstrated that dispersal  
520 limitation in root fungi was less determined by distance than by the spatial arrangements of  
host plants and their consequences on the permeability of the plant landscape to dispersion.  
Such permeability depends on the presence of specific host plants, thus confirming the  
occurrence of a host-preference effect (Vandenkoornhuysen et al. 2002). We found that this  
effect not only occurs in Glomeromycotina fungi, but that it also occurs in Ascomycota and  
Basidiomycota.

525 In agreement with the common consensus for macroorganisms, low connectivity  
between plant hosts is associated with a lower richness of the species pool of Basidiomycota  
fungi. However, in Glomeromycotina, localized host isolation promotes the effect of  
neighbouring patches, illustrating a possible edge effect, well-known in landscape ecology for  
promoting species richness (Ries et al. 2004). Such differences in response to degree of  
530 connectivity between fungi groups suggest that edge effect vs. dispersal drivers differ in their  
relative importance in shaping fungi assemblages. Beyond the effect of plant distribution and  
their interactions on the fungal assemblages, micro-scale changes in environment either or not  
due to plant effect is likely to interplay with the recruitment and selection of microorganisms  
isolates on plant roots. Further studies should explicitly quantify the effects of both local  
535 environmental conditions and host-plant isolation, and apply the theoretical framework  
developed for large spatial scales to the much smaller scale needed to examine assembly  
patterns of microorganisms.

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**Data Availability**

Data are available on Figshare: <https://doi.org/10.6084/m9.figshare.7893005.v1>

Table. 1: Summary of the models explaining the fungal microbiota richness at the plot scale (i.e. based on the richness on roots of five *B. pinnatum* plants sampled), which depended on mean isolation distance of host plants. Isolation was calculated as the mean pairwise distances between all sampled host plants. Richness was log-transformed in order to normalize the distribution. The provided model parameters are the intercept (Inter), independent variable with its coefficient (Est), the F statistic (F) and *p*-values from ANOVA and the adjusted R<sup>2</sup> (adj-R<sup>2</sup>). Significant models are in bold. Resistance distance in 2011 and in 2012 are related with a correlation coefficient of 0.63 (Pearson correlation test,  $t = 3.03$ ;  $p < 0.01$ ).

	Description Mean (SD) (min-max)	Resistance dist. (2011)				Resistance dist. (2012)			
		Inter	Est	F	Adj-R <sup>2</sup>	Inter	Est	F	Adj-R <sup>2</sup>
<i>All fungi</i>	171.6 (23.9) [131-206]	5.21	-0.003	2.15 (0.16)	0.07	5.20	-0.008	4.04 (0.06)	0.17
<i>Ascomycota</i>	116.4 (15.2) [90-143]	4.78	-0.001	0.37 (0.55)	-0.04	4.80	-0.007	3.33 (0.09)	0.13
<i>Basidiomycota</i>	46.5 (14.6) [20-72]	<b>4.04</b>	<b>-0.1</b>	<b>4.73</b> <b>(0.047)</b>	<b>0.20</b>	3.92	-0.02	2.50 (0.14)	0.09
<i>Glomeromycotina</i>	8.8 (3.6) [4-15]	1.80	0.01	3.55 (0.08)	0.15	1.91	0.02	2.76 (0.12)	0.11

Table. 2: Summary of the models explaining fungal microbiota richness and evenness on individual *B. pinnatum* plants, based on mean resistance distance between *B. pinnatum*. Isolation was calculated as the mean pairwise distance to the closest sampled *B. pinnatum*. Richness was log-transformed in order to normalize the distribution. Given model parameters are the intercept (Inter), independent variable with its coefficient (Est), the Chi-square statistic (Chisq) and *p*-values from ANOVA II and the marginal and complete R<sup>2</sup> (R<sup>2</sup>m; R<sup>2</sup>c). Significant models are in bold. Resistance distance in 2011 and in 2012 are related with a correlation coefficient equal to 0.55 (Pearson correlation test,  $t = 5.48$ ;  $p < 0.001$ ).

	Description Mean (SD) (min-max)	Resistance distance (2011)				Resistance distance (2012)			
		Inter	Est	Chisq	R <sup>2</sup> m(R <sup>2</sup> c)	Inter	Est	Chisq	R <sup>2</sup> m(R <sup>2</sup> c)
<b>Richness</b>									
All fungi	70.0 (11.7) [47-97]	4.21	0.001	0.71 (0.40)	0.015 (0.20)	4.24	0.003	0.008 (0.93)	0.0002 (0.21)
Ascomycota	47.6 (11.0) [20-73]	3.78	0.003	1.61 (0.21)	0.029 (0.13)	3.84	0.0006	0.02 (0.88)	0.0004 (0.16)
Basidiomycota	18.0 (6.3) [5-35]	2.89	-0.003	0.96 (0.33)	0.018 (0.14)	2.84	-0.002	0.17 (0.67)	0.003 (0.13)
Glomeromycotina	4.3 (2.3) [1-13]	<b>3.52</b>	<b>0.009</b>	<b>5.72</b> <b>(0.02)</b>	<b>0.077</b> <b>(0.079)</b>	<b>1.22</b>	<b>0.02</b>	<b>6.21</b> <b>(0.01)</b>	<b>0.083</b> <b>(0.08)</b>
<b>Evenness</b>									
All fungi	0.28 (0.06)	<b>0.26</b>	<b>0.001</b>	<b>4.06</b>	<b>0.080</b>	0.27	0.002	2.55	0.052

