Ecological corridors homogenize plant root endospheric mycobiota

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Summary

- Ecological corridors promote species coexistence in fragmented habitats where dispersal limits species fluxes. The corridor concept was developed and investigated with macroorganisms in mind, while microorganisms, the invisible majority of biodiversity, were disregarded.
- We analyzed the effect of corridors on the dynamics of endospheric fungal assemblages associated with plant roots at the scale of 1 m over 2 years (i.e. at five time points) by combining an experimental corridor-mesocosm with high-throughput amplicon sequencing.
- We showed that plant root endospheric mycobiota were sensitive to corridor effects when the corridors were set up at a small spatial scale. The endospheric mycobiota of connected plants had higher species richness, lower beta-diversity, and more deterministic assembly than the mycobiota of isolated plants. These effects became more pronounced with the development of host plants.
- Biotic corridors composed of host plants may thus play a key role in the spatial dynamics of microbial communities and may influence microbial diversity and related ecological functions.

Introduction

Anthropogenic activities have caused habitat destruction and degradation leading to habitat fragmentation and threatening biodiversity (Diamond, 1976; Hilty *et al.*, 2006; Haddad *et al.*, 2015). Ecological corridors are one way to mitigate the negative effect of habitat fragmentation (Rosenberg *et al.*, 1997; Haddad, 2015). When dispersal among habitat patches is limited, corridors facilitate the movement of animals and dispersal of plants between habitat patches (Tewksbury *et al.*, 2002) and reduce the probability of local extinction of isolated populations (Brown & Kodric-Brown, 1977). Corridors therefore have mostly positive effects on species richness at the patch scale (Damschen *et al.*, 2006) although there may be some negative effects, such as facilitation of dispersal by highly competitive species or pathogens (Haddad *et al.*, 2014). In metacommunities, sets of connected patches linked by dispersal fluxes, local community assembly involves both deterministic and stochastic processes such as competition, drift, and dispersal (Leibold *et al.*, 2004). The metacommunity framework predicts that local species diversity will increase when the dispersal rate is intermediate (a hump-shaped relationship among dispersal effects and local diversity). Patches with high dispersal may be less affected by drift and therefore more similar in species composition (Mouquet & Loreau, 2003). Beta-diversity among local communities is reduced (Jamoneau *et al.*, 2012) due to high migration rates (Arévalo *et al.*, 2010).

To date, research into the effect of ecological corridors on maintaining biodiversity has focused on macroorganisms, while microorganisms, the invisible majority of biodiversity (Staddon *et al.*, 2010), have largely been overlooked. Existing knowledge on ecological corridors applied to microorganisms focuses either on key pathogen species or on specific taxonomic groups such as mycorrhizal fungi (Groppe *et al.*, 2001; Yuen & Mila, 2015; Chagnon *et al.*, 2020) but does not account for the diversity, composition, and assembly process of microbial assemblages as a whole.

Microbial distribution has long been assumed to be subject to very little dispersal limitation due to the small size and high propagule production of many microbes (Baas-Becking's hypothesis; Baas Becking, 1934). If microbes indeed do not suffer from dispersal limitation, then community assembly would depend only on local conditions. However, microbe communities demonstrate biogeographical patterns (Martiny *et al.*, 2006; Hanson *et al.*, 2012; Nemergut *et al.*, 2013; Xu *et al.*, 2022) and strong distance–decay relationships (Xu *et al.*, 2021). Furthermore, mechanistic approaches, based on the metacommunity framework, have been used to quantify the importance of dispersal for species coexistence in microbes (Miller *et al.*, 2018; Langenheder & Lindström, 2019). Most studies that have analyzed

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microbial dispersal have focused on biogeographic distribution patterns based on metacommunity theory at a large scale (Powell *et al.*, 2015; Mansour *et al.*, 2018; Zhang *et al.*, 2018; Brown *et al.*, 2020), even dispersal studies of mycorrhizal fungal propagules (Correia *et al.*, 2019; Paz *et al.*, 2021).

However, local dispersal limitation has been shown to play a critical role in shaping the distribution and diversity of microorganisms (Telford *et al.*, 2006). Some of these microorganisms, including endospheric fungi, are preferentially associated with certain plants (Wagner *et al.*, 2016). The host preference effect results from close interactions among plants and their associated microbiota leading to the selective recruitment of particular microorganisms. Host plants are thereby the preferential microhabitats of these microorganisms, and microbial dispersal limitation may be linked to the distance between host plants (Mony *et al.*, 2020b). In endospheric fungi associated with plant roots, dispersal limitation seems to occur at scales of < 1 m (Mony *et al.*, 2020a), likely because of their low dispersal capacity. Local microbial dispersal among host plants is therefore assumed to be at least partially achieved via the dispersal of spores or hyphal fragments by soil fauna (Lilleskov & Bruns, 2005; Vašutová *et al.*, 2019), by the dispersal of stolons or rhizomes (Vannier *et al.*, 2018), or by inoculation of roots through contact with neighboring plant roots (Mony *et al.*, 2021). The effect of corridors provided by connected host plants may thus promote dispersal of microorganisms among plants.

In the initial stages of plant development, the colonization of microorganisms is mostly driven by priority effects (Debray *et al.*, 2022). Fungal species in the immediate vicinity colonize hosts sequentially rather than simultaneously with the first species to arrive possibly preventing further colonization by latecomers (e.g. as with arbuscular mycorrhiza; Werner & Kiers, 2015). This early microbial colonization process is determined by the composition of local microbial reservoirs and results in stochastic species dynamics in homogeneous environments (Bell, 2001). After this first phase of colonization, the host plants then regulate the colonization of microorganisms through selective recruitment of particular microorganisms potentially reducing the influence of stochasticity on colonization. Such root-associated microbial recruitment can be achieved by emitting secondary metabolites such as coumarins (Voges *et al.*, 2019) and volatile compounds (Schulz-Bohm *et al.*, 2018), and by rewarding the most beneficial symbionts in the endosphere (Kiers *et al.*, 2011). As such, the effect of corridors might be more pronounced over time as root-associated microbial communities become more specific to the host plant species.

Plant-associated microorganisms influence their host's performance (Trivedi *et al.*, 2020). Thus, if corridor connections can affect the distribution patterns of plant-associated microorganisms, then corridors might be important to consider as a driver for providing microbial functions (Mony *et al.*, 2022). Corridors between plants may enable favorable microorganisms to colonize plants such as mycorrhizal fungi which are particularly important in plant nutrition (Smith & Read, 2008), or detrimental microorganisms such as pathogens which may alter plant health. Understanding the dynamics of such corridors from the perspective of the microbes is therefore likely to contribute to building up barriers of pathogen dispersion or channels of beneficial microorganisms in agrosystems (Ampt *et al.*, 2022).

In this study, we analyzed the effect of corridors on the dynamics of plant root endospheric mycobiota at the scale of 1 m. We used carefully designed mesocosm systems to test two experimental treatments: two isolated patches of *Trifolium repens* growing in a matrix of *Brachypodium pinnatum*, one with a corridor of *T. repens* and one without (Fig. 1). After checking that *B. pinnatum* and *T. repens* contained distinct microbiota, we characterized root endospheric mycobiota using high-throughput amplicon sequencing for *T. repens* in connected or isolated patches and analyzed sequence cluster composition, richness, and dissimilarity under each treatment. In theory, in a community of sympatric organisms sharing the same habitat, species are neutral if the success of one is not dependent on the other. In this case, changes in the community composition are expected to be random. Alternatively, species can interact with one another, and these deterministic species interactions can alter community assembly. In natural communities, both neutral and deterministic processes take place simultaneously and can be partitioned. In this study, we used neutral community models (NCMs) (Sloan *et al.*, 2006) to predict whether connection would facilitate species fluxes between patches, thereby promoting species coexistence. To test for the effects of a corridor on fungal groups with particular ecological functions, we parsed fungal sequence clusters by ecological guilds or traits using an existing fungal trait database (Nguyen *et al.*, 2016; Zanne *et al.*, 2020).

Based on metacommunity theory, we made three specific predictions: (1) connected host plants display higher root endospheric mycobiota species richness than isolated host plants; (2) the root endospheric mycobiota of connected host plants are more similar than those of isolated host plants (i.e. lower beta-diversity); (3) the root endospheric mycobiota of connected host plants are less subject to stochasticity than the mycobiota of isolated host plants. Overrepresented and underrepresented sequence clusters detected in the NCM of connected plants are assumed to be actively promoted or filtered within the root mycobiota and to correspond to deterministic processes of assembly. We further expect that these patterns will become more pronounced over time with the development of host plants, because of the reduced importance of stochastic colonization of roots by prior species, compared with deterministic selection by plants in the assembly of root-associated mycobiota.

