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Comprehensive sampling of an isolated dune system demonstrates clear patterns in soil fungal communities across a successional gradient

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Summary

Coastal sand dunes are extremely dynamic ecosystems, characterized by stark ecological succession gradients. Dune stabilization is mainly attributed to plant growth, but the establishment and survival of dune-inhabiting vegetation is closely linked to soil microorganisms and to the ecological functions they fulfill. Fungi are particularly important in this context, as some interact intimately with plant roots, while others are critical to soil structure and nutrient availability.

Our study aimed to describe wholly fungal diversity and community composition in a secluded coastal dune ecosystem at eight different stages of succession. We comprehensively sampled a relic foredune plain, which is part of an archipelago in the Gulf of Saint Lawrence (Québec, Canada), by collecting soils from 80 sites and measuring soil characteristics. Soil fungal communities were characterized by pyrosequencing, followed by taxonomic classification and assignment of putative roles. Even though we did not observe clear patterns in diversity, we were able to detect distinct taxonomic and community composition signatures across succession stages, which seemed to translate into variations in fungal life strategies. Our results show that a taxonomically and functionally diverse fungal community exists at each dune succession stage, even in the barren foredunes.

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Introduction

Coastal dune ecosystems act as transition zones between marine and continental environments, and are globally distributed (Martinez and Psuty, 2008). Dune ecosystems are present on most major coastlines, and often reduce coastal erosion and protect littoral zones by providing a barrier against wave and wind action (Maun, 2009). They also contribute to water filtering, groundwater replenishment, maintenance of biodiversity, and are of cultural, aesthetic and recreational importance (Everard *et al.*, 2010). These services, however, depend on the integrity of the dune ecosystem and on the ecological interactions that take place within it.

Both fungi and bacteria perform crucial ecological functions in nearly barren habitats such as sand dunes, including nitrogen fixation, decomposition of organic matter into compounds that are easily assimilated by plants and rock degradation, which releases essential nutrients such as phosphorus (Landeweert *et al.*, 2001; Van Der Heijden *et al.*, 2008). Fungi have particularly important roles in decomposition and in affecting plant nutrition and diversity (Van Der Heijden *et al.*, 2008). Within the estimated 1.5×10^6 fungal species on Earth (Hawksworth, 2001), there are many known lifestyles that both directly and indirectly impact plant survival. For instance, mycorrhizal fungi are ubiquitous plant partners and increase plant nutrient absorption while protecting them against pathogen attacks and abiotic stresses (Read, 1992; Smith and Read, 2008). Saprotrophic fungi release a range of enzymes into soils, contributing to organic matter decomposition and nutrient cycling (Baldrian *et al.*, 2011) and converting nutrients into forms that are available to plants. Some fungi are parasites of plants and/or animals, while others are nematophagous, and may alter the impact of pathogenic nematode populations on plant performance. The combined activities of these functional guilds ultimately determine the suitability of soil habitats for ecosystem development.

In coastal dunes, the sequence of plant communities along the spatial gradient extending from upper beaches to fixed dunes is often used to study plant succession. The foredune is colonized by pioneer species with dense root systems, allowing dune stabilization, and facilitating the colonization of other herbs, shrubs and eventually, trees.

Yet, in these extreme environments, plant survival, growth and reproduction are greatly limited by environmental factors such as wind, salinity and drought. As a result, the development of pioneer vegetation is strongly linked to the presence of soil microorganisms, and fungi in particular, since these microbes help to offset the negative impacts of environmental constraints (Koske and Polson, 1984; Maun, 2009).

