Strong linkage between plant and soil fungal communities along a successional coastal dune system

Alice Roy-Bolduc[1], Etienne Laliberté[1], Stéphane Boudreau[2], Mohamed Hijri[1]

[1] Institut de recherche en biologie végétale, Département de sciences biologiques, Université de Montréal, 4101 Sherbrooke Est, Montréal, QC, H1X 2B2, Canada

[2] Département de biologie, Centre d'études nordiques, Université Laval, Pavillon Alexandre-Vachon, 1045 Av. de la Médecine, Québec, QC, G1V 0A6, Canada

Author for correspondence :

Alice Roy-Bolduc, Institut de recherche en biologie végétale

4101 Sherbrooke Est, Montreal, QC, H1X 2B2, Canada

Fax : +1 514 343 2288// Email : alice.roy-bolduc@umontreal.ca

Running title: Succession of plant and fungal communities in a coastal dune

Abstract

Complex interactions existing between plants and soil microorganisms drive key ecosystem and community properties such as productivity and diversity. In nutrient-poor systems such as sand dunes, plant traits and fungal symbioses related to nutrient-acquisition can strongly influence vegetation dynamics. We investigated plant and fungal communities in a relic foredune plain located on an archipelago in Québec, Canada. We detected distinct communities across the edaphic and successional gradient. Our results showed a clear increase in plant species richness, as well as in the diversity of nutrient-acquisition strategies. We also found a strong correlation between aboveground vegetation and soil fungal communities, and both responded similarly to soil physicochemical properties. Soil pH influenced the composition of plant and fungal communities, and could act as an important environmental filter along this relic foredune plain. The increasing functional diversity in plant nutrient-acquisition strategies across the gradient might favour resource partitioning and facilitation among co-occuring plant species. The coordinated changes in soil microbial and plant communities highlight the importance of aboveground-belowground linkages and positive biotic interactions during ecological succession in nutrient-poor environments.

Introduction

There is growing recognition that interactions between plants and soil microorganisms drive important community and ecosystem properties such as diversity and productivity (Bever *et al*., 2010; van der Putten *et al*., 2013; Barberán *et al*., 2015). In particular, soil fungi – as plant symbionts or as major regulators of decomposition, carbon sequestration and soil respiration – are key drivers of vegetation dynamics (Wardle *et al*., 2004; Boddy *et al*., 2007; van der Heijden *et al.*, 2008; Bever *et al*., 2010). Along with other microorganisms, they decompose dead organic material, ensure the recycling of essential nutrients in soils, and thus govern nutrient availability (Boddy *et al*., 2007; van der Heijden *et al*., 2008). Soil properties, particularly those related to nutrient availability, strongly influence plant growth and community composition (Lambers *et al*., 2008; van der Heijden *et al*., 2008).

Nutrient-acquisition by plants usually involves specialized root structures or symbiotic interactions with fungi (Lambers *et al*., 2008; Zemunik *et al*., 2015). These nutrient-acquisition strategies exist in the majority of plants species – over 90% of plant species have specialized features for nutrient uptake (Lambers *et al*., 2008). Some plants developed specialized strategies such as parasitism or carnivory, or display a specialized root morphology such as the brush-like cluster roots, while others form symbiotic associations with nitrogen-fixing bacteria such as *Rhizobia* or *Frankia* or with mycorrhizal fungi. Arbuscular mycorrhizas (AM) are by far the most common strategy, occurring in more than 80% of vascular plant species (Brundrett, 2009). This symbiosis is the result of the colonization of the cortex of plant roots by Glomeromycota fungal hyphae where they form typical structures such as arbuscules, vesicules and, sometimes, intraradical spores. Fungal hyphae extend beyond the roots where they take up nutrients – in particular phosphorus (P) – and translocate it through their hyphae into arbuscules where P is exchanged for carbon (Smith & Read, 2008). There exist other types of mycorrhizal symbioses: ectomycorrhizas involve N uptake and include a wide range of fungi in the Basidiomycota and Ascomycota in association with trees and shrubs, ericoid mycorrhizas are formed by specific soil ascomycetes partnered with Ericaceae, while orchid mycorrhizas are key to orchid seed germination (Smith & Read, 2008). Despite their ecological importance, soil fungal communities – and microbes in general – are often not considered in vegetation studies. However, a better understanding of plant-soil feedbacks and soil fungal community dynamics is imperative to improve our understanding of ecological processes such as succession (Wardle *et al*., 2004; Kardol & Wardle, 2010). Aboveground and belowground components of ecosystems have traditionally been studied separately, but they must be studied together to understand ecosystem functioning (Wardle *et al*., 2004).

In nutrient-poor systems such as sand dunes, plant traits and fungal symbionts related to nutrient-acquisition are expected to strongly influence vegetation dynamics and assembly (Lambers *et al*., 2008). As such, early work indicated close association between the development of vegetation and the abundance and identity of cultivable soil microbes (bacteria and fungi) along a sand dune successional gradient (Webley *et al*., 1952). Yet, little is known about these belowground traits and the specific mechanisms linking above and below ground biota remain at least partially unresolved (Barberán *et al*., 2015). Moreover, these feedbacks were only rarely considered in the context of ecological succession (Kardol *et al*., 2013; Walker & Wardle, 2014).

Coastal sand dunes are dynamic ecosystems presenting a sequence of vegetation spanning from low plant diversity in upper beach to fixed dunes with diverse vegetation. These habitats are typically characterized by different successional stages with distinct plant communities (Cowles, 1899; Olson, 1958; Lichter, 1998; Maun, 2009).

Here, we investigated the links between fungal and plant communities across a successional and edaphic gradient in a relic foredune plain (Giles & King, 2001). Building on our previous studies along this sequence where we investigated changes in soil microbial community composition (Roy-Bolduc *et al*., 2015), we explored linkages between soil microbial and plant communities across the dune system. Specifically, our study aimed to answer the following questions. Does the relative abundance of different plant nutrient-acquisition strategies vary along this edaphic and successional gradient? How does above-ground vegetation relate to belowground fungal communities in terms of diversity and community structure? Are changes in plant and/or fungal community structure related to variations in soil characteristics (e.g. pH, nutrients)? To address these questions, we performed a comprehensive sampling of soils and aboveground vegetation along a coastal dune system forming a successional and edaphic gradient (Giles & King, 2001; Roy-Bolduc *et al*., 2015). Vegetation cover by species was recorded at each sampling site and belowground fungal communities were characterized using 454-sequencing. We studied patterns of plant and fungal diversity, and of plant nutrition-acquisition strategies. We also related plant and fungal community structure using co-inertia analysis and explored the effect of soil properties using multivariate analyses. We expected to observe shifts in nutrient acquisition strategies along the dune sequence and a singnificant linkage of plant and fungal communities because of the plant-soil feedbacks described above.

Materials and methods

Study site

The study was conducted along a relic foredune plain known as 'Les Sillons' (Giles & King, 2001). This sequence of dunes is located within the Îles de la Madeleine archipelago, in the southern Gulf of St. Lawrence in Québec, Canada (47° 23′ N, 61° 52′ W). The region is characterized by a maritime cold temperate climate with a mean annual temperature of 4.5 $^\circ$C and

annual average precipitation of 987 mm, of which approximately one third falls as snow (Houle, 2008). 'Les Sillons' is composed of a series of shore-parallel ridges forming a crescent-shaped area of 10.6 km^2. This dune system is a sandy depositional barrier that formed during the Holocene and now connects two bedrock islands (Giles & King, 2001). This relic foredune plain is notable because it harbours a diversity of vegetation types over a short distance (generally < 1 km), and includes a series of habitats ranging from the beach to forests (on dune crests) and wetlands (in dune swales) (Fig. S1). The study site was divided into eight zones, moving away from the coastline: Z1) pioneer zone, Z2) front and Z3) back of the foredune, Z4) mobile dune, Z5) heathland, Z6) forest dune, Z7) inter-ridge swale, and Z8) lagoon wetland.