Materials and Methods

Experimental design and plant root sampling

The experimental design consisted of 20 mesocosms located in the common garden of the University of Rennes 1 (France). The mesocosms were filled with a homogenized substrate consisting of sand (20%) and agricultural silty clay soil (80%). We tested two treatments: one with and one without a corridor. In both treatments, we planted two perennial plant patches of *T.*

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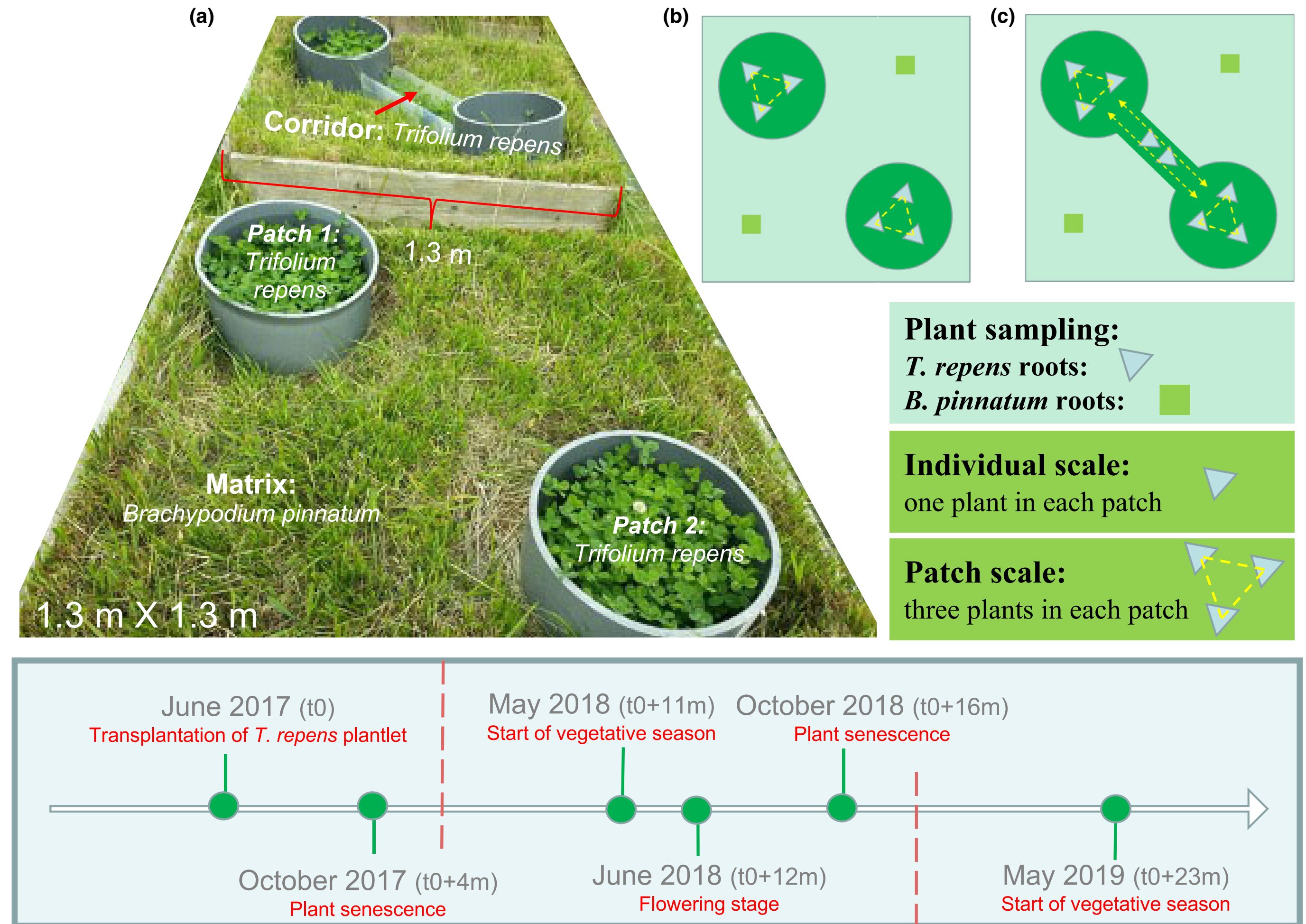

Fig. 1 Experimental design of a patch-matrix mesocosm. (a) Photograph of the designed mesocosms used in this study. Two patches (D: 0.40 m; H: 0.15 m) of *Trifolium repens* were embedded in a matrix (L: 1.30 m × W: 1.30 m × H: 0.25 m) of *Brachypodium pinnatum*; in the treatment including a corridor, *T. repens* patches were connected by a narrow corridor (L: 0.90 m × W: 0.15 m × H: 0.15 m) of *T. repens*. (b) Plant sampling design for mesocosms with no connecting corridor. (c) Plant sampling design for mesocosms with a connecting corridor. Triangles denote *T. repens* roots and rectangles denote *B. pinnatum* roots. Yellow dashed lines indicate potential belowground microbial interactions through plants. Data analyses were conducted at individual and patch scales. At the individual scale, the fungal structure of each individual plant was analyzed at each sampling time point. At the patch scale, the fungal structure of three *T. repens* plants in the same *T. repens* patch was pooled at each sampling time point (total sequence clusters were pooled with their mean abundances using the R function *aggregate*). In all, 10 mesocosms with a corridor and nine mesocosms with no corridor were created. The experiment was launched with the transplantation of *T. repens* plantlets in patches with or without a corridor in June 2017 (the first year), with five sampling time points: October 2017 (t0 + 4 months), May 2018 (t0 + 11 months), June 2018 (t0 + 12 months), October 2018 (t0 + 16 months), and May 2019 (t0 + 23 months). The corresponding growth stages of *T. repens* are indicated in red below each sampling time point. Red dashed lines denote the end of each year.

repens L. (Fabaceae) in a matrix of *B. pinnatum* (L.) P. Beauv. (Poaceae; Fig. 1a). In the corridor treatment, *T. repens* patches were connected by a narrow corridor planted with *T. repens*. *Trifolium repens* and *B. pinnatum* are phylogenetically different and likely associate with distinct root microbial assemblages. The experimental treatments thus corresponded to a patch-matrix mesocosm design. We used 10 mesocosms per treatment, which were all established on 9 June 2017. In the treatment with no corridor, one mesocosm was lost due to insufficient growth of *T. repens* in the patches. We sampled at five time points (October 2017, May 2018, June 2018, October 2018, and May 2019; Fig. 1). At each sampling time point, we randomly collected three ramets of *T. repens* in each of the two patches from all mesocosms, and two ramets in the corridor of mesocosms containing corridors for a total of 670 samples of *T. repens*. Two ramets of *B. pinnatum* were also collected randomly in the matrix at least 20 cm from the edge of the corridors and at least 10 cm from the edge of the mesocosm giving a total number of 190 samples of *B. pinnatum*. The root systems were carefully cleaned with tap water. For each sample, we then selected 100 mg of roots (fresh weight) from a representative subsample of the root system. The surface of the root was cleaned in a 2% Triton X100 solution for 5 min and then rinsed twice with tap water; finally, the roots were rinsed with sterile distilled water. The roots were then cut into small pieces and ground into powder for subsequent DNA extraction. More detailed information on the experimental design is available in Supporting Information Methods S1.

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DNA extraction, amplicon sequencing, and bioinformatics

DNA was extracted from 100 mg of fresh root powder using a standard protocol with magnetic beads (Sbeadex mini plant kit; LGC Genomics, Hoddesdon, UK) and an automated protocol (oKtopure robot platform; LGC Genomics) on the Gentyane platform. DNA concentrations were normalized to 12.5 ng μl^{-1} after a fluorometric Hoechst DNA assay for subsequent next-generation sequencing. A specific 550 bp of the fungal small subunit (SSU) rRNA gene fragment, including the V4 and V5 regions, was amplified from 50 ng of extracted total DNA using the primers SSU0817 and NS22B. We chose to work with an SSU rRNA gene fragment to limit overestimation of fungal richness in spite of lower taxonomic resolution, especially for higher fungi when compared to internal transcribed spacer (ITS). A negative control was also amplified and sequenced to detect possible cross-contamination. Library production and sequencing were performed on the EcogenO platform (https://osur.univ-rennes1.fr/ecogeno). Sequence data were analyzed using the Frogs pipeline (Escudié *et al.*, 2018). Within Frogs, the sequence clustering was performed using Swarm (Mahé *et al.*, 2014) enabling the production of sequence clusters that were not defined by a threshold, and hence a sequence cluster is close to an amplicon sequence variant which is one single DNA sequence or to a zero-radius operational taxonomic units which is one denoised sequence, but with the advantage of avoiding artificial overestimation of sequence-based diversity estimates. Sequence clustering was combined with a rigorous chimera removal step (Escudié *et al.*, 2018) followed by the removal of all sequence clusters detected in less than three independent samples and with a threshold of 0.005% of reads. As recommended in the Frogs guidelines (Escudié *et al.*, 2018), affiliation was made by Blastn+ for one representative sequence of each sequence cluster using herein PhymycoDB (Mahé *et al.*, 2012) as a reference database (threshold of at least 95% Blast identity and 95% coverage applied). In the particular case of getting identical Blastn+ scores for a given representative sequence to affiliate, the last convergent taxonomic rank was kept. The number of sequences per sample was normalized to 4292 sequences (Fig. S1). This contingency table was used for all subsequent statistical analyses. More detailed information is available in Methods S1.

