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Past spatial structure of plant communities determines arbuscular mycorrhizal fungal community assembly

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1. Due to the importance of arbuscular mycorrhizal fungi (AMF) in ecosystem productivity, a key ecological question is how do their communities assemble? As plant spatial patterns constitute a mosaic of AM fungi habitats, we hypothesized that AM fungal community assembly is determined by plant community structure, both in space and time.

2. We tested our hypothesis by sampling individuals of two host-plant species, *Brachypodium pinnatum* and *Elytrigia repens*, from experimental communities cultivated in mesocosms, and assessed their AM fungal root colonizers by mass-sequencing. We related AM fungal community structure to the distribution of neighbouring plant species at different spatio-temporal scales.

3. We demonstrated that AM fungal community assembly depends mostly on past plant spatial patterns at a small spatial scale (5 cm), indicating that plants growing at given locations leave a footprint on the AM fungi community. This spatial scale of response was also influenced by the host-plant species, probably by its clonal propagation.

4. **Synthesis.** Overall, we highlighted that processes involved in AM fungal community assembly do not operate at the rough scale of the overall plant community mosaic but are instead locally determined, delineating the AM fungal “eye-view” of the host-plant community.

**Keywords**

Assembly mechanisms; clonal host-plant; community dynamics; Glomeromycota; Plant-soil (belowground) interactions; spatio-temporal scale; symbiosis.
Introduction

Arbuscular mycorrhizal fungi (AM fungi) are present in soils of all ecosystems (Öpik, Moora, Liira, & Zobel 2006) and form symbiotic interactions with about 80% of all terrestrial plant species (Wang & Qiu, 2006; Brundrett, 2009; Davison et al., 2015). The symbiotic interactions between plants and AM fungi are known to drive ecosystem productivity (e.g. van der Heijden, Boller, Wiemken, & Sanders 1998; Wagg, Jansa, Stadler, Schmid, & van der Heijden 2011), in particular through their contribution to soil nutrient transfers (e.g. Duhamel & Vandenkoornhuyse, 2013) and their influence on resource sharing between plants (Klironomos, McCune, Hart, & Neville 2000). Because of the considerable importance of AM fungi and their key role in ecosystem functioning, the mechanisms driving AM fungal communities’ assembly have been a hot topic in recent years (see e.g. Davison et al., 2016; Lekberg & Waller, 2016; López-García et al., 2017; Sepp, Jairus, Vasar, Zobel, & Öpik 2018).

Patterns of AM fungi relative abundance and diversity are highly heterogeneous across multiple spatial scales (Bahram, Peay, & Tedersoo 2015). Indeed, fungal assemblages vary at a local scale (Brundrett & Abbott, 1995; Carbalho, Correia, Ryel, & Martins-Loucao 2003; Wolfe, Mummey, Rillig, & Klironomos 2007) and an autocorrelation of AM fungal species has been detected within a few meters (Bahram et al., 2015). Drivers of such local heterogeneity include a patchy distribution of nutrients (Oehl et al., 2005) and heterogeneous microedaphic and microclimatic conditions (Vályi et al., 2016). Besides, AM fungal assemblages have been suggested to be either independent (the Independence hypothesis) or to co-vary with the composition of the aboveground vegetation (Zobel & Öpik, 2014). Under variable environmental conditions, the Habitat hypothesis assumes that plant and AM fungal communities vary in parallel relatively to varying habitat conditions. In steady environments however, the relationship between AM fungal assemblages and aboveground vegetation would be related to the Driver-Passenger hypothesis (Hart, Reader, & Klironomos 2001; Zobel & Öpik, 2014). AM fungi would act as “passengers” of plant species, which favor their best symbionts by preferentially allocating carbon to these cooperators (Kiers et al., 2011). This process leads to a host-plant preference effect (Vandenkoolnuyse et al., 2002; Vandenkoolnuyse, Ridgway, Watson, Fitter, & Young 2003, Gollotte, van Tuinen, & Atkinson 2004). This plant filtering may also be fine-tuned by the plant species’ requirements over time (Vandenkoolnuyse et al., 2002). Conversely, AM fungi have been assumed to act as “drivers” of plant community structure (van der Heijden et al., 1998; Klironomos et al., 2000; Zobel & Öpik, 2014), and to promote some plant species over others.
(Hartnett, Hetrick, Wilson, & Gibson 1993; van der Heijden et al., 1998). Both processes suggest the existence of a spatial relationship between plant and AM fungi, which would change over time. Despite the support for the existence of a spatial relationship between plant and AM fungi (Horn, Hempel, Verbruggen, Rillig, & Caruso 2017), the hypothesis of a spatio-temporal relationship has never been thoroughly tested.

Studies dealing with the effect of plant communities on AM fungal assemblages should consider that plant individuals are not randomly distributed within communities but instead form aggregated patterns of different size and composition (Watt, 1947; Herben & Hara, 2003). This spatial aggregation results from seed dispersal (Zobel, Moora, & Herben 2010) and clonal propagation (Benot, Bittebiere, Ernoult, Clément, & Mony 2013), as in both processes, the offspring are preferentially produced close to the mother plants. Plant spatial patterns form a complex matrix of spaces for AM fungi growth, and can therefore be considered as a mosaic of potential habitats. A better understanding of the scale of plant influence on the AM fungal community could be obtained from spatial analyses of their relationships. Because the spatial structure of the plant community is defined very locally (Benot et al., 2013), the AM fungal community may respond to the overlying plant composition on a few centimetres scale, which would lead to considerable variability in AM fungal composition at the plant community level (meter).

Plant spatial patterns are dynamic due to species mobility, i.e. local extinctions or emergence through seeds or clonal growth (Watt, 1947; Thórhallsdóttir, 1990; Herben & Hara, 2003). The local turnover of plant species should subsequently result in changes in the spatial distribution of potential AM fungal habitats. Hausmann & Hawkes (2010) manipulated the order of plant species establishment experimentally and demonstrated that the first-established plant species filtered the initial AM fungal pool, thereby determining the symbiotic AM fungal assemblages of later-established plant species. This temporal sorting process would be reinforced by the effects of plant phenology and growth on AM fungal colonization rate, spore diversity and relative abundance (Johnson-Green, Kenkel, & Booth 1995; Schalamuk, Velazquez, Chidichimo, & Cabello 2006). Since AM fungal propagules (hyphae and spores) can survive and colonize plant roots even after one year of residence in the soil (McGee, Pattinson, Heath, Newman, & Allen 1997), we examined the hypothesis that the AM fungal community responds to past or present spatial patterns of the plant community.
We worked on two co-occurring Poaceae species, namely *Elytrigia repens* L. and *Brachypodium pinnatum* L., common in temperate grasslands. Individuals of both species were sampled from controlled experimental plant communities encompassing a large range of spatial structures. By accurately mapping the spatial distributions of plant species in each 1.7 m² mesocosm on several dates, we were able to relate the past and present spatial patterns of plant communities to the current structures of the *E. repens* and *B. pinnatum* AM fungal communities. A thorough analysis of the AM fungal communities associated with the roots of sampled individuals was carried out to test the following hypotheses:

(i) Past and present spatial patterns of the plant community drive the structure of the AM fungal community.