Îles-de-la-Madeleine is an archipelago in the Gulf of St Lawrence in Québec, Canada, containing different successional stages, which are each represented by characteristic plant communities over a short spatial gradient (generally < 1 km). The simplicity of island ecological systems and the compacted succession gradients of dunes make each a model system for ecological studies (Lichter, 1998a; Vitousek, 2002), and this system possesses both, reducing the confounding effects of geography and complex biotic interactions. The functional importance of fungi in coastal dunes is not fully understood, but the positive effect of some fungal groups – mainly mycorrhizal taxa – on plant establishment, survival and growth has been clearly demonstrated (e.g. Corkidi and Rincón, 1997; Gemma and Koske, 1997; reviewed in Read, 1989). In this study, we used pyrosequencing of the fungal internal transcribed spacer (ITS) region to describe comprehensively soil fungal composition and diversity across eight zones of an isolated foredune plain of *c*. 10 km^2 in the Îles-de-la-Madeleine archipelago. Based on previous research on soil microbial populations in forested ecosystems (Gao *et al*., 2015) and in successional habitats such as glacier retreats (Jumpponen, 2003; Blaalid *et al*., 2012; Brown and Jumpponen, 2013), we expected to observe important shifts in fungal community composition and in soil abiotic parameters across successional stages. In addition to shifts in taxonomy, we estimated shifts in functional guilds of fungi across dune zones by assigning our fungal genera to the most probable functional roles based on the literature and on existing databases. While the functional capacity of soil bacterial communities has been estimated from 16S rRNA gene data (Langille *et al*., 2013; Barberán *et al*., 2014), this type of approach has only been applied to fungi in a few recent studies (e.g. Clemmensen *et al*., 2013; Tedersoo *et al*., 2014; Clemmensen *et al*., 2015). We aimed to determine whether functional assignments of fungal taxa would reflect the taxonomic patterns we observed, or even strengthen them.

Results and discussion

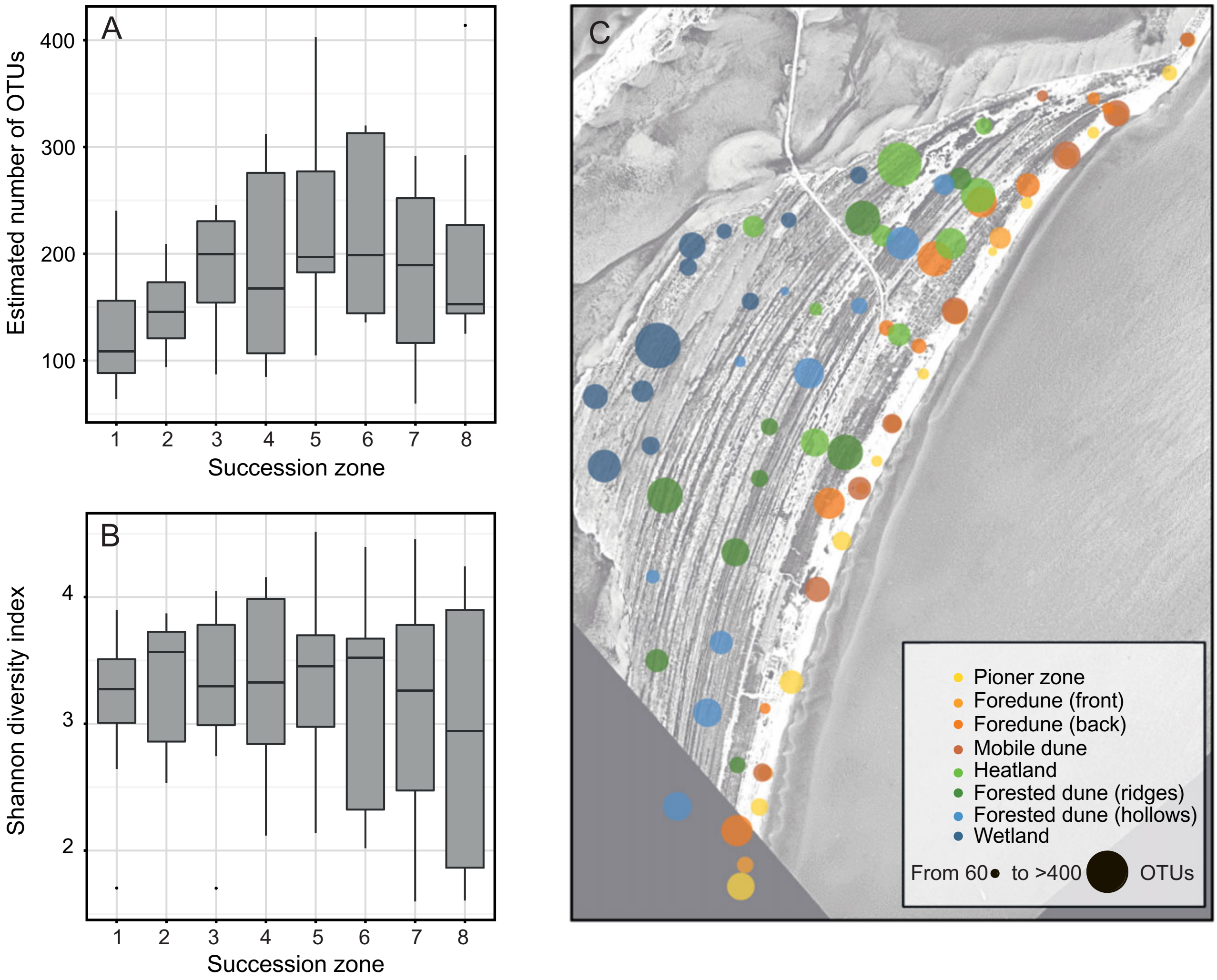
Study site description and soil properties