Field sampling

An extensive soil and vegetation field sampling was conducted in 'Les Sillons' in August 2011, in order to describe the plant and fungal communities associated with the different dune zones. We used a stratified sampling design by randomly selecting ten sampling points from each of the eight zones for environmental data collection, vegetation surveys, and soil sampling within 1 m x 1 m plots. The location of the plots was determined prior to field sampling by using maps and aerial images to divide the sampling points throughout each zone. Each sampling plot is located at a minimal distance of 50 meters from any other plot to reduce spatial autocorrelation. Slope, soil texture, and organic layer thickness were determined at each sampling point. All vascular plants encountered in the plots were identified and their cover was estimated visually. The complete species list is provided in the supporting information section (Table S2). In each plot, soil samples were composed of a mix of six soil cores of 0–25 cm depth that were collected randomly within the plot. The gravimetric water content was calculated as a ratio between the mass of water (weight of wet soil - weight of dry soil) and the dry weight of the sample. Conductivity, pH, extractable phosphorus (Mehlich-III), total phosphorus, carbon, and nitrogen were measured using an air-dried portion of the soil samples. Soil nutrients concentrations were converted from a dry weight basis to a soil volume basis in order to make the values comparable across the different soil types, which included mineral sandy and organic soils. Soil properties are summarized in Table S1, and details about the conversion to a soil volume basis can be found in Appendix S1 in supplementary information. Approximately 15 ml of previously homogenized soil samples were sub-sampled and frozen at -4°C within 6 hours of sampling for subsequent molecular analysis.

Soil fungal community description

We characterized soil fungal communities by 454-sequencing of the ITS region. The overall description of soil fungal diversity and community structure in this system was recently published (Roy-Bolduc *et al.*, 2015), and the detailed molecular analysis and sequence processing methodology can be found in the supplementary materials of this publication. We used these fungal community data in some analysis presented here and will therefore quickly summarize the molecular and bioinformatics approach that was used. DNA was extracted from the soil samples, amplified using the ITS1F and ITS4 primers (White *et al.*, 1990; Gardes and Bruns, 1993). The amplicons were multiplexed, purified and sequenced using Roche 454 GS-FLX+ chemistry at the Genome Quebec Innovation Center (McGill University, Montreal, Canada). We initially retrieved 435,124 reads and retained 182,242 after quality filtering in QIIME (Caporaso *et al.*, 2010) and Mothur (Schloss *et al.*, 2009). Sequences were clustered into Operational Taxonomic Units (OTUs) at a similarity level of 97% with Usearch v7.0 (Edgar, 2010). Taxonomy was assigned to each OTU consensus sequence using the UNITE database (Kõljalg *et al.*, 2013). Raw sequence data were deposited in the NCBI Sequence Read Archive and are available under the project number PRJNA269650.

Statistical analyses

All statistical analyses were conducted in R v.3.0.2 (R Development Core Team, 2014). The relative cover (%) of all vascular plants was computed by normalizing their absolute cover against total cover in each plot. We computed plant species richness as the number of species recorded in every given plot, and plant diversity using the Shannon and inverse Simpson diversity indices with the 'diversity' function in 'vegan' (Oksanen *et al.*, 2013). Our cover data and sampling design (i.e. no sub-plots) did not allow us to use individual or sample-based rarefaction to provide a better estimate of species richness (Gotelli & Colwell, 2001). We computed accumulation curves based on number of species per sample within each zone using the functions 'specpool' and 'poolaccum' in the 'vegan' library to ensure that our sampling design allowed to representatively capture plant richness (Fig. S2). We also assigned a nutritional strategy (facultative/obligate arbuscular mycorrhizal, ectomycorrhizal, ericoid mycorrhizal, arbutoid mycorrhizal, N-fixing, carnivorous, or unspecialized) to every plant species based on the literature (Table S3). We then computed the richness and diversity of the distinct plant nutritional

strategies. Diversity was quantified using the Simpson index that take into account the abundance distribution of the different categories.

To examine patterns in fungal richness, we conducted OTU rarefaction analysis for individual samples. This analysis suggested that our sequencing depth was adequate since all curves reached or were close to reaching an asymptote and all samples had a Good's coverage value close to 1. Good's coverage and the Chao total richness estimator (Chao, 1984) were both computed using the 'rarefaction.single' command in Mothur with 1000 iterations of re-sampling without replacement. Fungal diversity was evaluated the same way as plant diversity, using the Simpson and Shannon diversity indices. We tested the Pearson correlation between fungal and plant richness or diversity. We used one-way ANOVA and Tukey HSD *post-hoc* tests to compare plant, nutritional strategies, and fungal richness and diversity among vegetation zones.

The relationship between plant and fungal community structure was tested using a co-inertia analysis. The analysis was performed on Hellinger transformed data using the 'coinertia' function in the 'ade4' library (Dray & Dufour, 2007) and significance of coefficients was tested with the 'randtest' function of the same package. The vector correlation coefficient (called RV coefficient) is a multivariate generalization of the Pearson correlation coefficient which gives a measure of the consistency of the two data sets and takes a value between 0 and 1 (1 being perfectly identical).

Canonical redundancy analysis (RDA) based on Hellinger-transformed data was used to assess the influence of soil and topographic variables on community assemblages, using the 'vegan' library (Oksanen *et al.*, 2013). The tests were performed on plant and fungal communities separately. Adjusted-R^2 values (Borcard *et al*., 2011) were used to assess model fit. Statistical significance was assessed by permutation testing, using 999 permutations. The results of the RDAs were represented in a biplot using scaling type 2 in order to preserve the distances between sites. We also modeled community variance for plant and fungal communities with permutational analysis of variance (PERMANOVA) using the 'adonis' function in 'vegan'. The significance of the variance explained by the different parameters was tested with 999 permutations.

Results

Plant and fungal community succession

Each of the eight zones sampled supported distinct vegetation (Fig. 1; Supporting information, Table S2). The pioneer zone (Z1), foredunes (Z2-Z3) and mobile dunes (Z4) consisted of younger sites located close to the shore, which are characterized by dry, poorly-stabilised sandy soils and a plant community that was dominated by *Ammophila breviligulata* Fern., the American beachgrass. In areas that are not directly exposed to wind and salt spray, we also found a few herbaceous plants such as *Artemisia stellaria* L. and *Festuca rubra* L. Heathlands (Z5) are dry habitats and were dominated by shrubs such as *Myrica pensylvanica* Mirb., *Juniperus communis* L., and *Spiraea alba* Du Roi. This plant community transitioned progressively into black spruce, fir, and pine forests (Z6). The inter-ridge depressions and wetlands (Z7-Z8) harbour poorly-drained organic soils and support a diverse array of vegetation, including several Ericaceae, *Sphagnum*, and *Carex* species. We detected significant differences in plant richness and diversity across the eight zones (Richness: $F_{7,69}$ = 11.2, $P < 0.001$; Shannon: $F_{7,69}$ = 15.14, $P < 0.001$; Simpson: $F_{7,69}$ = 7.82, $P < 0.001$). Moving from the coastline to the wetland, we observed a generally increasing diversity with the heathland and lagoon wetland being the most diverse zones (Table 1). Observed plant richness by sampling plot ranged between 1 and 14. A total of 73 plant species were identified across all the plots sampled.