(ii) The AM fungal community responds to the overlying plant composition at a very local scale (centimetre).

**Materials & Methods**

**Host-plant species**

*Elytrigia repens* (L.) Desv. (syn. *Elymus repens* or *Agropyron repens*) and *Brachypodium pinnatum* (L.) Beauv. (syn. *Bromus pinnatus*) are two common, co-occurring perennial Poaceae species of grasslands in western Europe. These species grow laterally by producing sympodial plagiotropic rhizomes from which buds develop into erect shoots (ramets) [CLO-PLA database, (Klimešová & De Bello, 2009)]. *E. repens* and *B. pinnatum* respectively display long and short lateral dispersal (mean internode lengths of 2.8 cm and 1.0 cm respectively, Benote et al., 2013) i.e. either guerilla (loose clonal architecture) or phalanx growth (a packed front of ramets) (Lovett Doust, 1981). Both species produce roots of similar length (mean length = 6.9 cm ±2.5 in *E. repens* and 7.8 cm ±3.5 in *B. pinnatum*) and thickness (mean diameter = 0.05 cm ±0.005 in *E. repens* and 0.05 cm ±0.010 in *B. pinnatum*) as measured on individuals from our experimental design. These two target species are known to develop symbioses with AM fungi species and display a positive growth response (Rydllová & Vosátka, 2001; van der Heijden, Wiemken, & Sanders 2003). Literature studies have demonstrated different levels of mycorrhizal colonization in these species ranging from intermediate (*E. repens*, about 15% of root length) to high (*B. pinnatum*, about 50% of root length) (Rydllová & Vosátka, 2001; van der Heijden et al., 2003)
although root colonization levels can considerably vary within a growing season (Bohrer, Friese, & Amon 2004; Mandyam & Jumpponen, 2008).
Mapping plant community spatial structure over time

The spatio-temporal scale, at which the AM fungal community structure responds to the spatial patterns of the overlying plant community, was determined by randomly selecting experimental plant communities \textit{(i.e.} 19 per host-plant species\textit{)} of varying spatial structures from a wider outdoor mesocosm design (Fig. 1). This mesocosm design had been set up in 2009 in the experimental garden of the University of Rennes 1, to determine the effect of plant clonal growth strategies on spatial patterns (see Benot et al., 2013 for details).

The sampled plant communities were composed from a set of 12 species that are widely distributed in temperate grasslands in Western France (des Abbayes, Claustres, Corillion, & Dupont 1971) \textit{(E. repens, B. pinnatum, Agrostis stolonifera L., Holcus mollis L., Ranunculus repens L., Festuca rubra L., Agrostis tenuis Sibth., Anthemis nobilis L., Holcus lanatus L., Dactyliis glomerata L., Lolium perenne L., and Centaurea nigra L.)}, and known to form symbioses with arbuscular mycorrhizal fungi (West, 1996; Pawlowska, Blaszkowski, & Rühling 1997; Gollote et al., 2004; Wearn & Gange, 2007). These plant communities varied in both richness and composition (ranging from one to a mixture of all 12 selected plant species) (Tables 1, 2). Forty-eight plant units (one mature shoot with one internode of maternal connection – Stuefer & Huber, 1999) were initially transplanted 16 cm apart in a hexagonal pattern in each mesocosm of $1.30 \times 1.30 \times 0.25$ m (Birch, Oom, & Beecham 2007). The 48 transplanted plant units consisted of equal numbers of all the co-occurring species (including the host-plant species), and their positions within the plantation pattern were randomized for each mesocosm. The plants were grown on a homogeneous unsterilized substrate (initial chemical composition: $C/N = 7.2 \pm 3.2$, $NO_3^- = 32.5 \pm 18.6 \mu g$ g$^{-1}$ dry soil, $PO_4^{3-} = 28.5 \pm 16.7 \mu g$ g$^{-1}$ dry soil), composed of sand (20%) and soil from Western France (80%, collected in March 2009 and previously stored outside for a week). The mesocosms (boxes) were placed on a tarpaulin to isolate the substrate from the ground soil (Fig. 1). Weeds were regularly removed, and the mesocosms were watered every two days during the dry season. Above-ground vegetation was mown once a year at the end of summer and flowers were cut off to suppress sexual reproduction. The present spatial structure of the plant community was therefore solely due to vegetative growth, without any addition of new species or individuals through sexual reproduction, and directly resulted from the past structure.

Species cover changed over time due to the ongoing dynamics of the plant communities. The spatial distributions of the plant species in all mesocosms were recorded after two and three years of cultivation (early March 2011, and in May 2012 right before the host individual sampling).
(Fig. 2), by centring a $80 \times 80$ cm square lattice on the mesocosm. Presence/absence data were recorded in $5 \times 5$ cm cells of the lattice (256 cells total) and a plant species was considered present when at least one individual was rooted in the target cell, a given individual belonging to one cell only. Several plant species can co-occur in a cell. We then based our work on the hypothesis that aboveground plant species distribution can be a proxy of the belowground root distribution (the accuracy of this proxy is discussed in the Discussion section). The numbers of cells that were colonized by each plant species at different scales surrounding the positions of AM fungal community sampling, were calculated in 2011 (past) and 2012 (present) (Tables 1, 2) by GIS (ArcGIS ver. 9.3., ESRI) [Bittebier & Mony (2015) for more details on the method]. The following spatial scales i.e. 5, 10, 15, 20, and 25 cm from the sampled host individual, were tested (Fig. 2).