Statistical analysis

Root endospheric mycobiota diversity indices Root mycobiota diversity was indicated by sequence cluster richness and evenness for the entire community (hereafter termed 'global fungi'), and in each phylum group, for example, Ascomycota, Basidiomycota, Chytridiomycota, Glomeromycota, and Zygomycota, at individual and patch scales (e.g. population scale). At the patch scale, fungal sequence cluster richness and evenness were calculated based on the fungal structure pooled over all *T. repens* samples in the same experimental patch at each sampling time point (the abundance of each sequence cluster was calculated as the mean value of the three ramets sampled in the same patch at that sampling time point). This was calculated using the function *aggregate* in the R package stats. Sequence cluster richness was calculated as the total number of sequence clusters present in the whole community of root mycobiota. Evenness was assessed using Pielou's evenness index based on the original abundance at the individual scale and on the mean abundance of sequence clusters at the patch scale (Pielou, 1966). Both sequence cluster richness and evenness were calculated using the R package vegan (Oksanen *et al.*, 2022).

We used linear mixed-effects models (LMMs) or generalized linear mixed-effects models (GLMMs; Bates *et al.*, 2015) at individual and patch scales to test whether the presence of a corridor, time, and their interactions affected the root mycobiota diversity of global fungi and of each fungal phylum. In the models, the presence of a corridor (with vs without), time (five sampling time points), and their interactions were treated as fixed effects and mesocosm and patch (for individual scale analysis) and mesocosm (for patch scale analysis) as random factors. We used LMMs with the *lmer* function in the R package lme4 for evenness indices and GLMMs with a negative binomial distribution with the *glmer.nb* function in the R package lme4 for diversity indices, which is appropriate for count data and yielded GLMMs without overdispersion (Zuur *et al.*, 2009). We analyzed the significance of each variable within the model with analysis of variance (ANOVA) type II sums of squares. We calculated the proportion of variance explained by the model with only the fixed effects (marginal R^2) and by the model also including random effects (conditional R^2) for all analyses (Nakagawa & Schielzeth, 2013). We graphically checked for homoscedasticity, independence, and normality of residuals in the tested models. When the models were significant, group comparisons were tested using the *lsmeans* function in the R package LmerTest (Kuznetsova *et al.*, 2017).

Root endospheric mycobiota beta-diversity Fungal beta-diversity was assessed using the Bray–Curtis dissimilarity index and multivariate analysis. We computed Bray–Curtis distance matrices using the *vegdist* function in the R package vegan and then visualized them using principal coordinate analysis. Effects of the presence of a corridor, time, and their interactions on the structure of root mycobiota were tested with PERMANOVA using the *adonis* function in the R package vegan (Table S1). Because of significant interactive effects of the presence of a corridor and sampling time points, the effect of the presence of a corridor on root endospheric mycobiota composition at individual and patch scales was tested at each sampling time point (Table 1).

Bray–Curtis dissimilarity between paired root mycobiota communities of *T. repens* in the same mesocosm at the individual scale (all nine possible pairs between three individuals in patch 1 and three individuals in patch 2 of the same mesocosm) and at the patch scale (only one pair of the same mesocosm) was calculated using the *vegdist* function in the R package vegan. Individual-scale corridor effects and time effects on Bray–Curtis distances between root mycobiota of pairwise patches in the same mesocosm were tested using mixed linear models with the mesocosm as a random effect. At the patch scale, corridor effects and time effects were tested using *t*-tests and Tukey's honestly significant difference test, respectively.

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Table 1 Effect of the presence of a corridor on root endospheric mycobiota structure of *Trifolium repens* at each sampling time point at individual and patch scales.

Different scales/Sampling time points	Root mycobiota composition at the individual scale		Root mycobiota composition at the patch scale	
	F	*P*	*F*	*P*
October 2017	1.53	0.08	1.28	0.20
May 2018	1.80	**0.02**	0.81	0.68
June 2018	2.21	**0.01**	1.23	0.24
October 2018	2.42	**0.01**	2.20	**0.01**
May 2019	1.36	0.16	3.65	**0.002**

The effects were tested using a PERMANOVA with the *adonis* function in R. Significant results ($P < 0.05$) are shown in bold.

Neutral community model To detect the assembly process of the root mycobiota in the treatments with and without a corridor, we used a NCM proposed by Sloan *et al.* (2006) to predict the relationships between the detection frequency of detection sequence clusters and their relative abundance in a set of local communities (the community in either the treatment with a corridor or without a corridor) across the wider metacommunity (the community of both treatments, i.e. with a corridor and without a corridor). We used the pooled dataset as source data to calculate the null model: the source data differed among sampling time points to account for the possible change over time in the pool available for recruitment over time. The sequence clusters from each dataset were subsequently separated into three partitions depending on whether they occurred more frequently than (above the partition, overrepresented sequence clusters), less frequently than (below the partition, underrepresented sequence clusters), or within (neutral partition, stochastic sequence clusters) the 95% confidence interval of the NCM predictions (Fig. S2). In this model, Nm is an estimate of the dispersal of root mycobiota communities between patches with or without the presence of a corridor. The parameter Nm determines the correlation between the frequency of occurrence and regional relative abundance, where N represents the size of the metacommunity and m is the immigration rate. The parameter R^2 represents the overall fit to the neutral model. Detailed statistics for all the models are provided in Table S2. The 95% confidence intervals around all fitting statistics were calculated by bootstrapping with 1000 bootstrap replicates.

Functional prediction of root endospheric mycobiota

To test for the effects of a corridor on fungal groups with particular ecological functions, we combined information from the following databases: FUNGuild (Nguyen *et al.*, 2016), FUN^{FUN} v.0.0.3 (Zanne *et al.*, 2020), and FungalTraits v.1.2 (Põlme *et al.*, 2020) to parse fungal sequence clusters with ecological guilds or traits. First, we used FUNGuild.py script in the Python 3 environment to assign the functions of fungi by uploading our own file of taxa to the FUNGuild database, and then we manually added the complementary information about fungal traits and functions from databases FUN^{FUN} v.0.0.3 and FungalTraits v.1.2. An ecological guild is an index with the potential to indicate the functions of fungal species, but it is important to note that the assignment by the above databases to an ecological guild is currently largely at the genus level. In this study, we focused on three guilds: symbiotrophs, plant pathogens, and saprotrophs. The effect of the presence of a corridor, time, and their interactions on sequence cluster richness and on the abundance (cumulative reads of sequence clusters) of each guild was analyzed using GLMMs with a negative binomial distribution with the *glmer.nb* function in the R package lme4. Group comparisons of FUNGuild sequence cluster richness and abundance at the five sampling time points in treatments with or without a corridor were compared using the *lsmeans* function in the R package LmerTest.

Results

Plant species affected the structure of their associated root endospheric mycobiota

The dataset contained a total of 266 fungal sequence clusters within Ascomycota, Basidiomycota, Chytridiomycota, Glomeromycota, and Zygomycota (Fig. S3a,b). Even though *B. pinnatum* and *T. repens* shared most of their root endospheric mycobiota (Fig. S4), the abundance of those shared root endospheric mycobiota differed in these two plant species. The root mycobiota community composition of *B. pinnatum* differed from that of *T. repens* (Fig. 2a) across all sampling time points (Fig. S5), demonstrating that these two phylogenetically distant plant species are associated with specific fungal assemblages. The root endospheric mycobiota of *T. repens* were similar in the patches and corridors at each of the five sampling time points (Fig. S6). Despite some overlap, the structure of root-associated mycobiota of *T. repens* differed significantly among the five sampling time points at both the individual scale (Fig. 2b) and the patch scale (Fig. 2c). This result indicates that the composition of the mycobiota assemblages changed significantly between the beginning and the end of the 2-yr experiment. The composition of the mycobiota depended on the presence of a corridor (only at the individual scale), on the sampling time point, and on their interaction both at the individual scale and at the patch scale (Table S1).