Our unique study site is an isolated coastal dune system known as *Les Sillons*, and was selected because of the large habitat diversity that it supports, as described by Grandtner (1967). In this system, it is possible to observe a complete ecological succession sequence, from the beach to a relic foredune plain, including forested and wetland habitats (Fig. S1). The study site comprised eight different zones belonging to three larger regions: the coastal region encompassing the (i) pioneer zone, (ii) front of the foredune, (iii) back of the foredune and (iv) mobile dune; the forested region including the (v) heathland and (vi) forest dune, and the humid region with the (vii) inter-ridge swale and (viii) lagoon wetland. The coastal region consists of younger sites located close to the shore, which are characterized by dry sandy soils and a plant community that is largely dominated by *Ammophila breviligulata* Fern., the American beachgrass, as well as a few herbaceous plant such as *Artemisia stellaria* and *Fetusca rubra* in areas that are not directly exposed to wind and salt spray. Heathlands are dry habitats, dominated by shrubs such as *Myrica pensylvanica*, *Juniperus communis* and *Spirea alba,* and these transition into black spruce, fir and pine forests. The humid region encompasses the inter-ridge depressions and the wetlands that are located close to the lagoon and harbour an extremely diverse array of vegetation, including several *Ericaceae*, *Sphagnum* and *Carex* species. The dominant plant species for each zone are listed in Table S1. In terms of environmental variables, we observed variations in elevation, slope and soil type at sampling sites from different zones (Table S2). Physiochemistry also shifted substantially across the dune succession gradient, with significant increases in organic carbon, total nitrogen and bio-available phosphorus at later successional stages, as well as decreasing trends in pH and conductivity (Tables S3). A detailed description of sampling and experimental procedures is provided in Supporting Information (Appendix S1).

Fungal diversity

After sequence processing and quality filtering, we obtained 233 037 ITS reads that were clustered into 3406 operational taxonomic units (OTUs; after excluding singletons) using a 97% sequence similarity cutoff. Reads per sample ranged from 449 to 6556, but rarefaction curves were always close to saturation (Figure S2a–c). In support of this, the mean Good's coverage was 0.98 for all three zones. The sampling effort curve (Figure S2d) shows that the number of samples treated (77) allowed us to capture over 75% (3406 OTUs) of the estimated total diversity (4492 OTUs).

Although one might expect to find few coexisting fungal species in the nutrient-poor, water-limited and windy conditions of sand dunes, diverse bacterial and fungal communities have been observed in these environments (Webley *et al*., 1952) and other arid regions (e.g. Fierer and



Zones: 1-Pioneer zone; 2-Foredune(front); 3-Foredune(back); 4-Mobile dune; 5-Heathland; 6-Forested dune; 7-Inter-ridge swale; 8-Lagoon wetland

Fig. 1. Soil fungal diversity across succession stages based on rarefaction analysis with the Chao richness estimator (A and C) and the Shannon diversity index (B). The size of the circles on the map (C) is proportional to the number of estimated OTUs at each sampling point.