As a preliminary step, we checked for the absence of collinearity in our plant species abundances at the different spatial and temporal scales by testing pairwise correlations using non-parametric Spearman’s rank correlation tests. No strong correlations were found between the abundances of the different species at all spatial and temporal scales tested (i.e. correlation coefficients < 0.7 when significant) (Dormann et al., 2013).

**Sampling, DNA extraction and amplicon preparation**

In May 2012, one individual (a mature shoot and the associated roots) of the host-plant species, either *E. repens* or *B. pinnatum*, was randomly sampled from the centre of each mesocosm (Fig. 2). These sampled plant individuals had been initiated and grown in the mesocosms. The age of sampled roots was standardized by only harvesting roots directly attached to the shoot base. All individuals were sampled from independent plant communities. Nineteen samples of *E. repens* and as many of *B. pinnatum* were used in this study. The roots were separated from the rest of the plant, washed with a stringent detergent solution (Triton X100, 1% V/V), then rinsed with ultra-pure water before storage at -80°C and subsequent analysis of the root endosphere compartment (e.g. Lê Van et al., 2017).

The 38 root samples (~ 10 g each) were ground to powder using a pestle and mortar under liquid nitrogen. The total DNA was then extracted from a 100 mg sub-sample using the DNeasy plant kit (Qiagen) according to the manufacturer’s recommendations. A 520 bp DNA fragment of the AM fungi SSU rRNA gene was specifically amplified by PCR using NS31/AM1 primers (Simon, Lalonde, & Bruns 1992; Helgason, Daniell, Husband, Fitter, & Young 1998) with
PuReTaq Ready-to-go PCR beads (GE Healthcare). True technical amplicon replicates were obtained for each of the 38 samples (i.e. for a given DNA extract, two independent PCRs were never mixed together in any of the molecular studies). Each amplicon was tagged using a multiplex identifier (i.e. index). The 76 amplicons were purified using AMPure XP – PCR kit (Agencourt/Beckman-Coulter).

**Amplicon libraries preparation and sequencing**

The sequencing libraries were prepared by measuring the DNA concentration of each amplicon using a Picogreen assay (Invitrogen) and digital PCR (Fluidigm EP1). After equimolar mixing of the 76 purified amplicons, emPCR amplification (GS FLX Titanium emPCR Kit Lib-L) and sequencing (GS FLX Titanium Sequencing Kit XL+) were performed with a 454/Roche GSflx+ instrument, according to the manufacturer’s instructions. The 520 bp SSU rRNA fragments were fully sequenced.

**Sequence trimming and bioanalyses**

The sequence production and trimming strategy has been published previously (e.g. Ciobanu et al., 2014; Ben Maamar et al., 2015; Lê Van et al., 2017). Sequences shorter than 300 bp in length, with homopolymers longer than 8 bp or with ambiguous nucleotides, were removed from the dataset. Sequences containing errors in the MID (i.e. multiplex identifier) or primer sequences were discarded. The technical replicates were sequenced to filter sequencing errors. Only full length 100% identical sequences found in both technical replicates were kept in the dataset (Ciobanu et al., 2014; Lê Van et al., 2017). In addition to this first stringent filtering process, each sample was subjected to a search for chimeric sequences with the chimera.uchime command in MOTHUR (Kozich, Westcott, Baxter, Highlander, & Schloss 2013). Operational Taxonomic Units (OTUs) were delineated at a 97% identity threshold using DNACLUST, similarly to QIIME. After these steps, OTUs found in more than 10.5% of the samples (i.e. four samples) were kept. Thus an OTU was kept only if this one contained a minimum of eight identical sequences obtained independently (four sequences in each of the two independent PCR). The sequencing data were organized in a contingency matrix. Because of the different numbers of sequences per sample, the dataset was normalized to the lowest number (i.e. 1500 sequences for each sample) using the VEGAN package in R (Oksanen et al., 2016) and the frequencies of each OTU per sample were calculated. The resulting rarefied matrix was used for the statistical analyses (see below). The
most abundant sequence within each OTU was selected as the representative sequence for the phylogenetic analyses and taxonomic assignment using Phymyco-DB, a curated SSU rRNA fungal database (Mahé et al., 2012). At the last release this database contained 1400 non-redundant Glomeromycota SSU rRNA sequences and hundreds to thousands of sequences from other fungal phyla (see http://phymycodb.genouest.org/). All the OTUs detected belonged to the Glomeromycota. Nevertheless, taxonomic affiliations of OTU with a match at high taxonomic level only (i.e. phylum) remain speculative with no consequences for our analyses and results as they only represent 2% of all OTU. The taxonomic affiliation of the OTUs is summarized in Fig. 3. A Ward clustering analysis, using the Bray-Curtis dissimilarity index, was performed with SEED (Beck, Dennis, & Foster 2015). This analysis allowed us to compare the fungal communities between samples and more precisely, to determine their composition similarity.

OTUs phylogenetic analysis

The representative sequences of the 173 most abundant OTUs (i.e. 80% of the sequences) were aligned using SINA aligner v1.2.11 (Pruesse, Peplies, & Glöckner 2012) and imported into the non-redundant SILVA SSURef ARB database (release 115) (Ludwig et al., 2004). Alignments of the representative OTU sequences and their 15 closest phylogenetic relatives were exported from ARB. A putative Choanoflagellate, Acanthoeca spectabilis, was used as outgroup. Gaps and ambiguous positions were excluded by manually refining the alignments. The best possible model to explain the matrix (i.e. GTR+I+G), based on jModelTest v2.1.4 (Darriba, Taboada, Doallo, & Posada 2012) was used to compute the Maximum likelihood phylogeny in TREEFINDER (Jobb, von Haeseler, & Strimmer 2004) (Fig. S1). GenBank accession numbers of the most abundant OTU (Fig. S1) are MH429633 – MH429779.
Data analyses

The spatio-temporal scale of the AM fungal community response to the spatial distribution of overlying plant species, was determined from the AM fungal community indexes, and AM fungal species occurrences \textit{(i.e.} identities\textit{)} and relative abundances.