With regard to soil fungi, functionally and taxonomically distinct communities were found in each zone of the successional and edaphic gradient, as previously described (Roy-Bolduc *et al.*, 2015). Soil fungal richness was high across the succession gradient, with 3406 observed OTUs and a total richness of 4492 OTUs according the Chao estimator. Observed richness within individual sampling points ranged between 54 (in the pioneer zone) and 329 (in the lagoon wetland); however, there were no significant differences in either richness or diversity (Table 1). In addition, no significant correlations between fungal and plant richness nor alpha diversity were observed.

Plant nutrient-acquisition strategies

A clear increase in the number of strategies and their diversity was observed across the different zones (Fig. 2). The arbuscular mycorrhizal (AM) strategy was the only plant nutrient-

acquisition strategy present in all zones and the plants using this strategy were dominant in terms of relative cover in the first four successional stages, i.e. from the pioneer zone (Z1) to the mobile dune (Z4; Fig. 3). Despite a high relative cover, AM plants displayed low richness levels in the first zones because of the dominance of *Ammophila breviligulata* Fern. (Fig. S3). The ectomycorrhizal strategy was first observed in the mobile dune zone (Z4) and the relative cover of EM plants peaked in the forested zone (Z6). Even though it was represented by a low richness (Fig. S3), its relative cover was high especially in the forested zone. Ericoid mycorrhizal plants were only found in the last zones, i.e. from the heathland (Z5) to the lagoon wetlands (Z8). Plants forming symbioses with nitrogen-fixing bacteria were first observed in the mobile dune (zone 4) and their relative cover peaked in the inter-ridge swale (Z7). Plants without a specialized strategy were present mainly in the lagoon wetland, but also in zones 3, 5 and 7 but at very low densities (Fig. 3). Some strategies were rare and are therefore not shown in Figure 3, such as carnivorous and arbutoid mycorrhizal (Fig. S4). Two carnivorous plant species were identified in our sampling sites (*Drosera rotundifolia* L. and *Utricularia cornuta* Michx.) and were found mainly in the inter-ridge swale and in the lagoon wetland. *Arctostaphylos uva-ursi* L., a species from the Ericaceae forming arbutoid mycorrhizal symbiosis, was found in the heathland (Z5) and in the forested zone (Z6). Finally, we did not find any parasitic plants nor plants forming orchid mycorrhizas.

Linking plant and fungal communities

Despite the absence of a relationship between alpha diversity of plants and fungi, we observed coordinated changes in plant and soil fungal community structure. The RV coefficient measured in the co-inertia analysis was high (0.59) and highly significant ($P < 0.001$; after 9999 permutations), indicating a strong correlation between the two data sets (Fig. 4). Panel A of Figure 4 shows the projection of the sampling sites in the co-inertia space with regards to both datasets. Sites from the first four successional stages are mainly located on the right of co-inertia plot, while heathland (Z5) and forested sites (Z6) are grouped in the top left and the humid sites (Z7-Z8) at the bottom left. The first two axes represented respectively 73.85% and 7.12% of co-inertia. Arrows in panel B and C represent the importance and direction of the contribution of plant species and fungal genera to the distribution of sites in the co-inertia space. *Ammophila breviligulata* was the plant species with the most important contribution and was associated to the younger sites (Z1-Z4). *Juniperus communis*, *Myrica pensylvanica* and *Empetrum nigrum* were

associated to Z5-Z6, and *Myrica gale*, *Carex* sp., *Typha latifolia*, *Sphagnum* sp. and *Menyanthes trifoliata* to Z7-Z8. Amongst fungi, Sordariomycetes spp. had the most important contribution to site distribution and was associated with humid sites (Z7-Z8).

Influence of soil physico-chemical properties on community structure

Humus thickness, gravimetric water content, and organic carbon significantly increased along the successional and edaphic gradient, while pH and extractable phosphorus generally decreased (Supporting information, Table S1). We observed a significant correlation between soil properties and plant community structure (Fig. 5a). Our model explained 39.2% (adjusted R^2) of the variation in plant community ($F_{7,69}$ = 7.99; $P < 0.001$). Soil fungal community was also linked to soil properties but to a lesser extent, the model explaining 11.4% (adjusted R^2) of variation in soil fungal community composition ($F_{7,69} = 2.40$; $P < 0.001$) (Fig. 5b). The two first canonical axes explained 39.1% and 12.8% of the total model variance for plant and fungal communities respectively. Although soil physicochemical properties had a more important effect on vegetation (as determined by a higher adjusted R^2 value), their influence on plant and fungal communities were qualitatively similar (Table 2). Soil pH had the highest R^2 value in both models. The proportion of variation explained was significant for humus thickness, pH, soil water content, total nitrogen, and total carbon for fungi. Neither total nor extractable phosphorus explained a significant proportion of the variation in plant and fungal community composition.

Discussion

Our comprehensive sampling of the vegetation, combined with the characterisation of soil fungi using high-throughput sequencing, allowed us to explore changes in soil microbial and plant communities along a relic foredune plain forming a successional and edaphic gradient. We observed strong linkage between plant and fungal communities. Our results also showed a clear increase in plant richness and an increasing diversity of nutrient-acquisition strategies along the sequence. Finally, we found a significant correlation between plant and fungal communities, which were influenced by similar soil physicochemical properties.

First, we detected distinct plant and soil microbial communities across the successional and edaphic gradient. Given the contrasted abiotic conditions existing in the different dune zones

(e.g. in terms of wind, salinity and soil water content), variation in plant community composition was expected (Gurevitch *et al.*, 2002). We also expected that changes in soil fungal community structure would be strongly linked to vegetation dynamics (Roy-Bolduc *et al.*, 2015). Indeed, a co-inertia analysis produced a high and significant RV coefficient, demonstrating a strong correlation between plant and fungal community structure across the successional and edaphic gradient. These results are consistent with the current knowledge on the feedbacks existing between plants, microbes and soil (e.g. Barberán *et al.*, 2015; Wardle *et al.*, 2004). Vegetation, on one hand, is largely acknowledged as a key driver of soil microbial communities (e.g. Prescott & Grayston, 2013). Plants are hosts to many associations – both symbiotic and parasitic – with fungi (Broeckling *et al.*, 2008; Barberán *et al.*, 2015). Also, because saprophytic fungi are the main decomposers of plant dead material and rely directly on plant products such as leaf litter and root exudates, their community composition is often influenced by vegetation (Wardle *et al.*, 2004; Boddy *et al.*, 2007; Miniaci *et al.*, 2007). On the other hand, soil microbes have an impact on plant productivity, diversity and community composition through various processes implying direct (root-associated) or indirect (free-living) interactions that can be either positive or negative (van der Heijden, 2008). For example, soil pathogens such as *Fusarium* spp. can reduce plant productivity (a direct negative effect), while free-living soil saprotrophs are able to enhance soil nutrient availability by breaking down organic matter into inorganic forms available to plants (indirect positive effect) (Boddy *et al.*, 2007; van der Heijden, 2008). Several fungi involved in such interactions with plants were recorded in this dune system (Roy-Bolduc *et al.,* 2015). For example, the arbuscular mycorrhizal genus *Glomus* was often recorded in the foredune where it most likely associates to *Ammophila breviligulata* and improve its nutrition, growth and survival (Smith & Read, 2008). The occurence of other symbiotic fungi such as the EM *Scleroderma*, *Thelephora*, *Cortinarius*, and *Clavulina* indicates potential positive interactions occurring between fungi and their host plants. We also observed pathogenic fungi such as *Enthorrhiza* that might limit the growth of *Juncus* and Cyperaceae in the wetlands. Finally, several saprotrophs (e.g. *Dendryphiella*, *Emericellopsis*, *Neobulgaria* and *Schizothecium*) were observed along the dune sequence and might have indirect beneficial impact on plants by improving nutrient availability. The results of the co-inertia analysis did indicate strong linkage of plant and fungal communities by connecting presence of members from the two groups. However, further

experimental studies would be required to go beyond these co-occurrence patterns and to verify realized interactions and describe their ecological impacts and functions.