Jackson, 2006; Fukami *et al.*, 2010; Schmidt *et al.*, 2014; reviewed in Zak, 2005). We expected to see an increase in soil fungal richness with habitat development, soil complexity, and diversification of vegetation (Van Der Heijden *et al.*, 2008; Blaalid *et al.*, 2012; Zumsteg *et al.*, 2012). Brown (1958) investigated fungal diversity in coastal dunes of the UK by cultivation and isolation of fungi from sand samples, and found the greatest fungal diversity in the semi-fixed dune, where pioneer and late successional plants overlapped (reviewed in Frankland, 1998). In this study, richness ranged from 54 (in the pioneer zone) to 329 (in the lagoon wetland), with an average of 150 OTUs per sample, and analysis of variance (ANOVA) did not reveal significant differences in either the variance or means of the Chao richness estimator or the Shannon diversity index across the succession gradient. There was no clear pattern in OTU richness, but the highest mean number of OTUs was recorded in the heathland and forested dune zones (Fig. 1).

Fungal community structure and links to environmental variables

Redundancy analysis (RDA) indicated that fungal community composition varied across succession zones (Fig. 2A), with the objects (sites) clearly segregating into three groups that were fairly consistent within the three regions. The cluster located at the right of the graph consisted mainly of the younger sites of the coastal region. The sites located at the left of the ordination

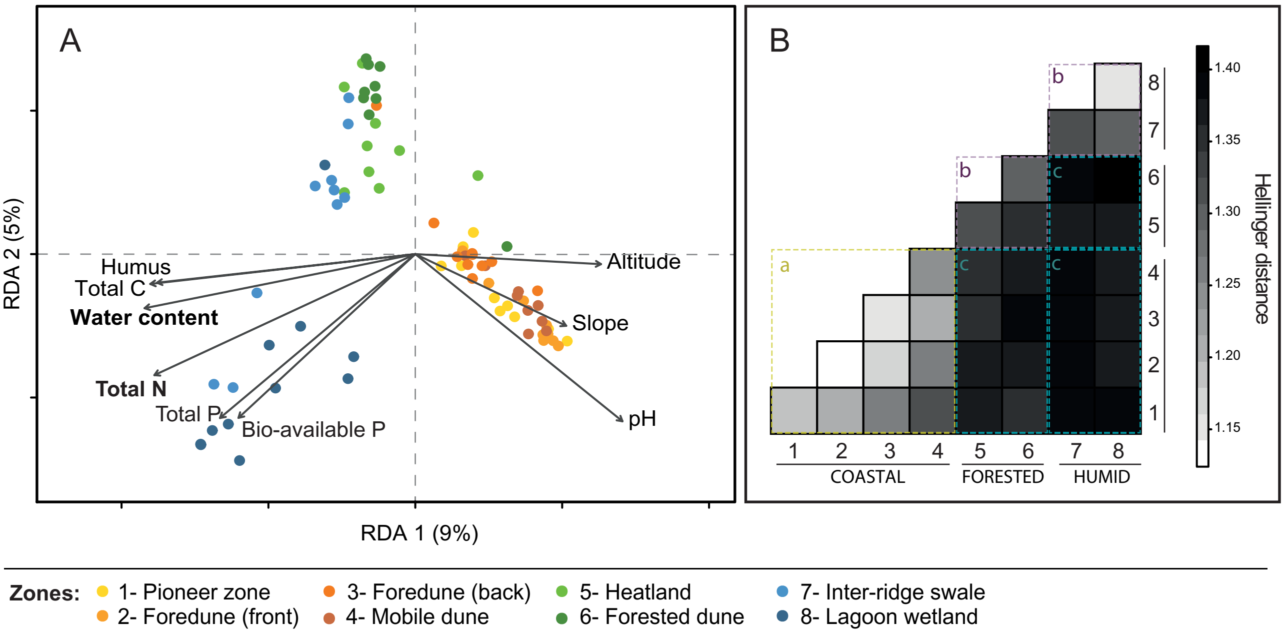


Fig. 2. (A) Transformation-based RDA biplot of the fungal communities in relation to soil properties and site topography using the Hellinger distance and displaying the Hellinger distance in terms of OTU composition using soil properties. (B) Heat map displaying inter-plot variability as the mean Hellinger distance within and between succession zones. Different letters indicate significant differences between pairs of dune regions (for $P < 0.05$).