AM fungal community indexes were calculated using the VEGAN package in R (Oksanen et al., 2016): (i) the AM fungal richness (S, \textit{i.e.} the number of OTUs), (ii) the Simpson diversity index (H'), and (iii) the equitability index (J). Pairwise correlations between AM fungal community indexes were tested using non-parametric Spearman’s rank correlation tests. OTU richness and equitability were poorly correlated \textit{(i.e.} correlation coefficients < 0.65\textit{)} while OTU Simpson diversity was strongly correlated with the two other indexes \textit{(correlation coefficients between OTU Simpson diversity and equitability > 0.9, and between OTU Simpson diversity and richness > 0.7). We therefore removed OTU diversity from subsequent analyses to avoid collinearity between the AM fungal community indexes.\textit{

The influence of plant community spatial patterns on AM fungal community indexes in \textit{B. pinnatum} and \textit{E. repens} at all spatial and temporal scales was determined by multiple regression analyses with plant species abundances as explanatory variables in linear model (LM) procedures. Data was log-transformed, when necessary, to satisfy the assumption of a normal distribution of model residuals. One model was developed for each date and spatial scale. Thus, ten models were constructed per index, each of which was optimized by backward stepwise selection of the explanatory variables. The information-theoretic model comparison approach based on Akaike’s Information Criterion (AIC) was used to compare all the optimized models for each index through second-order AIC corrected for small sample sizes (AICc) (Burnham & Anderson, 2002). In our analyses, those models with smaller AICc values and with a substantial level of empirical support \textit{(i.e., a difference of AICc > 2 compared to other models)} were considered the most probable (Burnham & Anderson, 2002). The coefficients and the proportion of index variation that was accounted for by the regression (R²) were then calculated for these most probable models. The significance of each explanatory variable was determined by ANOVA analyses.

AM fungal OTUs occurrences and relative abundances were used as two response matrices in Canonical Correlation Analyses (CCA) with the plant species abundances as the environmental matrix. These CCA were performed at all spatial and temporal scales tested with the VEGAN package (Oksanen et al., 2016), to determine the effects of plant species abundances on the AM fungal community composition \textit{i.e.} the identities and relative abundances of the fungal species
present. One model was developed for each date and spatial scale. Thus, ten models were
constructed per response matrix, each being optimized by backward stepwise selection of the
explanatory variables. The percentage of variance of the AM fungal species occurrences explained
by the environmental matrix was calculated (constrained CCA inertia) and an ANOVA was
carried out based on permutation test to determine the significance of the CCA.

All statistical tests were performed using R 3.2.3 (R Development Core Team, 2008).

Results

AM fungi in E. repens and B. pinnatum roots, γ-diversity

After the trimming steps, the rarefied dataset contained 58,920 sequences corresponding to 602
OTUs (i.e. formed with a minimum of eight sequences). Only 21 OTUs were present in more than
80% of the samples, and 113 OTUs were found in more than 50% of the samples, showing that
our samples contained a majority of rare OTUs. 544 and 22 OTUs were found within the
Glomerales and Diversisporales orders respectively (Fig. 3). No sequence belonging to other
Glomeromycota orders (i.e. Archaeosporales, Paraglomerales) was detected (Fig. 3). At higher
taxonomic ranks, 24 and 12 OTUs could not be identified at the class and phylum levels,
respectively (Fig. 3). *Rhizophagus* and unclassified Glomerales were dominant, accounting for
93.6 % of the total number of sequences and 87.0 % of all the OTUs (Fig. 3). Phylogenetic
analysis of the OTUs representing 5/6 of the sequences dataset (i.e. the 173 most abundant OTUs)
demonstrated that 39 of these OTUs could not be identified at the order level (i.e. Glomeromycetes). The 30 most abundant OTUs accounted for 46.7% of the sequences (Fig. S2).

In these AM fungal communities, Glomeromycota OTU richness per sample ranged from
59 to 128 in *E. repens*, and from 61 to 123 in *B. pinnatum* with no difference in the mean
Glomeromycota richness (mean=95, ±SD=17) or equitability (mean=0.56, ±SD=0.04) between the
host-plants. However, the Ward clustering analyses demonstrated that the AM fungal communities
colonizing the roots of one of these two host-plant species were closer to each other than to those
of the other host-plant species (*P*<0.05) (Fig. S3), although only 10 OTUs were host-plant species
specific.

Response of AM fungal community indexes to plant community spatial structure

Our procedure of model selection based on the AICc allowed us to delineate the most probable
models adjusted to our data. These models were described in Table 3, while all other models were
discarded from further analyses. These most probable models indicated that the richness and
equitability of the AM fungal community associated with *E. repens* and *B. pinnatum* (with the exception of AM fungal equitability in *E. repens* at 5-10 cm scale) were significantly determined by plant community spatial patterns (Table 3, Fig. 4). Our results demonstrated that past plant spatial patterns alone explained about 70% and 58% of the variations in AM fungal richness in *E. repens* and *B. pinnatum* roots respectively, and from 27% to 59% of the equitability variations, depending on the host-plant species.

Overall, the AM fungal community indexes responded at a rather small scale to past plant spatial patterns (i.e. 5 cm radius, except for AM fungal equitability in *E. repens*) (Table 3, Fig. 4). An effect of past plant spatial patterns was also observed at a larger spatial scale (10-15 cm radius) on the AM fungal richness recorded in *E. repens* roots.

AM fungal community indexes were influenced by the presence and abundance of only two to four plant species (Table 3, Fig. 4). These plant species decreased the richness and equitability of the AM fungal communities associated with both host species, with the exception of *Anthemis nobilis* and *E. repens*. These latter species had positive effects on the richness and equitability of the *E. repens* symbiotic community at 15 cm, and on the richness of the *B. pinnatum* symbiotic community at 5 cm, respectively.

Response of AM fungal occurrences and relative abundances to plant community spatial structure

Canonical Correlation Analyses demonstrated that the abundances of the overlying plant species did not drive AM fungal species occurrences and relative abundances in the roots of *Brachypodium pinnatum* (ANOVAs, p-values > 0.05 for all CCA). In *Elytrigia repens* however, both AM fungal species occurrences and relative abundances responded to plant community spatial structure (Table 4). Our CCA results demonstrated in this host-plant species that past plant spatial patterns explained at least 30% and 20% of the variations in AM fungal occurrences and relative abundances respectively, with an additional effect of the present plant spatial patterns. Overall, the AM fungal community structure responded at a fine spatial scale to plant spatial patterns (i.e. from 5 cm to 15 cm radius) (Table 4) although an effect of present plant spatial patterns was also observed at a larger spatial scale (20 cm radius) on the AM fungal relative abundances in *E. repens* roots. AM fungal occurrences and relative abundances were influenced by the presence and abundance of only two to five plant species in particular *Holcus lanatus* (Table 4).
Discussion

Plant landscape affects fungal community structure

Our results are consistent with the Passenger hypothesis (Hart et al., 2001; Zobel & Öpik, 2014) and previous studies demonstrating that AM fungal community structure can be related to the composition of the overlying plant community (e.g. Bever, Morton, Antonovics, & Schultz 1996; Eom, Hartnett, & Wilson 2000; Vandenboornhuyse et al., 2002; 2003; Pivato et al., 2007; Davison, Öpik, Daniell, Moora, & Zobel 2011; Ji, Gehring, Wilson, Miller, & Johnson 2013).