Ecological corridors promoted plant root endospheric mycobiota diversity

At the individual scale, fungal assemblages of connected *T. repens* individuals displayed higher richness than isolated individuals, even though the positive effect of the presence of a corridor on sequence cluster richness was only perceptible in June 2018 and October 2018 (i.e. 12 and 16 months after the start of the experiment; Fig. 3a). At the individual scale, sequence cluster richness depended on the presence of corridors for all phyla for at least one sampling time point (Fig. S7a–e). Higher sequence cluster

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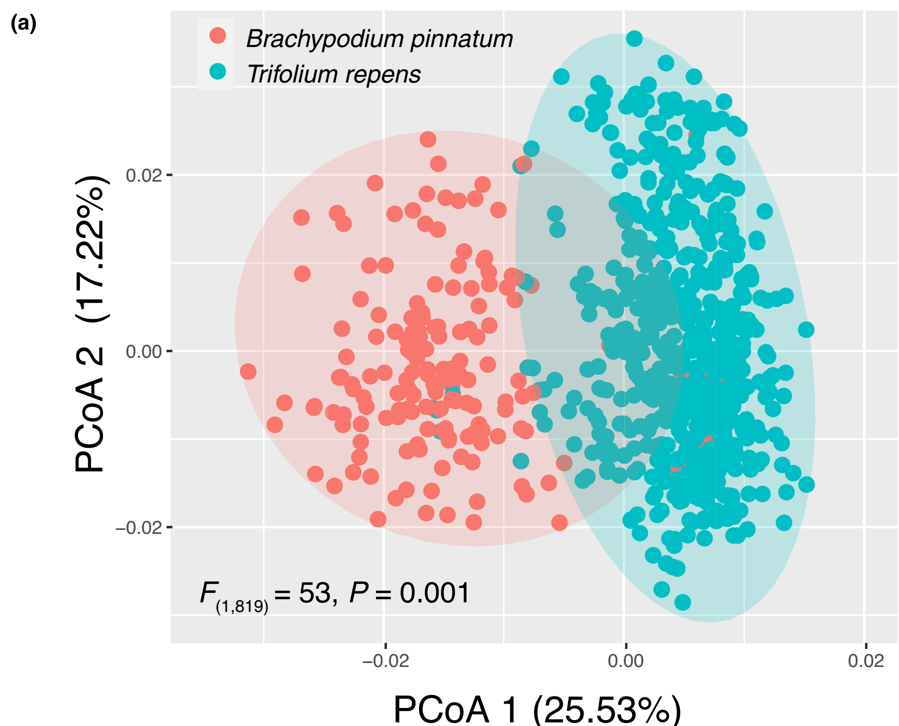

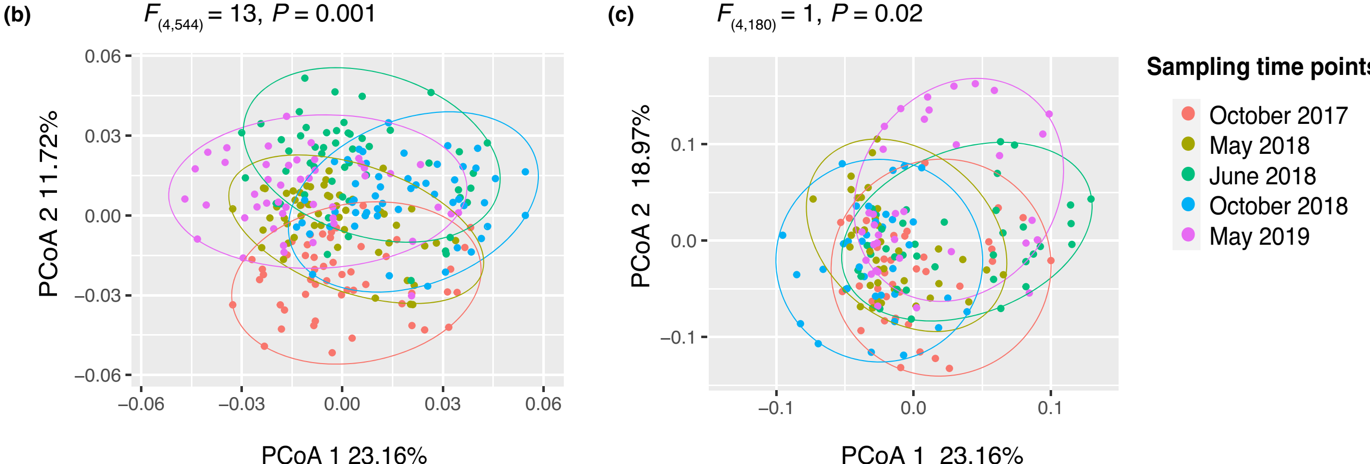

Fig. 2 Root endospheric mycobiota structure of *Trifolium repens* and *Brachypodium pinnatum* visualized by principal coordinate analysis. (a) Root mycobiota community composition of both *T. repens* and *B. pinnatum* at all sampling time points. (b) Root mycobiota community composition of only *T. repens* in patches along with five sampling time points at the individual scale. (c) Root mycobiota community composition of only *T. repens* in patches along with five sampling time points at the patch scale. One dot corresponds to the root mycobiota of one *T. repens* sample in (a) and (b), one dot corresponds to the root mycobiota of three *T. repens* samples from the same patch in (c). Ellipses in each figure represent the 95% confidence interval. Statistics indicated with F and P values obtained from a PERMANOVA with the *adonis* function in R: (a) using the two plant species as factors and (b, c) using five sampling time points as factors.

richness was detected in connected *T. repens* than in isolated *T. repens* in Ascomycota (June 2018, October 2018, and May 2019), Chytridiomycota (October 2018), and Zygomycota (June and October 2018). However, and unlike the other phyla, we observed lower sequence cluster richness in Basidiomycota (October 2017), and in Glomeromycota (May 2019) in connected *T. repens* plants than in isolated ones (Fig. S7d,e). Evenness was higher in June 2018 and lower in October 2018 in connected *T. repens* (Fig. 3b), with changes only detected in the Chytridiomycota phyla in October 2018 (Fig. S7m). At the patch scale, the positive effect of the presence of a corridor on root mycobiota sequence cluster richness was only significant in October 2018 (Fig. 3c), while no effect was detected on evenness (Fig. 3d). An increase in richness was found in all phyla except Glomeromycota in plants growing in connected patches in October 2018 (Fig. S7f–j).

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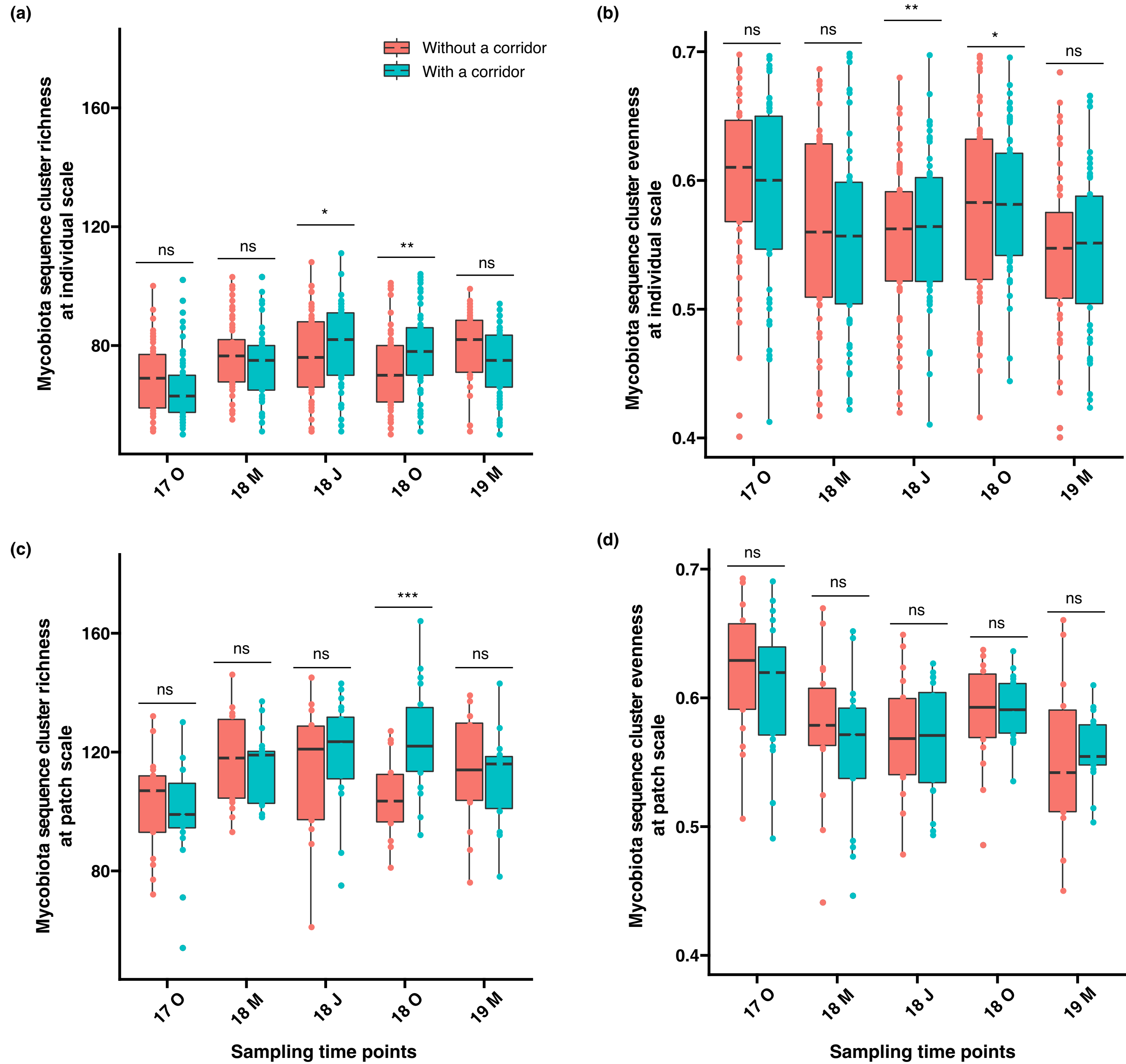