correspond to sites from the more advanced succession zones and the wetland lagoon, the upper part primarily including the drier sites of the forested region, and the lower portion the humid region. In fact, permutational ANOVA revealed a significant grouping of sites with respect to the three regions as well as to the eight succession zones (both P-values = 0.0001, R^2 of 0.083 and 0.079 respectively). The pairwise comparisons indicated significant groupings for all pairs of regions and succession zones at a significance level of $P < 0.05$ (adjusted P-values), with the exception of the back foredune, which is not significantly different from the front foredune or the mobile dune. The canonical axes explained 9% and 5% of the response data variance, with a total R^2 of 0.356, and of 0.141 when adjusted for the number of observations and degrees of freedom. The F-statistic ($F = 1.654$) was significant ($P = 0.001$). Despite the clear groupings, compositional shifts were not easily explained by environmental descriptors alone. Of all explanatory variables included in the model, only total nitrogen ($F = 1.388$, $P = 0.024$) and water content ($F = 1.34$, $P = 0.041$) significantly influenced fungal composition, and total phosphorus ($F = 1.22$, $P = 0.082$) had a nearly significant influence (at the 0.05 level). This suggests that soil properties alone did not have an overwhelming role in shaping soil fungal communities. Unlike bacteria, which appear less limited by dispersal and are shaped heavily by soil parameters, fungi may be somewhat disconnected from the abiotic environment (Schmidt *et al.*, 2014). For example, correlations to soil pH, as has so often been shown for bacteria (Fierer and Jackson, 2006), are less clear for fungi, possibly because they have wider pH optima (Rousk *et al.*, 2010). Although changes in the community structure of some fungal groups (e.g. EM fungi) have been attributed to shifts in pH, soil moisture and nitrogen availability (Erland and Taylor, 2003), the factors determining soil fungal communities remain poorly understood, especially in natural and undisturbed environments (Van Der Heijden and Sanders, 2002). Our results could suggest, as previously supported in microbial studies of glacier retreats (Jumpponen, 2003; Blaalid *et al.*, 2012; Brown and Jumpponen, 2013; Schmidt *et al.*, 2014), that fungal communities assemble in a less deterministic way than bacteria, and that they might be more sensitive to stochastic processes such as history and dispersal limitation.

In comparison to bacteria, fungi, as exclusively heterotrophic organisms, are more dependent on the presence of organic matter, or on living organisms in the case of obligate parasites or symbionts. Vegetation could therefore play a key role in shaping soil fungal communities in this dune system. A more substantial shift in the composition of fungal communities than bacterial communities following plant host introduction, and based on plant identity, was already described in contaminated soils (Bell *et al.*, 2014). Because of the specificity of root exudates and litter quality, plant communities have been acknowledged as important drivers of soil microbial community structure (Edwards *et al.*, 2006; Miniaci *et al.*, 2007). In fact, we found that plant species composition did explain a substantial proportion of the variation in fungal community data in this study that was left unexplained by soil parameters (unpublished data).

Visual examination of inter-plot variability of fungal communities within and between succession zones (Fig. 2B) revealed that community structure was much more similar within the primary succession zones, and indicated increasing fungal community divergence at later succession stages. An ANOVA revealed highly significant differences in inter-plot distance ($P < 2\text{e-}16$) across the different dune successional zones and regions. The post-hoc Tukey HSD test performed between pairs of regions confirmed that community structure is more variable in the latter succession stages. Inter-plot variability was lowest within the coastal region and highest within the humid region and between the forested and humid regions. We also observed distinct fungal taxonomic signatures in each of the designated dune zones, with substantial variation in the relative abundance of most major fungal groups (Fig. S3). For example, the relative abundance of *Glomeromycota* (in terms of number of reads) was higher in the coastal region, chytrids were found only in the coastal and humid regions, and the *Basidiomycota* (especially the *Agaricomycetes*) were more abundant in the heathland and dune forest.