Additionally, those results newly indicated that the heterogeneous spatial distributions of plant individuals, forming a mosaic of potential habitats, determine the species pool and competitive balance of the AM fungal community. Indeed, in both host-plant species, we found an effect of plant spatial patterns on the AM fungal richness and equitability, with an additional effect on AM fungal species occurrences and relative abundances recorded in $E. repens$. The discrepancy in the responses of AM fungal community descriptors in $B. pinnatum$, would indicate a fungal pool with a varying number of rare species and subsequently a varying equitability, but with no shift in the dominant species identities and insignificant variations in their relative abundances.

In the two host-plant species studied, AM fungal communities responded to the plant aboveground spatial patterns, which were used as a proxy of belowground root distribution. The accuracy of this proxy would vary with the plant species. Plant species richness can be 1.5 times higher belowground than aboveground due to the root distribution of rhizomatous species (Hiiesalu et al., 2014), for example $E. repens$, $B. pinnatum$ or $H. mollis$ in our system. Based on their aboveground spatial patterns, the actual root distributions of these three plant species may have been underestimated in our study. Future investigations relating the spatial patterns of AM fungi and plant species should therefore be based on spatially explicit root sampling to improve precision.

Our findings strongly suggest that the processes involved in the coexistence of AM fungal species (OTUs) are spatially determined locally. By analogy with the work of Turkington & Harper (1979) on plant communities, this allows us to determine the AM fungal “eye-view” of the host-plant community. Landscape ecological studies (e.g. Burel & Baudry, 1999) have clearly shown that the biodiversity of macro-organisms is strongly influenced by the habitat mosaic (i.e. composition and relative abundances of habitat types, e.g. forests or crops) defined at the square kilometre scale (e.g. Michel, Burel, Legendre, & Butet 2007). By analogy, we argue that the
spatial structure of plant communities can similarly be considered as a mosaic of AM fungal potential habitats and would therefore constitute a centimetric “plant landscape”. Hereafter, we refer to this conceptual analogy to investigate the assembly processes in action within the AM fungal community.

Our results emphasize the importance of the host-plant species in modulating the strength of the plant landscape effect on AM fungal community assembly. The processes involved in AM fungal community assembly therefore result from an interplay between the plant landscape and the host-plant species identity.

Spatio-temporal scale of AM fungal community response to plant landscape

In *E. repens*, AM fungal occurrences and relative abundances were shown to depend on present but also on past plant landscape. Moreover, in the two host-plant species, OTU richness and equitability depended on the past, rather than the present plant landscape. While there is literature support for the existence of a spatial relationship between present plant landscape and AM fungal community structure (Horn et al., 2017), we demonstrated for the first time that this spatial relationship varies through time with a dominant effect of the past plant landscape.

This ‘footprint’ of the past plant landscape on current AM fungal community structure can be explained by two non-exclusive processes. First, after a growing season, AM fungal spore composition in soil varies according to the overlying plant species (Johnson, Zak, Tilman, & Pfleger 1991; Bever et al., 1996; Eom et al., 2000). Bever (2002) showed that this differentiation increased over successive growing seasons, due to the filtering of AM fungi by their hosts. In particular, because plants are able to provide a higher carbon flux to their best cooperators (Kiers et al., 2011), the expected consequence of this selective rewarding would be a better fitness for these particular symbionts, and thus the possible exclusion of certain colonizers (Duhamel & Vandenkornhuyse, 2013). Overall, this suggests that the very early presence of plant species within the plant landscape is important. Second, a recent study has confirmed the importance of the arrival order of AM fungal species for colonization of plant seedlings (Werner & Kiers, 2015). This ‘priority effect’, *i.e.* early arrival conferring an advantage to a particular AM fungus in occupying the root habitat, might provide an ability to exclude one that arrives later (Werner & Kiers, 2015) and thus impact the assembly processes of future AM fungal communities. Nevertheless, the persistence of past plant landscape effects could be related to the rather short period of time investigated in this study (15 months). As most fungal spores and propagules are
able to survive in soil, a longer term study, mapping plant communities e.g. five years before AM fungal community sampling, would allow to determine the temporal limits of the past plant landscape footprint on current AM fungal community structure.

Within each host-plant species, fungal OTU richness and equitability generally responded to plant landscape at the same very narrow spatial scale (5 cm radius). Similarly, in *E. repens*, the OTU occurrences was influenced by past and present plant landscapes within a 5 cm radius neighbourhood. This could be explained by two complementary hypotheses related to dispersal and recruitment mechanisms of AM fungi involving spores, hyphae, and colonized root fragments (Allen, 1991). First, spores are not randomly distributed in soil but can occur in patches of about 4 cm² (Allen & MacMahon, 1985), in agreement with observations of dispersal limitation (Friese & Koske, 1991), in ectomycorrhizal fungi, for example (Peay, Bidartondo, & Arnold 2010). Second, the dispersal of AM fungi through hyphae and colonized root fragments may have been influenced by the clonal growth strategy of the two studied host-plants, although this hypothesis remains to be thoroughly tested with several plant species per clonal growth strategy. Indeed, the roots of daughter ramets, developing less than 5 cm from their mother and displaying the same host-plant preferences, will likely be colonized, through hyphae and root contacts, by a similar AM fungal community (*i.e.* pseudo-vertical transmission of the AM fungal community to ramets). Thus, the additional effect of a larger plant landscape (10-20 cm radius) on AM fungal community structure observed in *E. repens*, could be related to its more diffuse clonal growth (Benot et al., 2013), allowing AM fungal transmission from parent to offspring through the hyphal growth, over greater distances (Friese & Allen, 1991; Jakobsen, Abbott, & Robson 1992). Moreover, this hypothesis would suggest that clonal plant networks serve as privileged dispersal pathways for AM fungi. AM fungi will disperse step by step from the older to the younger ramets (Vannier et al., 2018).