Second, we observed a significant increase in plant richness and diversity along the gradient. This pattern of increasing plant diversity with soil age has been observed in several chronosequences under various climates (Laliberté *et al.*, 2013). For example, Wardle *et al.* (2008) studied tree and plant diversity through succession and ecosystem retrogression (i.e. the decline phase of succession that is characterised by decreasing soil fertility and plant biomass) at six different locations. They found a significant increase in vascular plant diversity at all locations but one, and observed an increasing number of plants with smaller statured life forms. Zemunik *et al.* (2015) also observed a clear increase in plant richness and diversity along a well-studied Australian dune chronosequence. Possible explanations for this pattern of increasing plant diversity with greater soil age are the decline of competition intensity among co-occurring plants during ecosystem retrogression, greater spatial heterogeneity of soil nutrients, and increased light availability because of fewer trees (Wardle *et al.*, 2008). However, most of these mechanisms remain incompletely understood and still need to be tested empirically (Laliberté *et al.*, 2013). Our data does not allow us to describe the mechanisms responsible for the pattern of plant diversity we observed. Still, the complementarity in belowground functional traits linked to resource acquisition along the successional gradient, which coincided with increasing plant richness and diversity, is consistent with results of a recent study (Zemunik *et al.*, 2015) and suggests a potential importance of belowground resource partitioning (Laliberté *et al*., 2013). Indeed, access to distinct nutrient pools and diverse nutrient-uptake strategies can reduce interspecific competition for soil resources and thus promote local plant coexistence (Turner, 2008; Laliberté *et al*., 2013). Complementarity of nutritional strategies could also perhaps facilitate the growth of neighbour plant species, which might promote plant species diversity. For example, Teste *et al.* (2014) observed the best growth of *Melaleuca* seedlings in the presence of both ectomycorrhizal host plants and nutrient mining plants with cluster roots, suggesting a synergistic effect between the two strategies. The trends we observed in nutrient-acquisition strategies are consistent with the model proposed by Lambers *et al.* (2008), where AM plants are more abundant in young soils, followed by ectomycorrhizal, ericoid mycorrhizal and then non-mycorrhizal plants. The observation of AM as the dominant nutrient uptake strategy is consistent with the fact that the majority of plant species associate with AM fungi (Smith & Read, 2008).

Zemunik *et al.* (2015) also identified the AM strategy as the dominant one along a dune chronosequence. Most plant nutrient acquisition strategies linked to fungal symbionts followed the distribution of associated fungal functional guilds. Ectomycorrhizal fungi and ericoid mycorrhizal fungi displayed high abundance in Z5-Z6 (Roy-Bolduc *et al.*, 2015), a pattern similar to the associated plant nutrional strategy. However, occurences of EM and ericoid fungi were also recorded in the coastal zone without the presence of associated hosts (Roy-Bolduc *et al.*, 2015). That might be explained by the fact that many of these fungi are facultative saprotrophs (Tedersoo *et al.*, 2003; Cullings & Courty, 2009). The distribution and abundance of AM fungi seemed limited to Z2-Z3, which contrast with the fact that AM plants were present in all zones of the dune sequence. However, ITS markers do not allow to adequately capture Glomeromycota diversity and the observed abundance of AM fungi is most likely an underestimation (Lee *et al.*, 2008).

We also found significant correlations of both plant and fungal community composition to soil physicochemical properties. From all soil properties investigated, pH was the one explaining the highest proportion of variance in plant and fungal community composition. Soil pH has previously been identified as one of the key drivers of plant community composition along an Australian dune chonosequence (Zemunik *et al.*, 2015; Laliberté *et al.*, 2014). Even though fungi are thought to have a wide pH optima (Rousk *et al.*, 2010), some fungal groups such as arbuscular mycorrhizal fungi are described as highly responsive to variations in soil pH (Dickie *et al.*, 2013) which could have an indirect effect on the vegetation. Also, the impact of soil pH on nutrient solubilisation and availability can explain the extent of its influence on plant communities (Gobat *et al.*, 2004). Soil water content is another property that we found to be influential, as expected. Cornwell and Ackerly (2009) also found that gravimetric soil water content strongly affected plant functional trait distribution. For example, they predicted an increase in plant richness following increasing soil water, as we observed here. Also, fungi are known to be very sensitive to anoxic conditions (Bragazza *et al*., 2015; Lin *et al*., 2012). Water content was the second-most important environmental factor explaining variation in fungal community structure. The various and complex factors shaping plant diversity along a gradient, such as the one we studied here, remain unresolved. One potential mechanism, namely environmental filtering, explains local plant richness by the filtering of the species poorly adapted to local conditions from the regional species pool. Previous studies suggested that environmental

filtering driven by soil acidification was responsible for plant assembly across a dune chronosequence (Laliberté *et al*., 2014; Zemunik *et al*., 2015). Similarly, our results also indicate that soil pH could act as an environmental filter along this relic foredune plain. Furthermore, we detected similar responses of plants and fungi to the same soil parameters. This could be the result of indirect interactions between above and belowground communities through modification of the soil environment. In other words, vegetation might be influencing (at least partially) soil microbiota through modification of soil properties. However, our data does not allow us to discriminate between this hypothesis or that of similar independent reactions to environmental variables.

In conclusion, our study highlighted the strong linkage between plants and the soil biota in a nutrient-poor successional environment. The patterns we observed in nutrient-acquisition strategies indicate that symbiotic interactions with fungi have an influence on vegetation dynamics. As the positive effects of microbes are known to be most common in nutrient-poor habitats where they enhance the supply of growth limiting nutrients (van der Heijden *et al*., 2008; Jonsson *et al*., 2001; Teste *et al*., 2014), plant-fungi symbiotic interactions could be important for plant establishment and survival in this relic foredune plain. The diversification of belowground traits promoted functional diversity, and might favour resource partitioning and facilitation among co-occuring plant species, which could be one of the mechanisms favoring local plant diversity (Zemunik *et al*., 2015). Hence, local plant community assembly could be partly determined by mechanisms of resource competition and/or facilitation between co-occurring plant species. On the other hand, we also observed that soil properties, such as pH and water content, shapes community structure, which suggests that environmental filtering is also important. Our results suggest that both environmental filtering (exclusion of species outside their viable range) and limiting similarity (coexistence of functionally dissimilar species due to competition for limiting resources) can together drive plant community assembly. Our finding that changes in soil microbial and plant communities were strongly coordinated along this sequence highlights the importance of aboveground-belowground linkages during ecological succession (Walker & Wardle, 2014). Such aboveground-belowground linkages should be considered in conservation and ecological restoration practices (Kardol & Wardle, 2010). This can be particularly important for ecosystems that are vulnerable to disturbance, such as coastal sand dunes.

Acknowledgments

Conflict of Interest Statement

The authors declare no conflict of interest.