Functional guilds and indicator taxa

Although we have learned a great deal from high-throughput taxonomic studies of microbial communities, we need to better understand how these shifts relate to ecosystem functioning. One approach is to correlate microbial taxa with measures of ecosystem productivity, while another is to project functions from previous data annotations. Metagenomic estimates of the functional diversity in bacterial communities rely heavily on such methods, as tools such as MG-RAST assign gene sequences to known functional categories (e.g. nitrogen fixation). While powerful and informative, metagenomics (which produces detailed functional and metabolic profiles by sequencing the vast majority of abundant genes in a community) requires deep sequencing and substantial analysis, and is still prohibitive due to its cost. The computational capacity required to assemble such datasets may limit its application to complex natural soil systems. In contrast, taxonomic profiles can sometimes be used to reliably predict major shifts in soil microbial functional potential with minimal financial investment, allowing processing of many more samples, and the major functional abilities of bacterial communities can be predicted from 16S rRNA marker sequences in various environments such as soil or the gut microbiome (Langille *et al.*, 2013; Barberán *et al.*, 2014). In addition, fungal reads are severely under-sampled in metagenomes due to the overwhelming dominance of prokaryotic genes. For example, gene annotation of soil metagenomes from the site of a former petrochemical plant showed that over 95% of annotated genes were prokaryotic, while less than 2% were fungal genes (Y. Terrat, unpubl. data) and examination of public MG-RAST metagenomes from various soil environments reveals similar trends.

The ecological roles of many fungal genera have been described in the literature, and some ecological guilds are even catalogued in publically available databases (e.g. unite.ut.ee/EcM_lineages.php). Here, we have projected fungal ecological roles from ITS-derived taxonomic information, as was done by Clemmensen and colleagues (2013; 2015) in boreal forests and Tedersoo *et al.* (2014) at a global scale. We aimed to determine how changes in fungal communities across a dune gradient are likely to correspond to changes in ecosystem function. By putatively assigning functions to each classified fungal genus based on the best-available literature (Table S4), we found clear patterns in fungal lifestyles across dune zones (Fig. 3). For example, the relative number of reads assigned to ectomycorrhizal (EcM) and ericoid mycorrhizal fungi was higher in the forested region, while plant pathogens displayed the reverse pattern. Variations in the abundance of EcM and ericoid fungi generally followed shifts in their preferred hosts. Ectomycorrhizal fungi are associated with a wide range of trees and shrubs, while ericoid mycorrhizal fungi form symbioses with the roots of plants from the *Ericaceae* family. All of these plants are found more frequently in the forested area of the dune, while the *Ericaceae* were also more abundant in the humid zone. In contrast, the arbuscular mycorrhizal fungi (AMF) reach their maximum relative abundance in the coastal region. The presence of AMF in the foredune of coastal dunes is well documented in the literature, and patterns similar to what we observed have been described in other studies (e.g. Koske and Halvorson, 1981; Gemma and Koske, 1997; Kowalchuk *et al.*, 2002). The decline in AMF abundance after the foredune and the subsequent increase in EM and ericoid fungi correspond to the pattern described by Read (1989) and by Lambers and colleagues (2008) in their review of variations in plant nutrient-acquisition strategies with soil age. Microbial patterns indeed reflect soil age, habitat maturation and soil nutrient status. Very few nematode predator genera were present, and there was no clear pattern in their relative abundance in terms of number of reads. Lichenized fungi were also uncommon, but peaked in the forested zone. Saprotrophy appears to be by far the dominant fungal lifestyle in almost all dune zones. Since we did not quantify fungal deoxyribonucleic acid with quantitative polymerase chain reaction, it is not possible to draw