Considering the importance of clonal growth in grassland ecosystems [70% of plant species, (van Groenendaal & de Kroon, 1990)], this dispersal mechanism would be of particular importance for AM fungal species fitness and thus AM fungal community dynamics.

**Determinant species of the plant landscape**

In our study, we were interested in the scale of AM fungal community response to the overall plant landscape. We therefore considered that the various plant species composing this landscape had similar spatial scales of influence. Actually, this might not be the case, particularly for plant
species with different clonal growth strategies (guerilla, phalanx or tussock – Lovett Doust, 1981), but this requires further investigation.

Interestingly, the AM fungal community response was triggered by only a few of the plant species composing the plant landscape, regardless of their abundances. With the exception of Holcus mollis for community indexes, this set of plant landscape species varied according to the host-plant species. The species in our landscapes impacted AM fungal richness or equitability with different strengths and generally negatively, except for E. repens and Anthemis nobilis. Thus the intensity of the filtering process will vary within the plant landscape, which therefore represents a mosaic of AM fungi microhabitats of heterogeneous quality. These findings are consistent with previous reports demonstrating that AM fungal diversity in Plantago lanceolata is dependent on the specific identity of its neighbours (Johnson et al., 2003), or is reduced by the sole presence of Centaurea maculosa in the plant community (Mummey & Rillig, 2006). The positive effect of E. repens and A. nobilis on AM fungal species coexistence likely indicates that their roots are easily reached and colonized by AM fungi. Indeed, our work with mesocosms revealed that these plant species produce superficial and diffuse roots, which increases the probability of between-root contacts (pers. obs.). This highlights the major role of interspecific differences in root architecture for root colonization (Friese & Koske, 1991) and hence micro-habitat reachability.

**AM fungal community in B. pinnatum and E. repens roots**

Since its publication in 1998, the primer pair NS31/ AM1 (Helgason et al., 1998) amplifying a fragment of the 18S SSU rRNA gene of Glomeromycota has frequently been used to detect and analyse AM fungal communities despite the known bias in favour of Glomerales and Diversisporales (i.e. Paraglomerales and Archaeosporales not being amplified) (Lee, Lee, & Young 2008). This bias was the same across all the samples studied here. Despite the stringent sequence trimming, we found a higher OTU richness within the Glomeromycota, than in other studies (e.g. Johansen et al., 2015; Varela-Cervero et al., 2015). However, direct comparisons between published studies of the total number of OTUs are difficult as this value depends on the number of analysed samples, the sequencing depth, the targeted sequence (i.e. primer used), and also the trimming strategy. Our high OTU richness is likely due to our delineation method. Application of a 97% sequence identity from the SSU rRNA gene for OTU detection and delineation, has been shown to be a good proxy of AM fungal species, but is accompanied by an increase in species richness compared to the Monophyletic Clade Approach, for example...
Nevertheless, as confirmed by an additional multivariate cut-off level analysis (MultiCoLA) (Fig. S4) and the existing literature, our delineation method has no impact on the detected AM fungal community patterns (Lekberg et al., 2014; Hart et al., 2015), and thus on the relevance of our results. However, the number of Glomeromycota OTUs observed in this single study, which is higher than the known morphospecies (for an updated AM fungal species list see URL http://schuessler.userweb.mwn.de/amphylo/amphylo_species.html), and the relationships with known taxonomic groups, suggests that our understanding of Glomeromycota diversity is still very fragmented.

Conclusion and prospects

This study demonstrated that the plant community constitutes a dynamic centimetric landscape that deeply influences AM fungi community assembly. As in macro-organisms (e.g. birds, insects, mammals) (Fahrig et al., 2011), the coexistence of AM fungi species is determined by the interplay between the landscape (i.e. spatial heterogeneity and temporal variability of habitats distribution) and the suitability of the habitat (i.e. host-plant identity). Clonal networks of host-plants may moreover contribute to the plant landscape permeability to AM fungi species i.e. facilitating their dispersal from one host species to another.

This study originally involves the use of landscape ecology concepts at a centimetric scale to address questions related to the community assembly of microorganisms. We argue that transposition of this biogeographic framework which involves powerful tools to study spatially determined processes at a very fine scale, would provide a new understanding of the community structure and dynamics of microorganisms. In particular, and for the first time, this framework provided us with an AM fungal “eye-view” of the host-plant community. Considering the spatial dimension in future studies should lead to a better appraisal of plant microorganism interactions, a current key concern in deciphering the plant-holobiont (i.e. the whole organism formed by the plant and the associated microbiota, see e.g. Vandenkoornhuyse, Quaiser, Duhamel, Lê Van, & Dufresne 2015; Vannier, Mony, Bittebiere, & Vandenkoornhuyse 2015).

Acknowledgements

This project benefited from two grants from the French National Agency for Research ANR-08-SYSC-012 and the ANR-10-STRA-002. We thank Pierrick Boulard for assistance with the experiment and data collection and Alexis Dufresne, who developed the pipeline for amplicon data.
analysis. We additionally thank Qicheng Zu, who performed the MultiCoLA. We are grateful to Diana Warwick for comments and suggested modifications on previous versions of the manuscript, to Biogenouest Genomics and the Human and Environmental Genomics platform (https://geh.univ-rennes1.fr) for sequencing, and the Genouest Bioinformatics facilities.
Data availability statement

The study accession number in the European Nucleotide Archive is PRJEB26688 (ERP108695).
Authors’ contributions

AKB, CM, PV conceived the experimental design. AKB performed the experiment. AKB, EM, AG, AD, and SC collected data. MB and Alexis Dufresne designed and ran the pipeline for amplicon sequences analyses. PV performed phylogenetic analyses. AKB and CM performed statistical analyses. AKB, CM and PV wrote the manuscript, and all authors contributed to revisions.
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**Supporting Information**

Additional supporting information may be found in the online version of this article:

**Figure S1.** Maximum likelihood phylogeny of the 173 most abundant OTUs.

**Figure S2.** Cumulated Glomeromycota OTUs frequencies from the rarified contingency matrix.

**Figure S3.** Ward clustering of the 38 AM fungal communities.

**Figure S4.** Results from the MultiCoLA.

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Table 1. Mean percentages of abundance (±SD) of each plant species at different scales surrounding the sampling position of the AM fungal community of *E. repens* in 2011 (past) and 2012 (present). Abundance of plant species in a mesocosm was calculated as the ratio between the number of species occurrences and the number of grid cells (256). Because several plant species could occur in one cell of the grid, the summed abundances for a single mesocosm can be greater than 100%.