	[0.14-0.36]			<b>(0.04)</b>	<b>(0.28)</b>			(0.11)	(0.32)
Ascomycota	0.35 (0.09)	0.32	0.001	2.37	0.052	0.34	0.002	2.34	0.049
	[0.17-0.52]			(0.12)	(0.32)			(0.13)	(0.35)
Basidiomycota	0.32 (0.17)	<b>0.26</b>	<b>0.003</b>	<b>4.64</b>	<b>0.063</b>	0.31	0.0006	0.07	0.001
	[0.04-0.75]			<b>(0.03)</b>	<b>(0.063)</b>			(0.80)	(0.04)
Glomeromycotina	0.38 (0.18)	<b>0.46</b>	<b>-0.004</b>	<b>7.90</b>	<b>0.130</b>	0.41	-0.004	2.60	0.049
	[0.06-0.92]			<b>(0.005)</b>	<b>(0.22)</b>			(0.11)	(0.21)

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Table. 3: Summary of the models explaining fungal microbiota Beta diversity (Bray-Curtis indices) on individual *B. pinnatum* plants, based mean resistance distance between *B. pinnatum*. Isolation was calculated as the mean pairwise distance to the closest sampled *B. pinnatum*. Given model parameters are the intercept (Inter), independent variable with its coefficient (Est), the Chi-square statistic (Chisq) and *p*-values from ANOVA II and the marginal and complete R<sup>2</sup> (R<sup>2</sup>m; R<sup>2</sup>c). Significant models are in bold. Resistance distance in 2011 and in 2012 are related with a correlation coefficient equal to 0.55 (Pearson correlation test,  $t = 5.48$ ;  $p < 0.001$ ).

	Description	Euclidean distance				Resistance distance (2011)				Resistance distance (2012)			
		Inter	Est	Chisq	R <sup>2</sup> m(R <sup>2</sup> c)	Inter	Est	Chisq	R <sup>2</sup> m(R <sup>2</sup> c)	Inter	Est	Chisq	R <sup>2</sup> m(R <sup>2</sup> c)
All fungi	0.51 (0.17) [0.16-0.84]	0.55	-0.11	1.41 (0.23)	0.007 (0.57)	<b>0.55</b>	<b>-0.002</b>	<b>3.92</b> <b>(0.048)</b>	<b>0.053(0.56)</b>	0.51	-0.001	0.21 (0.64)	0.003 (0.57)
Ascomycota	0.45 (0.17) [0.12-0.77]	0.51	-0.16	3.64 (0.06)	0.018(0.48)	<b>0.49</b>	<b>-0.003</b>	<b>5.35</b> <b>(0.02)</b>	<b>0.068 (0.48)</b>	0.45	-0.002	0.59 (0.44)	0.006(0.46)
Basidiomycota	0.63 (0.24) [0.08-0.99]	0.69	-0.14	1.26 (0.26)	0.006(0.62)	0.69	-0.003	3.21 (0.07)	0.043(0.64)	0.62	0.002	0.29 (0.59)	0.003(0.61)
Glomeromycota	0.51 (0.24) [0.05-0.96]	0.53	-0.05	0.09 (0.76)	0.007(0.18)	0.53	-0.0009	0.37 (0.54)	0.004(0.18)	0.48	0.005	3.16 (0.08)	0.030(0.21)



**Figure legends**

**Figure 1** - Distribution of fungal microbiota assemblages for *Ascomycota*, *Basidiomycota*, and *Glomeromycotina* based on OTU composition recorded in total. Scales on the *Y* axis differ by fungal groups: *Ascomycota*, *Basidiomycota* and *Glomeromycotina* represented 71.6%, 27.8% and 0.6% of the sequences respectively. Left graphs depict the percentage of sequences and the right graphs depict OTU richness for the main taxonomic groups represented within *Ascomycota*, *Basidiomycota*, and *Glomeromycotina*.

**Figure 2** - Mean isolation distances at the plot scale for the four mixtures of plants we analyzed. 1sp. denotes the *B. pinnatum* monocultures, whereas the other 'sp.' abbreviations denote *B. pinnatum* in mixed plant mesocosms of 2, 4, and 8 plant species. Bar graphs provide isolation distances, Euclidean distances (green), Resistance distances calculated in 2011 (yellow) and in 2012 (red).