References

Barberán A, McGuire KL, Wolf JA, *et al.* (2015) Relating belowground microbial composition to the taxonomic, phylogenetic, and functional trait distributions of trees in a tropical forest. *Ecol Lett* **18**: 1397–1405.

Bever JD, Dickie IA, Facelli E, *et al*. (2010) Rooting theories of plant community ecology in microbial interactions. *Trends Ecol Evol* **25**: 468-478.

Boddy L, Frankland JC & van West P (2007) *Ecology of saprotrophic basidiomycetes.* Elsevier Academic Press, Amsterdam.

Borcard D, Gillet F & Legendre P (2011) *Numerical ecology with R*. Springer, New York.

Bragazza L, Bardgett RD, Mitchell EA & Buttler A (2015) Linking soil microbial communities to vascular plant abundance along a climate gradient. *New Phytol* **205**: 1175-1182.

Broeckling CD, Broz AK, Bergelson J, Manter DK & Vivanco JM (2008) Root exudates regulate soil fungal community composition and diversity. Appl Environ Microbiol 74: 738-744.

Brundrett M (2009) Mycorrhizal associations and other means of nutrition of vascular plants: understanding the global diversity of host plants by resolving conflicting information and developing reliable means of diagnosis. *Plant Soil* **320**: 37-77.

Caporaso JG, Kuczynski J, Stombaugh J*, et al.* (2010) QIIME allows analysis of high-throughput community sequencing data. *Nat Methods* **7:** 335-336.

Chao A (1984) Nonparametric Estimation of the Number of Classes in a Population. *Scand J Stat* 1984*;* **11**: 265-270.

Cornwell WK & Ackerly DD (2009) Community assembly and shifts in plant trait distributions across an environmental gradient in coastal California. *Ecol Monogr* **79**: 109-126.

Cowles HC (1899) The Ecological Relations of the Vegetation on the Sand Dunes of Lake Michigan. Part I.-Geographical Relations of the Dune Floras. *Botanical Gazette* **27**: 95-117.

Cullings K & Courty PE (2009) Saprotrophic capabilities as functional traits to study functional diversity and resilience of ectomycorrhizal community. *Oecologia* **161***:* 661-664.

Dickie IA, Martínez-García LB, Koele N*, et al.* (2013) Mycorrhizas and mycorrhizal fungal communities throughout ecosystem development. *Plant Soil* **367**: 11-39.

Dray S & Dufour AB (2007) The ade4 package: implementing the duality diagram for ecologists. *J Stat Softw* **22**: 1-20.

Edgar RC (2010) Search and clustering orders of magnitude faster than BLAST. *Bioinformatics* **26**: 2460-2461.

Gardes M & Bruns TD (1993) ITS primers with enhanced specificity for basidiomycetes - application to the identification of mycorrhizae and rusts. *Mol Ecol* **2**: 113-118.

Giles PT & King MC (2001) Les Sillons: a relict foredune plain. *Can Geogr* **45**: 437-441.

Gobat JM, Aragno M & Matthey W (2004) *The living soil: fundamentals of soil science and soil biology.* Science Publishers, Enfield.

Gotelli NJ & Colwell RK (2001) Quantifying biodiversity: procedures and pitfalls in the measurement and comparison of species richness. *Ecol Lett* 2001; **4**: 379-391.

Gurevitch J, Scheiner SM & Fox GA (2002) *The ecology of plants.* Sinauer associates incorporated, Sunderland.

Houle G (2008) Plant species richness and its determinants on a coastal dune system at Îles de la Madeleine, Québec (Canada). *Ecoscience* **15**: 113-120.

Ihrmark K, Bödeker ITM, Cruz-Martinez K, *et al.* (2012) New primers to amplify the fungal ITS2 region – evaluation by 454-sequencing of artificial and natural communities. *FEMS Microbiol Ecol* **82**: 666-677.

Jonsson L, Nilsson M, Wardle D & Zackrisson O (2001) Context dependent effects of ectomycorrhizal species richness on tree seedling productivity. *Oikos* **93**: 353-364.

Kardol P & Wardle DA (2010) How understanding aboveground–belowground linkages can assist restoration ecology. *Trends Ecol Evol* **25**: 670-679.

Kardol P, De Deyn GB, Laliberté E, Mariotte P & Hawkes CV (2013) Biotic plant–soil feedbacks across temporal scales. *J Ecol* **101**: 309-315.

Kõljalg U, Nilsson RH, Abarenkov K, *et al.* (2013) Towards a unified paradigm for sequence-based identification of fungi. *Mol Ecol* **22**: 5271-5277.

Laliberté E, Grace JB, Huston MA, *et al.* (2013) How does pedogenesis drive plant diversity? *Trends Ecol Evol* **28**: 331-340.

Laliberté E, Zemunik G & Turner BL (2014) Environmental filtering explains variation in plant diversity along resource gradients. *Science* **345**: 1602-1605.

Lambers H, Raven JA, Shaver GR & Smith SE (2008) Plant nutrient-acquisition strategies change with soil age. *Trends Ecol Evol* **23**: 95-103.

Lee J, Lee S & Young JPW (2008) Improved PCR primers for the detection and identification of arbuscular mycorrhizal fungi. *FEMS Microbiol Ecol*, **65:** 339-349.

Lichter J (1998) Primary succession and forest development on coastal Lake Michigan sand dunes. *Ecol Monogr* **68**: 487-510.

Lin X, Green S, Tfaily M, *et al.* (2012) Microbial community structure and activity linked to contrasting biogeochemical gradients in bog and fen environments of the Glacial Lake Agassiz Peatland. *Appl Environ Microbiol* **78**: 7023-7031.

Maun MA (2009) *The biology of coastal sand dunes.* Oxford University Press, Oxford.

Miniaci C, Bunge M, Duc L, *et al.* (2007) Effects of pioneering plants on microbial structures and functions in a glacier forefield. *Biol Fert Soils* **44**: 289-297.

Mitchell RJ, Hester AJ, Campbell CD, *et al.* (2010) Is vegetation composition or soil chemistry the best predictor of the soil microbial community? *Plant Soil* **333**: 417-430.

Olson JS (1958) Rates of succession and soil changes on southern Lake Michigan sand dunes. *Botanical Gazette* **119**: 125-170.

Oksanen J, Blanchet FG, Kindt R, *et al.*(2016) *vegan: Community Ecology Package*. R package version 2.3-3. https://CRAN.R-project.org/package=vegan.

Prescott CE & Grayston SJ (2013) Tree species influence on microbial communities in litter and soil: current knowledge and research needs. *Forest Ecol Manag* **309**: 19-27.

Rousk J, Bååth E, Brookes PC, Lauber CL, Lozupone C, Caporaso JG *et al.* (2010) Soil bacterial and fungal communities across a pH gradient in an arable soil. *ISME J* **4**: 1340-1351.

Roy-Bolduc A, Bell TH, Boudreau S & Hijri M (2015) Comprehensive sampling of an isolated dune system demonstrates clear patterns in soil fungal communities across a successional gradient. *Environ Microbiol Rep* **7**: 839-848.

Schloss PD, Westcott SL, Ryabin T, *et al.* (2009) Introducing mothur: Open-Source, Platform-Independent, Community-Supported Software for Describing and Comparing Microbial Communities. *Appl Environ Microbiol* **75**: 7537-7541.

Smith SE & Read DJ (2008) *Mycorrhizal symbiosis*, 3rd edn. Academic Press, London.