<table>
<thead>
<tr>
<th>Spatial scales</th>
<th>Time scales</th>
<th>Erep</th>
<th>Asto</th>
<th>Hmol</th>
<th>Rrep</th>
<th>Bpin</th>
<th>Frub</th>
<th>Aten</th>
<th>Anob</th>
<th>Dglo</th>
<th>Hlan</th>
<th>Lper</th>
<th>Cnig</th>
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<tbody>
<tr>
<td>5 cm Past</td>
<td>95.9 ±11.2</td>
<td>20.5 ±33.4</td>
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<td>5.8 ±13.5</td>
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<td>&lt;0.5</td>
<td>&lt;1.0 ±5.1</td>
<td>1.2 ±13.5</td>
<td>4.1 &lt;0.5</td>
<td>&lt;0.5 &lt;0.5</td>
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<tr>
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<td>7.3 ±13.8</td>
<td>7.5 ±13.2</td>
<td>4.3 ±8.1</td>
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<td>0.5 ±1.5</td>
<td>1.0 ±4.4</td>
<td>3.5 ±10.6</td>
<td>&lt;0.5 &lt;0.5</td>
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<tr>
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<td>7.3 ±12.5</td>
<td>6.4 ±9.8</td>
<td>4.8 ±10.0</td>
<td>2.0 ±5.1</td>
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<td>0.7 ±2.5</td>
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<td>0.6 ±2.6</td>
<td>2.7 ±6.4</td>
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<td>6.7 ±8.5</td>
<td>5.2 ±9.0</td>
<td>3.2 ±6.3</td>
<td>&lt;0.5</td>
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<td>0.5 ±1.7</td>
<td>2.7 ±6.0</td>
<td>&lt;0.5 ±2.0</td>
<td>0.8 ±2.0</td>
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<td>±29.7</td>
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<td>±23.3</td>
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<td>±11.1</td>
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<td>4.6</td>
<td>±10.8</td>
<td>23.2</td>
<td>±27.9</td>
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<td>±22.9</td>
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<td>±20.1</td>
<td>4.3</td>
<td>±9.6</td>
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<td>±25.1</td>
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<td>±4.0</td>
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<td>±21.5</td>
<td>6.7</td>
<td>±12.0</td>
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<tr>
<td>25</td>
<td>Present</td>
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<td>±8.7</td>
<td>21.2</td>
<td>±22.7</td>
<td>2.8</td>
<td>±3.9</td>
<td>15.4</td>
<td>±19.5</td>
<td>6.6</td>
<td>±10.5</td>
</tr>
</tbody>
</table>

Notes: Erep, Elytrigia repens; Asto, Agrostis stolonifera; Hmol, Holcus mollis; Rrep, Ranunculus repens; Bpin, Brachypodium pinnatum; Frub, Festuca rubra; Aten, Agrostis tenuis; Anob, Anthemis nobilis; Dglo, Dactylis glomerata; Hlan, Holcus lanatus; Lper, Lolium perenne; Cnig, Centaurea nigra.
Table 2. Mean percentages of abundance (±SD) of each plant species at different scales surrounding the sampling position of the AM fungal community of *B. pinnatum* in 2011 (past) and 2012 (present). Abundance of plant species in a mesocosm was calculated as the ratio between the number of species occurrences and the number of grid cells (256). Because several plant species could occur in one cell of the grid, the summed abundances for a single mesocosm can be greater than 100%.

<table>
<thead>
<tr>
<th>Spatial scales</th>
<th>Time scales</th>
<th>Erep</th>
<th>Asto</th>
<th>Hmol</th>
<th>Rrep</th>
<th>Bpin</th>
<th>Frub</th>
<th>Aten</th>
<th>Anob</th>
<th>Dglo</th>
<th>Hlan</th>
<th>Lper</th>
<th>Cnig</th>
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</thead>
<tbody>
<tr>
<td>5 cm Past</td>
<td></td>
<td>34.5</td>
<td>22.8</td>
<td>7.6 ±19.3</td>
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<td>&lt;0.5</td>
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<tr>
<td>10 cm Past</td>
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<td>35.6</td>
<td>20.8</td>
<td>6.8 ±13.8</td>
<td>4.0 ±9.2</td>
<td>46.1 ±32.5</td>
<td>6.3 ±14.6</td>
<td>14.8 ±27.5</td>
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<td>12.1 ±21.6</td>
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<td>4.0 ±7.8</td>
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<td>8.1 ±12.0</td>
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<td>19.2</td>
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</tbody>
</table>
Table 3. Results from the most probable linear models (ΔAICc < 2) linking the indexes characterizing AM fungal community structure with plant species abundances in the neighborhood of the sampled individual (see Materials and Methods section for the model selection). The parameters represent the regression slopes associated with each explanatory variable, the model intercept, and the proportion of variance accounted for by the regression (R²).

<table>
<thead>
<tr>
<th>Time scale of response</th>
<th>Spatial scale of response (Radius)</th>
<th>R²</th>
<th>Selected model formula</th>
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</thead>
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<tr>
<td><strong>E. repens</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Richness†</td>
<td>Past 5 cm</td>
<td>0.70***</td>
<td>5.53 – 0.07 Hmol – 0.29 Dglo – 0.06 Hlan</td>
</tr>
<tr>
<td></td>
<td>Past 10 cm</td>
<td>0.70***</td>
<td>5.54 – 0.03 Hmol – 0.15 Dglo – 0.03 Hlan</td>
</tr>
<tr>
<td></td>
<td>Past 15 cm</td>
<td>0.75***</td>
<td>5.52 – 0.02 Hmol – 0.09 Dglo – 0.27 Cnig + 0.14 Anob</td>
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<tr>
<td>Equitability</td>
<td>Past 5 cm</td>
<td>0.14†</td>
<td>0.80 – 0.02 Hlan</td>
</tr>
<tr>
<td></td>
<td>Past 10 cm</td>
<td>0.06ns</td>
<td>0.80 – 7.0 × 10⁻³ Hlan</td>
</tr>
<tr>
<td></td>
<td>Past 15 cm</td>
<td>0.27*</td>
<td>0.80 – 0.11 Cnig – 9.0 × 10⁻³ Dglo + 0.05 Anob</td>
</tr>
<tr>
<td><strong>B. pinnatum</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Richness†</td>
<td>Past 5 cm</td>
<td>0.58**</td>
<td>5.41 – 0.05 Hmol – 0.06 Frub + 0.03 Erep</td>
</tr>
<tr>
<td>Equitability</td>
<td>Past 5 cm</td>
<td>0.59***</td>
<td>0.84 – 0.02 Frub – 0.03 Rrep – 0.006 Aten</td>
</tr>
</tbody>
</table>