Fig. 3 Root endospheric mycobiota diversity for 'all fungi' associated with *Trifolium repens* in treatments with and without a corridor over five sampling campaigns at individual and patch scales. Root endospheric mycobiota diversity was calculated as sequence cluster richness and Pielou's evenness. Indices were calculated and tested at two different biological scales: the individual plant (i.e. one root sample; upper panels a and b) and the patch (i.e. group of three *T. repens* root samples pooled from the same patch; lower panels c and d). (a, c) Sequence cluster richness of root endospheric mycobiota at individual and patch scales, respectively. (b, d) Pielou's evenness of root endospheric mycobiota at individual and patch scales, respectively. Boxplots showing the middle 50% of scores (i.e., the range between the 25th and 75th percentile), horizontal lines inside boxplots indicate the median scores, the upper and lower whiskers represent scores outside the middle 50%, data points that are located outside the whiskers of the boxplots are outliers. 17 O, October 2017; 18 M, May 2018; 18 J, June 2018; 18 O, October 2018; 19 M, May 2019. Asterisks indicate the significance level of the presence of a corridor on root endospheric mycobiota diversity: *, $0.01 < P < 0.05$; **, $0.001 < P < 0.01$; ***, $P < 0.001$; ns, not significant.

Ecological corridors homogenized plant root endospheric mycobiota composition

The effect of the presence of a corridor on the similarity of *T. repens* root mycobiota composition between patches was assessed using the Bray–Curtis dissimilarity index at the individual and patch scales. While there was no significant effect of the presence of a corridor on the Bray-Curtis dissimilarity in October 2017 and May 2018, the composition of *T. repens* root endospheric mycobiota was more similar in connected individuals and patches than in isolated individuals and patches at the other sampling time points (June 2018, October 2018, and May 2019; Figs 4,

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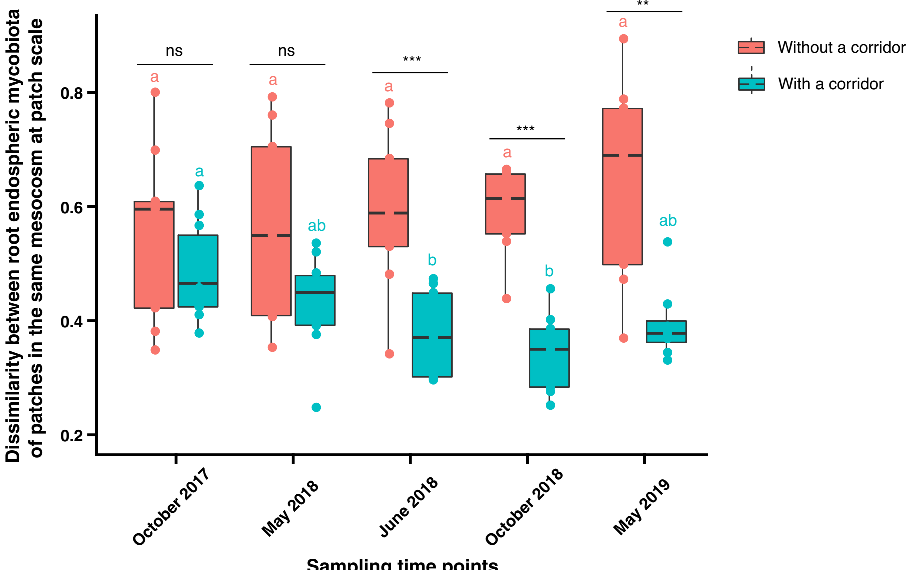

Fig. 4 Effect of the presence of a corridor on *Trifolium repens* root endospheric mycobiota dissimilarity at the patch scale. *Trifolium repens* root endospheric mycobiota dissimilarity was calculated as Bray–Curtis dissimilarity between the root mycobiota of pairwise *T. repens* patches (i.e. at the patch scale) for the same mesocosms with and without a corridor and for the five sampling time points. Boxplots showing the middle 50% of scores (i.e., the range between the 25th and 75th percentile), horizontal lines inside boxplots indicate the median scores, the upper and lower whiskers represent scores outside the middle 50%, data points that are located outside the whiskers of the boxplots are outliers. Pairwise comparisons were conducted on Bray–Curtis dissimilarity between treatments with a corridor and without a corridor using a *t*-test, and the significance is indicated by asterisks at the top of the bar plots: *, $0.01 < P < 0.05$; **, $0.01 < P < 0.001$; ***, $P < 0.001$; ns, not significant. Multiple group comparisons were conducted on Bray–Curtis dissimilarity across sampling campaigns with and without corridors, and the significance is indicated by lowercase letters.

S8). In the mesocosms without corridors, the Bray–Curtis dissimilarity index remained stable over time (Fig. 4), whereas it decreased significantly over time in the presence of a corridor (Fig. 4). Connected patches showed lower Bray–Curtis dissimilarity in Ascomycota only at the individual scale (May 2018, June 2018, and October 2018), in Basidiomycota only at the individual scale in June 2018, in Chytridiomycota at both the individual (June 2018, October 2018, and May 2019) and patch scales (May 2019; Fig. S9c,h), in Glomeromycota at both the individual (June 2018) and patch scales (June 2018 and October 2018; Fig. S9d,i), and in Zygomycota only at the individual scale in May 2019 (Fig. S9e).

Ecological corridors boosted the deterministic assembly of plant root endospheric mycobiota

To assess the relative contribution of stochastic and deterministic assembly processes on the root mycobiota communities, we applied the Sloan neutral model to root endospheric mycobiota sequence data (Sloan *et al*., 2006; described in the Materials and Methods section; Fig. S2). We expect sequence clusters to be more represented or less represented than in the calculated neutrality envelope if deterministic processes drive assembly. Alternatively, if stochastic processes drive assembly, then sequence clusters are expected to fall inside the neutrality envelope.

Our results suggest that the stochastic assembly process predominated over the deterministic assembly process (i.e. measured stochasticity in the richness and abundance of fungal sequence clusters of 46–51% and 53–78%, respectively; Figs 5a, S10a). The estimated stochastic assembly process was higher for endospheric mycobiota of plants growing in patches with no corridor, with a higher percentage of stochastic sequence clusters recorded at all sampling time points in plants growing in patches with no corridor (51–57%) than in patches with a corridor (46–51%; Fig. 5a). A higher proportion of sequence clusters (calculated based on their abundance) showing stochastic behavior was detected at two of the five sampling time points (October 2017 and October 2018) in plants growing in patches with no corridor (Fig. S10a). This observation is likely linked with the higher richness and abundance of neutral sequence clusters detected in patches without a corridor (Figs 5, S10, S11). Sequence clusters that occurred more frequently than expected under a null model (i.e. overrepresented sequence clusters) dominated in plants growing in patches with a corridor (Fig. 5b). This was potentially due to improved maintenance of biodiversity in patches via dispersal and by limiting the effect of drift. Overrepresented sequence clusters were more frequent in relatively rare fungal species (by comparing proportion of sequence cluster richness and their corresponding abundance, minimum 35% of unique sequence clusters only constituted maximum 20% of sequence cluster abundance) with corridor connections except in October 2017 (Figs 5b, S10b). Sequence clusters that occurred less frequently than expected under a null model (i.e. underrepresented sequence clusters) were more frequent in plants growing in isolated patches both in richness (Fig. 5c, May 2018, June 2018, and May 2019) and in abundance (Fig. S10c, at all sampling time points except October 2017) suggesting greater drift. The presence of a corridor increased *Nm* (Table S2), where *N* is the