R Development Core Team (2014) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria. http://www.r-project.org/

Tedersoo L, Kõljalg U, Hallenberg N & Larsson KH (2003) Fine scale distribution of ectomycorrhizal fungi and roots across substrate layers including coarse woody debris in a mixed forest. *New Phytol* **159**: 153-165.

Tedersoo L, Nilsson RH, Abarenkov K *et al.* (2010) 454 Pyrosequencing and Sanger sequencing of tropical mycorrhizal fungi provide similar results but reveal substantial methodological biases. *New Phytol* **188**: 291-301.

Teste FP, Veneklaas EJ, Dixon KW & Lambers H (2014) Complementary plant nutrient-acquisition strategies promote growth of neighbour species. *Funct Ecol* **28**: 819-828.

Turner BL (2008) Resource partitioning for soil phosphorus: A hypothesis. *J Ecol* **96**: 698-702.

van der Heijden MGA, Bardgett RD & van Straalen NM (2008) The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. *Ecol Lett* **11**: 296-310.

van der Putten WH, Bardgett RD, Bever JD, *et al*. (2013) Plant–soil feedbacks: the past, the present and future challenges. *J Ecol* **101**: 265-276.

Walker LR & Wardle DA (2014) Plant succession as an integrator of contrasting ecological time scales. *Trends Ecol Evol* **29**: 504–510.

Wardle DA, Bardgett RD, Klironomos JN, *et al.* (2004) Ecological linkages between aboveground and belowground biota. *Science* **304**: 1629-1633.

Wardle DA, Bardgett RD, Walker LR, Peltzer DA & Lagerström A (2008) The response of plant diversity to ecosystem retrogression: evidence from contrasting long-term chronosequences. *Oikos* **117**: 93-103.

Webley DM, Eastwood DJ & Gimingham CH (1952) Development of a Soil Microflora in Relation to Plant Succession on Sand- Dunes, Including the `Rhizosphere' Flora Associated with Colonizing Species. *J Ecol* **40**: 168-178.

White TJ, Bruns T, Lee S & Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *PCR protocols: a guide to methods and applications* (Innis MA, Gelfand DH, Sninsky JJ & White TJ, eds.), pp.315-322. Academic Press, New York.

Zemunik G, Turner BL, Lambers H & Laliberté E (2015) Diversity of plant nutrient-acquisition strategies increases during long-term ecosystem development. *Nat Plants* **1**: 15050.

Data Accessibility

The raw .sff files from 454 sequencing have been deposited in the Sequence Read Archive with Accession no. SRP059280. Available at:

http://www.ncbi.nlm.nih.gov/Traces/sra/?study=SRP059280

Table 1 Variation in plant and fungal alpha diversity across succession stages. Plant richness was measured as the number of observed species, while fungal richness was evaluated as the number of observed species, total richness Chao estimator, and rarefied richness (subsampling of 200 reads per sample). Diversity was measured with the Shannon and the inverse Simpson indices. Values are mean ± standard error (n=10). Letters indicate significant differences between means assessed with Tukey HSD *post hoc* tests ($P \leq 0.05$). The last rows indicate the total richness, i.e. the total number of different plant species or fungal OTUs recorded in each zone.

ABOVE-GROUND VEGETATION								
	Z1	**Z2**	**Z3**	**Z4**	**Z5**	**Z6**	**Z7**	**Z8**
	Pioneer zone	Foredune front	Foredune back	Mobile dune	Heathland	Forest dune	Inter-ridge swale	Lagoon wetland
Richness	1.00 ± 0.00 (a)	1.11 ± 0.11 (a)	2.44 ± 0.60 (ab)	2.6 ± 0.56 (ab)	6.44 ± 0.80 (cd)	4.56 ± 0.69 (abc)	5.20 ± 0.98 (bcd)	8.36 ± 1.30 (d)
Shannon	0.00 ± 0.00 (a)	0.07 ± 0.07 (a)	0.41 ± 0.18 (ab)	0.32 ± 0.12 (ab)	1.25 ± 0.11 (cd)	0.71 ± 0.16 (bc)	0.89 ± 0.18 (bcd)	1.39 ± 0.16 (d)
Simpson	1.00 ± 0.00 (a)	1.08 ± 0.08 (a)	1.66 ± 0.35 (ab)	1.29 ± 0.13 (a)	3.09 ± 0.43 (bc)	1.80 ± 0.29 (ab)	2.19 ± 0.32 (abc)	3.45 ± 0.56 (c)
Total richness	1	2	10	12	28	23	23	43

SOIL FUNGI								
	Z1	**Z2**	**Z3**	**Z4**	**Z5**	**Z6**	**Z7**	**Z8**
Observed richness	107.30 ± 14.59	121.44 ± 9.74	151.00 ± 15.57	150.00 ± 22.80	181.67 ± 24.93	173.56 ± 22.69	152.80 ± 21.50	164.73 ± 23.07
Rarefied richness	99.28 ± 8.60	106.40 ± 6.78	112.40 ± 11.13	114.32 ± 13.17	124.93 ± 11.02	125.28 ± 13.62	118.32 ± 16.05	118.44 ± 12.88
Chao total richness	129.83 ± 18.24	148.06 ± 12.23	182.88 ± 18.65	187.38 ± 28.79	227.61 ± 30.65	223.29 ± 26.05	183.36 ± 26.18	198.94 ± 26.64
Simpson	12.78 ± 2.36	13.55 ± 2.58	13.12 ± 2.72	14.05 ± 2.92	15.55 ± 4.49	13.23 ± 4.44	13.18 ± 3.87	11.38 ± 3.50
Shannon	3.16 ± 0.20	3.31 ± 0.18	3.28 ± 0.25	3.33 ± 0.23	3.35 ± 0.25	3.15 ± 0.31	3.14 ± 0.29	2.92 ± 0.32
Total richness	607	519	685	892	1039	920	942	1157

Table 2 Community variance (portion of the model non-adjusted R^2) explained by soil physiochemical properties. Significant relationships (at $P \leq 0.05$) are in bold.