**Notes:** As obtained through ANOVA analyses, bold indicates the significance of the model slopes i.e. *P < 0.05*, and asterisks indicate the significance level of R²: ns = not significant; t 0.1 > P > 0.05; * P < 0.05; ** P < 0.01; *** P < 0.001. † log-transformation. The explanatory variables in the selected model formula correspond to the abundances of the plant species at the different spatial scales studied: Erep *E. repens*; Hmol *H. mollis*; Rrep *R. repens*; Frub *F. rubra*; Aten *A. tenuis*; Anob *A. nobilis*; Dglo *D. glomerata*; Hlan *H. lanatus*; Cnig *C. nigra*. 

|
Table 4. Results from the significant Canonical Correlation Analyses linking the AM fungal occurrences and relative abundances with plant species abundances in the neighborhood of the sampled *E. repens* individuals. No CCA performed in *B. pinnatum* were significant. Constrained CCA inertia indicated the proportion of variance accounted for by the environmental matrix.

<table>
<thead>
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<th>Scale of response</th>
<th>ANOVA test</th>
<th>CCA inertia</th>
<th>Significant plant species</th>
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<td>Df</td>
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<tr>
<td>OTU occurrences</td>
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<td>Present</td>
<td>5 cm</td>
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<td>OTU occurrences</td>
<td>Present</td>
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<td>OTU abundances</td>
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<td>2</td>
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<td>OTU abundances</td>
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<td>3</td>
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Notes: Constrained CCA Inertia is expressed as a proportion of Total CCA inertia. Asterisks indicate the significance level of the CCA: * $P < 0.05$; ** $P < 0.01$. The explanatory variables in the CCA correspond to the abundances of the plant species at the different spatial scales studied: Erep, *Elytrigia repens*; Asto, *Agrostis stolonifera*; Rrep, *Ranunculus repens*; Frub, *Festuca rubra*; Aten, *Agrostis tenuis*; Anob, *Anthemis nobilis*; Hlan, *Holcus lanatus*; Lper, *Lolium perenne*; Cnig, *Centaurea nigra*. 

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Figure legends

Figure 1. Mesocosm design set up in the experimental garden of the University of Rennes 1 in May 2009, to study the role of plant clonal traits for the spatial dynamics and functioning of prairial systems. 14 types of plant communities were tested, differing in richness, nature and number of occurring functional groups based on plant clonal traits (from one to 12 plant species in mixture). Ten replicates of each community type were randomly positioned in the experimental design, comprising 140 square mesocosms. a) the 140 mesocosms in late April 2011, b) focus on mesocosms. The experimental plant communities studied in our work are part of this wider mesocosm design: we selected for each target species *B. pinnatum* and *E. repens*, 19 mesocosms out of the 140, in which they occur.

Figure 2. Characterization of the plant species spatial distributions. The black arrow represents the sampling point of the AM fungal community (in the roots of an *Elytrigia repens* or *Brachypodium*...
pinnatum individual). The above two maps represent examples of the present and past spatial distributions of plant species (different species corresponding to different shades of green. Although more than one plant species can actually occur per cell, this has not been indicated here to simplify the figure. Circles surrounding the sampling point of the AM fungal community represent the different spatial scales that were tested in the analyses (5, 10, 15, 20, and 25 cm).
**Figure 3.** Taxonomic distribution of the sequence reads. This simplified cladogram resulted from a Maximum likelihood reconstruction using a small sample of each taxonomic group identified. The proportions of OTUs and sequences are shown beside the cladogram.
**OTUs**

- **Unclassified Glomeromycota** (Phylum)
  - 2.0 %
  - <0.05 %

- **Unclassified Glomeromycetes** (class)
  - 4.0 %
  - <0.05 %

- **Unclassified Glomerales** (order)
  - 60.7 %
  - 30.5 %

- **Claroideoglomus** (genus)
  - 0.05%
  - 1.1 %

- **Funneliformis**
  - 1.9 %
  - 2.2 %

- **Glomus**
  - 0.05%
  - <0.05 %

- **Rhizophagus**
  - 26.2 %
  - 63.1 %

- **Sclerocystis**
  - 1.2 %
  - 1.6 %

- **Unclassified Diversisporales** (order)
  - 1.2 %
  - <0.05 %

- **Diversispora** (genus)
  - 1.3 %
  - 0.07 %

- **Acaulospora**
  - 1.1 %
  - 0.05 %

- **Scutellospora**
  - 0.05 %
  - 1.2 %

- **Gigaspora**
  - <0.05 %
  - <0.05 %

- **Racocetra**
  - <0.05 %
  - <0.05 %

**Sequences**

- **Unclassified Glomeromycota** (Phylum)
  - 2.0 %
  - <0.05 %

- **Unclassified Glomeromycetes** (class)
  - 4.0 %
  - <0.05 %

- **Unclassified Glomerales** (order)
  - 60.7 %
  - 30.5 %

- **Claroideoglomus** (genus)
  - 0.05%
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  - 0.05 %
  - 1.2 %

- **Gigaspora**
  - <0.05 %
  - <0.05 %

- **Racocetra**
  - <0.05 %
  - <0.05 %

**90.4 % of the OTUs**

**98.5 % of the sequences**

**3.7 % of the OTUs**

**1.3 % of the sequences**
Figure 4. Past plant spatial patterns determine the AM fungal community structure. Only the most probable models describing a significant relationship between AM fungal richness or equitability and neighboring plant species abundances are plotted (neighboring species with no influence on AM fungal community indexes do not appear on this figure). Arrows thickness and style represent the significant model slopes i.e. respectively the strength and the sign (solid: negative; dashed: positive) of the influence of neighboring plant species abundances on *E. repens* or *B. pinnatum* AM fungal community structure. Values in brackets indicate the spatial scale of AM fungal community response (radius in cm) to neighboring plant species abundances. For *H. mollis*, underlined radius values are only for *E. repens* AM fungal community richness.