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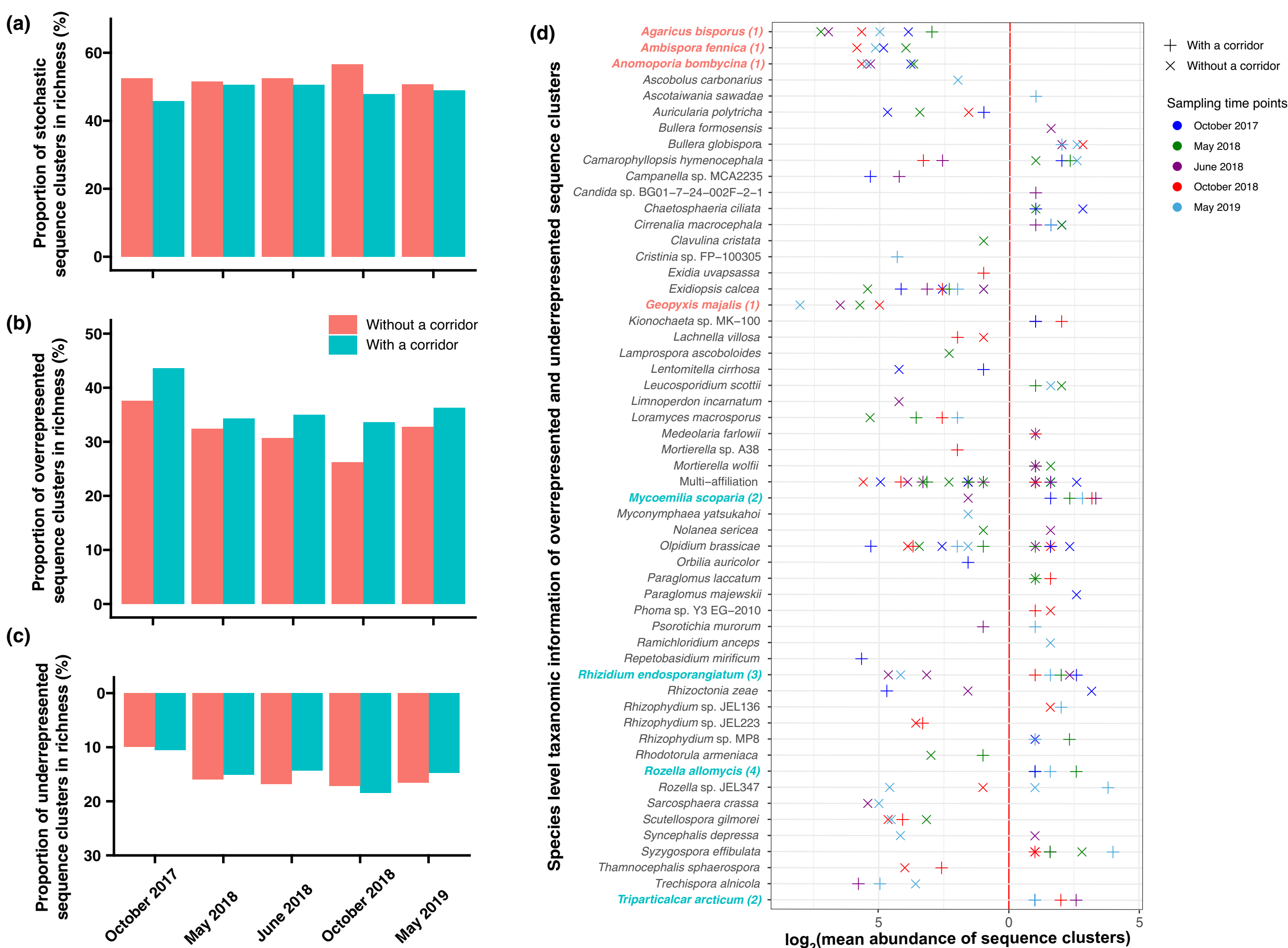

Fig. 5 Summary of root endospheric mycobiota assembly patterns with and without corridors at five sampling time points. (a) Proportion of stochastic sequence clusters among all the sequence clusters based on their richness in both treatments, that is with and without a corridor. (b) Proportion of overrepresented sequence clusters among all sequence clusters based on their richness in both treatments, that is with and without a corridor. (c) Proportion of underrepresented sequence clusters among all the sequence clusters based on their richness in both treatments, that is with and without a corridor. (d) Species-level taxonomic information of overrepresented and underrepresented sequence clusters. In (d), underrepresented sequence clusters are shown on the left of the vertical red dashed line. Overrepresented sequence clusters are shown on the right of the vertical red dashed line; the symbol '+' indicates fungal species belonging to treatments with a corridor, the symbol 'x' indicates fungal species belonging to treatments without a corridor, symbol colors correspond to the different sampling time points indicated in the figure key on the right. The colors of names of the fungi on the y-axis in (d) correspond to treatments with or without a corridor in (a–c), the numbers in parentheses after the names of the fungi give the number of sequence clusters belonging to this species.

size of the metacommunity and m is the immigration rate, thereby demonstrating the role of the corridor in fungal dispersal. Most neutral sequence clusters in patches both with and without a corridor were assigned to Ascomycota. The highest proportion of underrepresented sequence clusters in plants growing in patches with no corridor was assigned to Basidiomycota at all sampling time points except May 2019 (Fig. S12a,c,e,g,i). In plants growing in patches with corridors, the highest proportion was assigned to different phyla depending on the sampling time points (Fig. S12b,d,f,h). The highest proportion of overrepresented sequence clusters shifted from Ascomycota to Zygomycota and Basidiomycota depending on the sampling time points for plants growing in isolated patches, and were assigned to Ascomycota and to a lesser extent to Zygomycota (particularly in May and October 2018) in plants growing in patches with a corridor (Fig. S12b,d,f,h,j). At the highest taxonomic resolution (i.e. species level), 11 different sequence clusters closely related to four fungal species, that is, *Mycoemilia scoparia* (Zygomycota; two closely related sequence clusters), *Rhizidium endosporangiatum* (Chytridiomycota; three closely related sequence clusters), *Rozella allomycis* (Chytridiomycota; four closely related sequence clusters), and *Triparticalcar arcticum* (Chytridiomycota; two closely related sequence clusters) were the most overrepresented root endospheric sequence clusters with corridor connections detected from the NCMs (Fig. 5d). Conversely, four sequence clusters closely related to *Agaricus bisporus* (Basidiomycota), *Anomoporia bombycina* (Basidiomycota), *Geopyxis majalis* (Ascomycota), and *Ambispora fennica* (Glomeromycota) were the most

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underrepresented root endospheric sequence clusters without corridor connections (Fig. 5d). The molecular target used, that is the V4–V6 SSU rRNA gene fragment, offers a good taxonomic resolution for lower fungi but a limited resolution for Ascomycota resulting in a possible bias if interpreting sequence data with species-level affiliation only.

Ecological corridors did not promote the spread of plant pathogens

After combining three databases (FUNGuild, FUN^{FUN}, and FungalTraits) to predict fungal functions, we were able to assign 138 of the 266 sequence clusters detected in the *T. repens* root endosphere to ecological guilds, that is, plant pathogens (16 sequence clusters), symbiotrophs (18 sequence clusters), and saprotrophs (77 sequence clusters; Table S3). Sequence cluster richness and the abundance of plant pathogens did not depend on the presence of a corridor, nor on its interaction with a sampling time point at either the individual or the patch scale (Fig. S13a,d; Table 2). Plant pathogen abundance decreased with time at both individual and patch scales (Table 2). Symbiotroph richness decreased with time without a corridor connection at the individual scale (Fig. S13b), but increased with time with a corridor connection at the patch scale (Fig. S13e). It was higher with corridor connections in June 2018 at the individual scale and in October 2018 at the patch scale (Fig. S13b,e). There was no corridor effect on symbiotroph abundance at either the individual or the patch scale (Fig. S13h,k; Table 2). Saprotroph richness increased with time at both the individual and patch scales (Fig. S13c,f; Table 2), while saprotroph abundance decreased with time at both the individual and patch scales (Fig. S13i,l; Table 2). Saprotroph richness was higher with a corridor connection in June 2018 and October 2018 at the individual scale and in October 2018 at the patch scale (Fig. S13c,f). There was no corridor effect on saprotroph abundance at either the individual or patch scale (Fig. S13i,l; Table 2).