	PLANT COMMUNITY R^2	P-value	FUNGAL COMMUNITY R^2	P-value
Humus thickness	**0.11458**	0.001	**0.03766**	0.001
pH	**0.20532**	0.001	**0.05063**	0.001
Water content	**0.07731**	0.001	**0.04830**	0.001
Total carbon	0.01459	0.085	**0.01722**	0.022
Total nitrogen	**0.01698**	0.040	**0.01673**	0.018
Total phosphorus	0.01232	0.141	0.01289	0.235
Extractable phosphorus	0.00658	0.548	0.01238	0.291
Residuals	*0.55232*		*0.80418*	
Total	*1.00000*		*1.00000*	
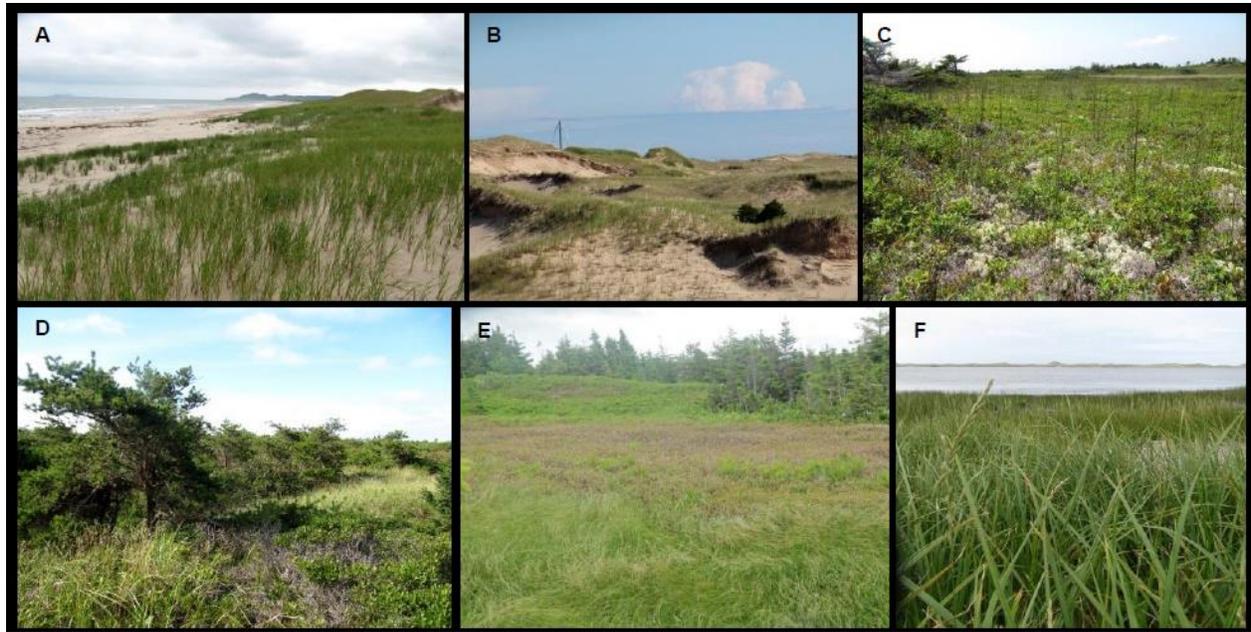
Figure 1 The different zones of our study site, from the pioneer zone and the foredune (A), to the mobile dune (B), the heathland (C) and the forested dune (D). At the end of the edaphic gradient, we can also observe wetlands in the inter-ridge swales (E) and the lagoon wetlands (F).
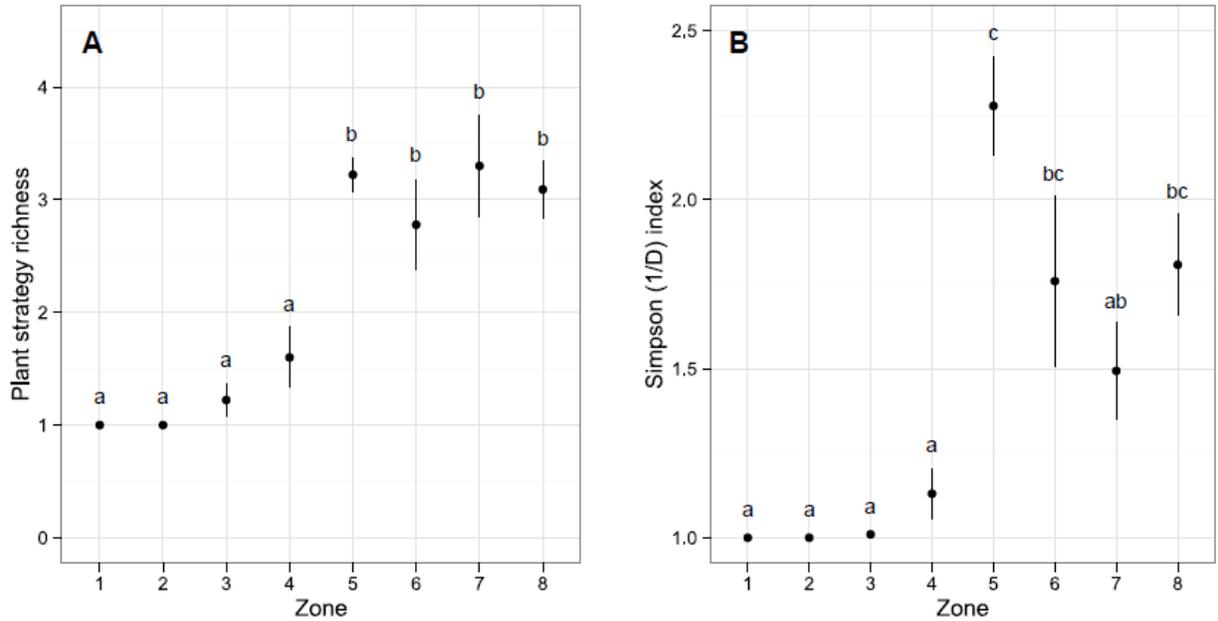
Figure 2 Richness (A) and diversity (B) of plants nutrient-acquisition strategy by sampling plots along the successional and edaphic gradient. Obligate and facultative AM are considered as the same strategy, and unknown were removed from the dataset for this analysis. Points are means and bars standard errors (n=10). Different letters indicate significant differences ($P \leq 0.05$).