Discussion

Plant root endospheric mycobiota were shaped by the presence of corridors

While the structure of sequence clusters of the *T. repens* root endosphere was marked by strong temporal dynamics, we demonstrated that the plant mycobiota community was shaped by connectivity between patches of individual plants. Depending on the sampling time points, the presence of a corridor influenced root endospheric fungal structure, richness and/or evenness, and fungal diversity at all scales from the individual plant to plant patches and between patches. Our experimental design was based on the assumption that two phylogenetically distant plants such as *B. pinnatum* and *T. repens* were associated with different root fungal communities and could thus be used to simulate the isolation or connectivity of fungal assemblages associated with *T. repens* individuals. This assumption was confirmed throughout the experiment with contrasted fungal assemblages in the two plant species, but similar assemblages between *T. repens* mycobiota originating from patches or corridors, suggesting a strong host preference effect. Edge effects (Tscharntke *et al.*, 2012) were limited with little species spillover from the *B. pinnatum* matrix

Table 2 Effects of the presence of a corridor, time, and their interaction on root endospheric mycobiota sequence cluster richness and abundance in each FUNGuild of *Trifolium repens* at individual and patch scales.

	Corridor effect	Time effect	Interactive effect (Corridor × Time)	R^2_m	R^2_c
At the individual scale					
Richness					
Plant pathogen	0.22 (ns)	**14.07** (**) ↑	8.02 (ns)	0.04	0.05
Symbiotroph	1.53 (ns)	**25.50** (***) ↓	7.30 (ns)	0.06	0.09
Saprotroph	1.14 (ns)	**62.93** (***) ↑	**24.13** (***) ↓	0.13	0.16
Abundance					
Plant pathogen	0.94 (ns)	**17.08** (**) ↓	10.39 (*) ↓	0.04	0.07
Symbiotroph	1.58 (ns)	**50.94** (***) ↑	1.87 (ns)	0.04	0.10
Saprotroph	0.90 (ns)	**46.12** (***) ↓	6.10 (ns)	0.02	0.03
At the patch scale					
Richness					
Plant pathogen	0.01 (ns)	2.94 (ns)	0.60 (ns)	0.03	0.09
Symbiotroph	0.01 (ns)	16.62 (**) ↓	7.29 (ns)	0.12	0.12
Saprotroph	2.45 (ns)	**30.05** (***) ↑	5.36 (ns)	0.16	0.27
Abundance					
Plant pathogen	0.51 (ns)	**11.22** (*) ↓	**11.11** (*) ↓	0.004	0.006
Symbiotroph	1.11 (ns)	**26.39** (***) ↑	0.51 (ns)	0.13	0.20
Saprotroph	0.54 (ns)	**29.46** (***) ↓	5.15 (ns)	0.07	0.09

Random effects of different mesocosms and patches were tested at the individual scale, and random effects of different mesocosms were tested at the patch scale. R^2_m stands for marginal R^2, and denotes total variance explained by regression, coefficient of determination in the model with only fixed effects; R^2_c stands for conditional R^2, and denotes total variance explained by regression coefficient of determination in the full random model. Significant results are shown with asterisks in parentheses after the chi-squared value: *, $0.01 < P < 0.05$; **, $0.001 < P < 0.01$; ***, $P < 0.001$; ns, not significant. Upward and downward arrows denote positive or negative effects of explanatory variables in all the models, respectively. Significant results ($P < 0.05$) are shown in bold.

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to *T. repens* patches despite the likely existence of contacts between the roots of the two plant species. Such a spillover edge effect was also absent in corridors, despite their longer edges and linear forms that result in a higher ratio between the length of edges to the amount of habitat that might have made them more prone to the influence of *B. pinnatum* growing outside the corridors. The rooting system of the host plants may provide available niches for fungal species with a certain host preference effect and correspond to particular habitats to colonize, or through which to disperse. Dispersal may involve not only a given organism or species but also small or large parts of communities leading to community coalescence (Rillig *et al.*, 2015), that is, the mixing of different communities, from which homogenization of species composition and community structure would be expected.

The expected homogenization influence of corridors was not detected at the very beginning of the experiment but was detected 10–12 months later. This time-lag response may be explained by the succession process involved in the assembly of fungi associated with plants throughout the course of their development from germination to the mature stage. Indeed, newly developing roots are initially randomly colonized by the microbial species present in the soil where the roots are developing (Dini-Andreote & Raaijmakers, 2018; Hu *et al.*, 2020). This colonization can take place through fungal propagules, hyphal fragments, or spores that are present locally in the soil (Smith & Read, 2008). Then, during the course of their development, individual plants progressively select among the fungi colonizing the endosphere those that are the most favorable for their own development through active recruitment or filtration processes, such as the carbon rewarding process (Kiers *et al.*, 2011). As found in this study, plant filtering modifies species composition over time, with a decrease in fungal species evenness in fungal assemblages associated with individual plants. Our results suggest that in addition to this plant filtering process, the spatial configuration of the plant influences the process of fungal succession. The influence of the host plant configuration on root-associated mycobiota has already been demonstrated in *B. pinnatum* plant species (Mony *et al.*, 2020b), especially due to the functional connectivity it provided among plants (Mony *et al.*, 2020a). The present study clearly demonstrates that this connectivity effect exists. To validate the assumptions about the dispersal and colonization process, it would be interesting to undertake complementary experiments with tagged fungi that could be surveyed over time in the presence and absence of corridors. This could be carried out using a range of fungi selected for their contrasted colonization abilities in order to obtain more detailed information to explain the community-level dispersal we addressed here.

Connected host plants displayed higher root endospheric mycobiota species richness and more similar species structure than isolated host plants

Confirming our first prediction, we demonstrated that the presence of a corridor increased the endospheric fungal sequence cluster richness associated with *T. repens* roots, and that this effect was more pronounced with increasing age and in later phenological stages, notably after the plants had flowered. This effect on fungal species richness was accompanied by a change in species composition that was already detected at the first sampling time point and remained significant until the end of the experiment. Changes were observed earlier at the individual scale (in composition in October 2017, in richness in June 2018) than at the patch scale (in composition and richness in October 2018), reflecting a progressive process that eventually reached the entire population of host plants. In addition, we confirmed our second prediction of a more similar endospheric mycobiota among *T. repens* individuals in connected patches than in isolated ones. This was evidenced by a decrease in root endospheric mycobiota dissimilarity associated with connected *T. repens* individuals and patches compared with isolated ones. This effect on beta-diversity was detected after June 2018, that is, 1 yr after the start of the experiment, and until the end of the experiment. This result confirms that, despite starting from a different and stochastic root endospheric mycobiota, corridors tend to homogenize root-associated fungal composition among connected plants. Such time-lag effects in response to landscape structure are referred to as a colonization credit and have been repeatedly demonstrated in the case of macroorganisms (Kuussaari *et al.*, 2009). There have only been a few demonstrations of such processes in microorganisms, especially in response to habitat connectivity (Mony *et al.*, 2022), probably because of the limited number of studies based on time-series analysis of microbial composition. The present work demonstrates the importance of analyzing temporal dynamics in microbial response to host landscapes.

In landscape ecology, the positive effect of the presence of corridors on diversity in the landscape is often explained by two main processes: facilitated dispersal among habitat patches (Rosenberg *et al.*, 1997; Beier & Noss, 1998) and/or an increase in habitat availability, which refers to the habitat amount hypothesis (Fahrig, 2013). Our results suggest that these processes may also apply to fungi, at least at the metric scale considered in this study. Dispersal of mycorrhizal fungi among patches via our experimental corridor may be driven by root contacts (Smith & Read, 2008), but endophytic fungi also disperse through the clonal development of stolons (Vannier *et al.*, 2018, 2019). Additional dispersal processes may involve litter decomposition and a legacy effect on the soil reservoir after the plant leaf senescence stage that influenced the microbial composition of the sampling campaign in October, showing an obvious seasonal effect. Indeed, litter composition has a strong impact on soil fungal composition (Habtewold *et al.*, 2020).

In addition to improved species coexistence, corridors led to species homogenization among patches by promoting preferential dispersal of particular fungi along the corridor, leading to more deterministic assemblages. As we predicted, root endospheric mycobiota of *T. repens* in connected patches were subjected to more deterministic processes than in isolated *T. repens* patches, suggesting a buffering effect of drift through corridors. Despite an existing fungal host plant preference confirmed herein, the drift effect in isolated patches could result from a priority effect. The first fungal colonizers have a major influence on

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subsequent community assembly, leading to divergent root endospheric mycobiota composition, with the strength of the drift effect expected to be positively correlated with the heterogeneity of available fungal propagules among disconnected patches. This is in line with the observation that among the most underrepresented fungi in patches with no corridor, we found an arbuscular mycorrhizal fungus (*Ambispora fennica*, Fig. 5d) for which an existing priority effect has already been demonstrated (Werner & Kiers, 2015). The other underrepresented fungi in the unconnected patches were mainly presumed saprotrophs (Table S3).

The importance of habitat availability for microscopic fungal species is questionable, as many processes that define the carrying capacity of the roots of individual plants for fungi remain unknown. Interactions among microbial components (e.g. fungus–fungus and fungus–bacteria) that constitute the plant microbiota may indeed impact the carrying capacity of plants for microorganisms. Interactions include competition for habitat, available nutrients, exclusion through antimicrobial compounds and reciprocally, via facilitation processes such as cross-feeding, cometabolism, evolution of dependencies (Mataigne *et al.*, 2021), but these ideas are still mostly theoretical and empirical support is needed. Beyond these microbial interactions that putatively at least partially explain microbial community assembly, we cannot exclude possible indirect effects of *T. repens* on soil characteristics and thereby habitat conditions for fungi. It has long been known that, like other Fabaceae species associated with a rhizobium, *T. repens* could enrich the soil in bioavailable nitrogen (Ruschel *et al.*, 1979). Changes in soil characteristics, and especially in nitrogen, are known to affect both fungal and bacterial activity in soil (Demoling *et al.*, 2008; Wang *et al.*, 2021), which possibly result in a change in root endospheric microbiota (Mareque *et al.*, 2018). Further work is necessary to disentangle the direct effect of *T. repens* corridors on microbial dispersal from changes in soil abiotic conditions resulting in changes in the soil microorganism reservoir.

The presence of a corridor affected the fungal species that form the plant root endospheric mycobiota differently

Corridors increased fungal richness in all phyla except Glomeromycota, both at the individual scale (except Basidiomycota) and at the patch scale, despite a wide range of dispersal strategies among phyla. The absence of an effect on Glomeromycota for the first four sampling occasions may result from a less pronounced host preference effect on this phylum, thereby increasing the influence of plants developing in the vicinity (Hausmann & Hawkes, 2009; Mony *et al.*, 2020a). Because the roots of focal and neighboring plants may intermingle, exchanges of the entire set of the fungal assemblages may occur between the two habitats illustrating a potential coalescence process among host plants (Rillig *et al.*, 2015). At the last sampling occasion, the reduced richness in Glomeromycota in patches with a corridor may be related to a strong effect of competitive interactions among fungi belonging to Glomeromycota within roots (Maherali & Klironomos, 2007) driving species assembly. By contrast, symbiotroph richness increased over time in the presence of a corridor, probably because the disappearance of some Glomeromycota species released ecological niches for other symbiotic species. Certain components of the plant mycobiota at small spatial scales could be important, considering the wide range of functions fulfilled by fungi for plants (Vandenkoornhuyse *et al.*, 2015).

The general results we obtained based on functional guilds revealed no significant effect of connectivity on plant pathogens, and only a weak effect on symbiotrophs and saprotrophs; however, these results should be interpreted with caution due to the lack of available information for almost half of the sequence clusters in our dataset. We found that specific taxa were depleted in isolated (four closely related sequence clusters and four fungi taxa) or selected in connected conditions (11 closely related sequence clusters and four fungi taxa). These taxa that were promoted by corridor connections were pathotrophs, symbiotrophs, and saprotrophs, while those that were disfavored by isolation were symbiotrophs and saprotrophs. This may be a consequence of competitive exclusion and competitive behavior for root colonization. More specifically, bad competitors may escape from their competitive superiors through better dispersal and can persist by finding new habitat; this could explain the coexistence of functionally redundant fungi (Smith *et al.*, 2018). Fungal competition–colonization trade-offs could be seen as an important hypothesis to be further explored to link ecological functions and traits to community assembly and dynamics, possibly through experiments using fungal synthetic fungal communities to colonize plants. Future studies may focus more on specific taxa that are selected by connection or depleted by isolation, and develop *in vitro* complementary analysis to better describe their biological traits in order to understand their specific responses to connectivity.

Corridors as a key to understanding plant-associated mycobiota assembly: toward a concept of biotic corridors

Through this work, which was based on a simple but robust experimental design with two patches of plants isolated or connected by a linear corridor of the same plants, we demonstrated that plants provide biotic corridors for fungi, with effects on alpha- and beta-diversity of root-associated fungal communities. Our results followed the same predictions as those concerning corridors for macroorganisms (Pardini *et al.*, 2005). Importantly, we detected such corridor effects at the community level despite the wide range of modes of dispersal. Community-wide assessment of corridor effects is a recent topic in landscape ecology for macroorganisms (Uroy *et al.*, 2019), while this is one of the very first pieces of evidence for microorganisms (see Mony *et al.*, 2022 for a review on habitat corridor studies for microbes). Because such effects are demonstrated at the community level, these results also demonstrate that most fungi are limited to short spatial scales for their dispersal. In addition, ecological corridors are often defined by a habitat (Beier & Noss, 1998; Gilbert-Norton *et al.*, 2010; Fletcher *et al.*, 2016). Here, we demonstrated that the corridors provided by host plants may provide suitable habitats for certain fungi to promote their exchanges between two connected patches, and mitigate the negative influence of isolation on the structure of endospheric mycobiota. The corridors could comprise biotic corridors that differ from the classical

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corridor concept because of the tight interactions of a host and its microbiota that override the effect of the characteristics of the physical environment (e.g. here habitat is better defined by the host plants than by the abiotic conditions). Such a biotic corridor concept could widely apply to microbes due to the large number of host-associated microbes. Whether the corridor affects dispersal independently of a potential indirect effect of *T. repens* on soil characteristics or not remains an open question. Plants are known to be associated with preferential fungal assemblages. Here, we demonstrated that, for fungi, a host preference effect can also influence processes at the larger spatial scale of the micro-landscape, thereby promoting connectivity of fungi from one patch of host plants to another. Such an effect could explain the marked spatial heterogeneity of fungi recorded even at small spatial scales (Bahram *et al*., 2015). This paper illustrates a new concept of biotic corridors for microorganisms that offers opportunities to advance our theoretical understanding of fungal assembly, and for the manipulation of *in situ* mycobiota structure and its application in agriculture through plant configuration.

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Competing interests

None declared.

Author contributions

CM and PV conceived the study and the methodology. CM, PV and FK collected the data. FK and RC-V performed the sequence analyses. JH performed statistical analysis of the data and wrote the manuscript with the help of CM and PV. All the authors contributed critically to interpreting the results and to the drafts and gave their final approval for publication.

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Data availability

Sequencing reads from root endospheric mycobiota have been deposited in the Sequence Read Archive (SRA: https://www.ncbi.nlm.nih.gov/bioproject/PRJNA793385/) and are accessible under accession number PRJNA793385. Data that are relevant to this manuscript and the scripts used in the computational analyses are available at https://github.com/HuJamie/Microbial.Corridor. All other study data are included in the article and/or in the Supporting Information.

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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 Rarefaction curves for sequence data of all samples.

Fig. S2 An example of Sloan's neutral model.

Fig. S3 Proportion of each phylum in root endospheric mycobiota sequence clusters of *Trifolium repens* and *Brachypodium pinnatum.*

Fig. S4 Venn diagram of shared and unique root endospheric mycobiota of *Trifolium repens* in patches or corridors and *Brachypodium pinnatum* across all sampling campaigns at the individual scale.

Fig. S5 Root endospheric mycobiota community structure of *Trifolium repens* and *Brachypodium pinnatum* across all sampling campaigns at the individual scale.

Fig. S6 Root endospheric mycobiota community structure of *Trifolium repens* from corridors and patches at the individual scale.

Fig. S7 Root endospheric mycobiota diversity for each fungal phylum associated with *Trifolium repens* in treatments with and without a corridor over five sampling campaigns at individual and patch scales.

Fig. S8 Effect of the presence of a corridor on *Trifolium repens* root endospheric mycobiota dissimilarity at the individual scale.

Fig. S9 Effect of the presence of a corridor on *Trifolium repens* root endospheric mycobiota dissimilarity of each phylum at individual and patch scales.

Fig. S10 Summary of root endospheric mycobiota assembly patterns based on their abundance with and without corridors at five sampling time points.

Fig. S11 Sloan neutral community model for root endospheric mycobiota in treatments without and with corridors at each sampling time point.

Fig. S12 Taxonomic identity of the Sloan neutral community model for root endospheric mycobiota in treatments without a corridor and with a corridor at each sampling time point.

Fig. S13 Dynamics of root endospheric mycobiota sequence cluster richness and abundance within each fungal guild over time in the treatments with a corridor and without a corridor at individual and patch scales.

Methods S1 Supporting materials and methods.

Table S1 Effects of the presence of a corridor, time, and their interaction on root endospheric mycobiota composition of *Trifolium repens* at individual and patch scales.

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Table S2 Statistics for 10 Sloan neutral models.

Table S3 Taxonomic identifications and ecological functions of 138 root endospheric sequence clusters.

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