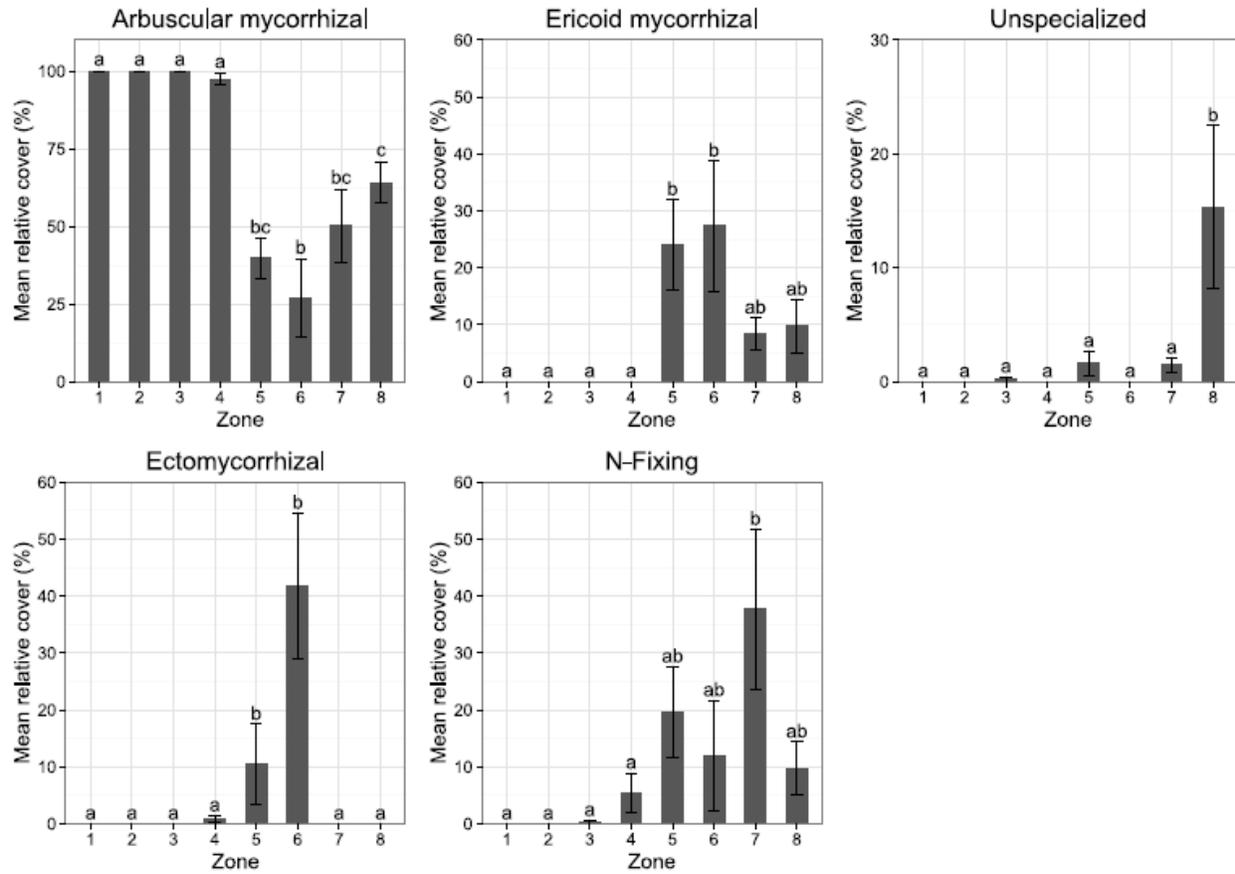
Figure 3 Mean relative cover of the main nutrient-acquisition strategies along the successional and edaphic gradient. Bars represent standard errors (*n*=10) and different letters indicate significant differences (*P*≤0.05).
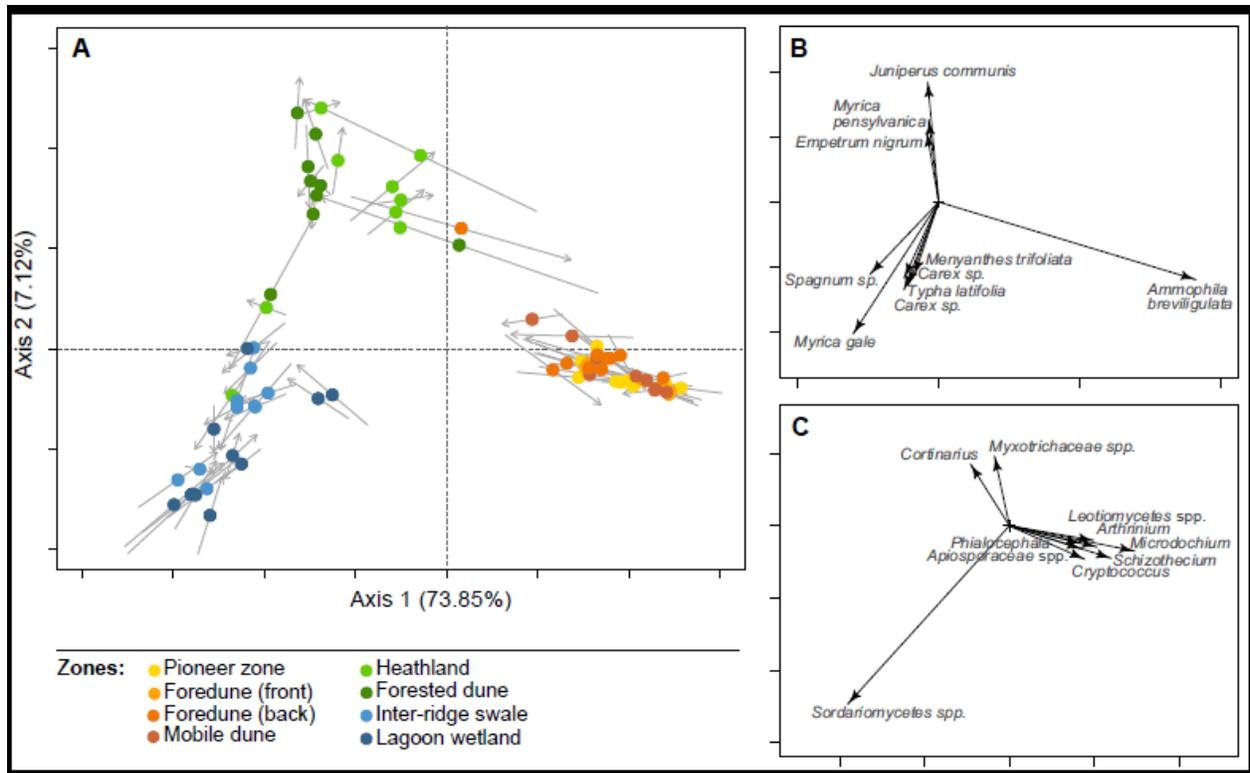
Figure 4 Joint site plot (a) depicting consensual ordination of sites in function of both plant and fungal community compositions preserving the Hellinger distances, and projection of the ten plant species (b) and fungal genera (c) with the highest scores. The two first canonical axes account for 80.97% of total co-inertia and the RV coefficient is 0.59 (*P*=0.001). Arrow length is proportional to the difference between the ordinations of the two data sets: position of the arrow tails is determined by the ordination of plant community data, and the head by fungal community data.
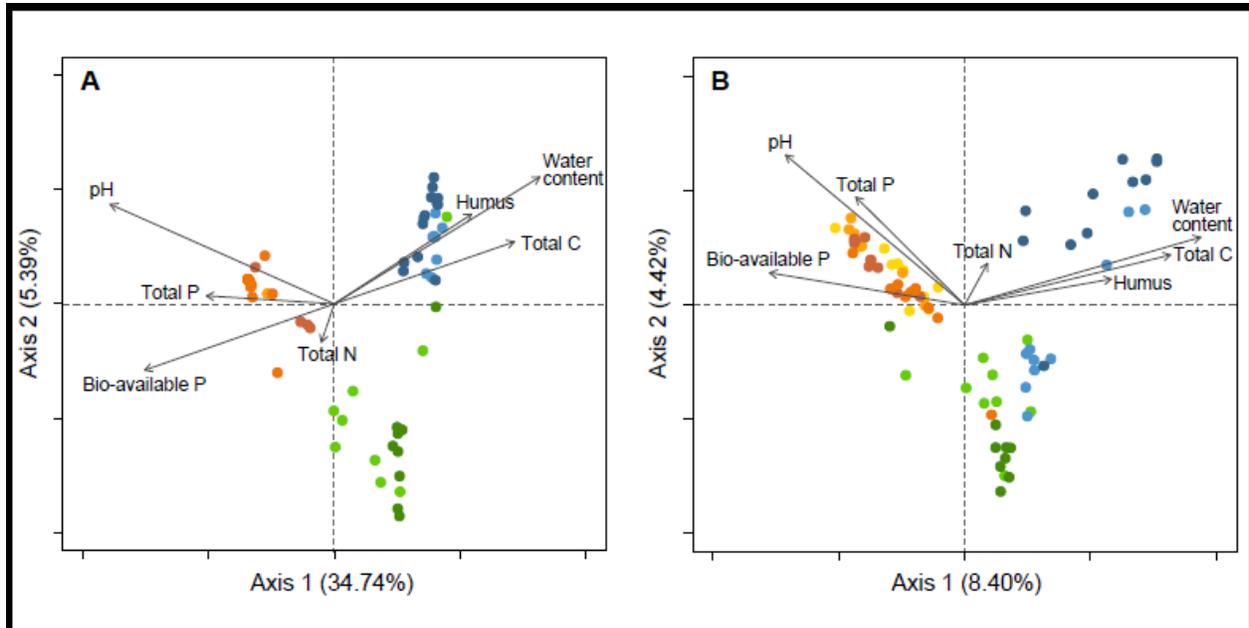
Figure 5 Transformation-based RDA biplots of plant (a) and fungal (b) communities in relation to soil properties using the Hellinger distance. Scaling type = 2. Adjusted R^2 is of 0.3916 (*P*=0.001) for the analysis on vegetation and of 0.1142 (*P*=0.001) for fungi.