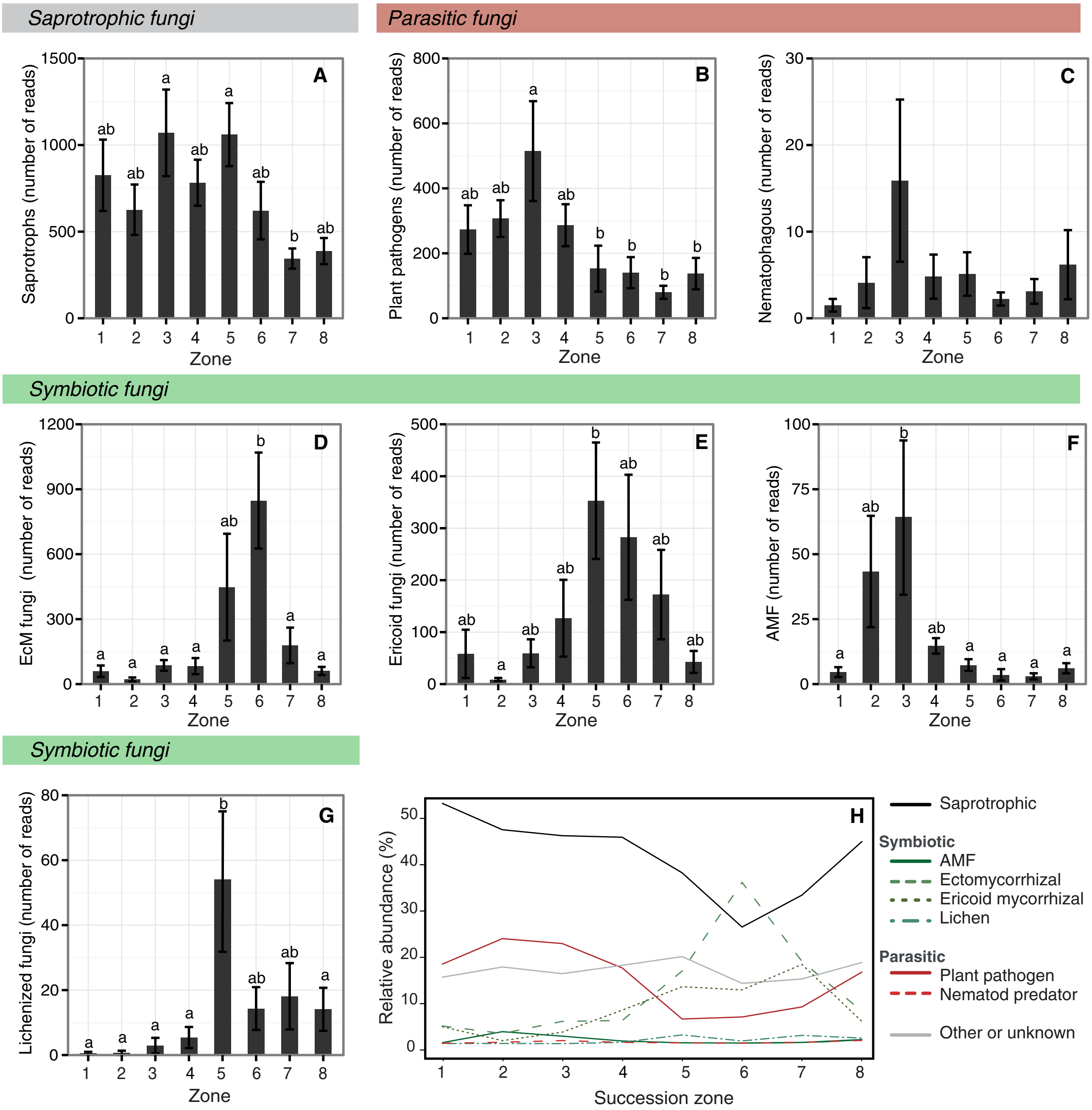


Fig. 3. Changes in fungal life strategies across dune succession stages. A–G show mean and standard error of the number of reads found in samples for each functional guild. Different lower case letters indicate significant differences across succession zones. H combines the relative abundance of all functional guilds.

conclusions about absolute fungal abundance, but our approach indicates which functional groups are likely to be most important at each successional stage. Analysis of the mean number of genera for each functional guild displayed very similar patterns (Fig. S4).

We also conducted an indicator species analysis and selected the best indicator genera (top three according to indicator value) for sites clustered into the eight designated zones and into the three regions. We calculated their relative abundance, and averaged these over each succession zone (Fig. 4). All selected genera have high indicator values (ranging from 0.473 to 0.916), and these values were statistically significant, with a maximum *P*-value of 0.05. Within the selected genera, most ectomycorrhizal symbionts (*Thelephora*, *Cortinarius* and *Clavulina*) were indicators of sites in the forested region.

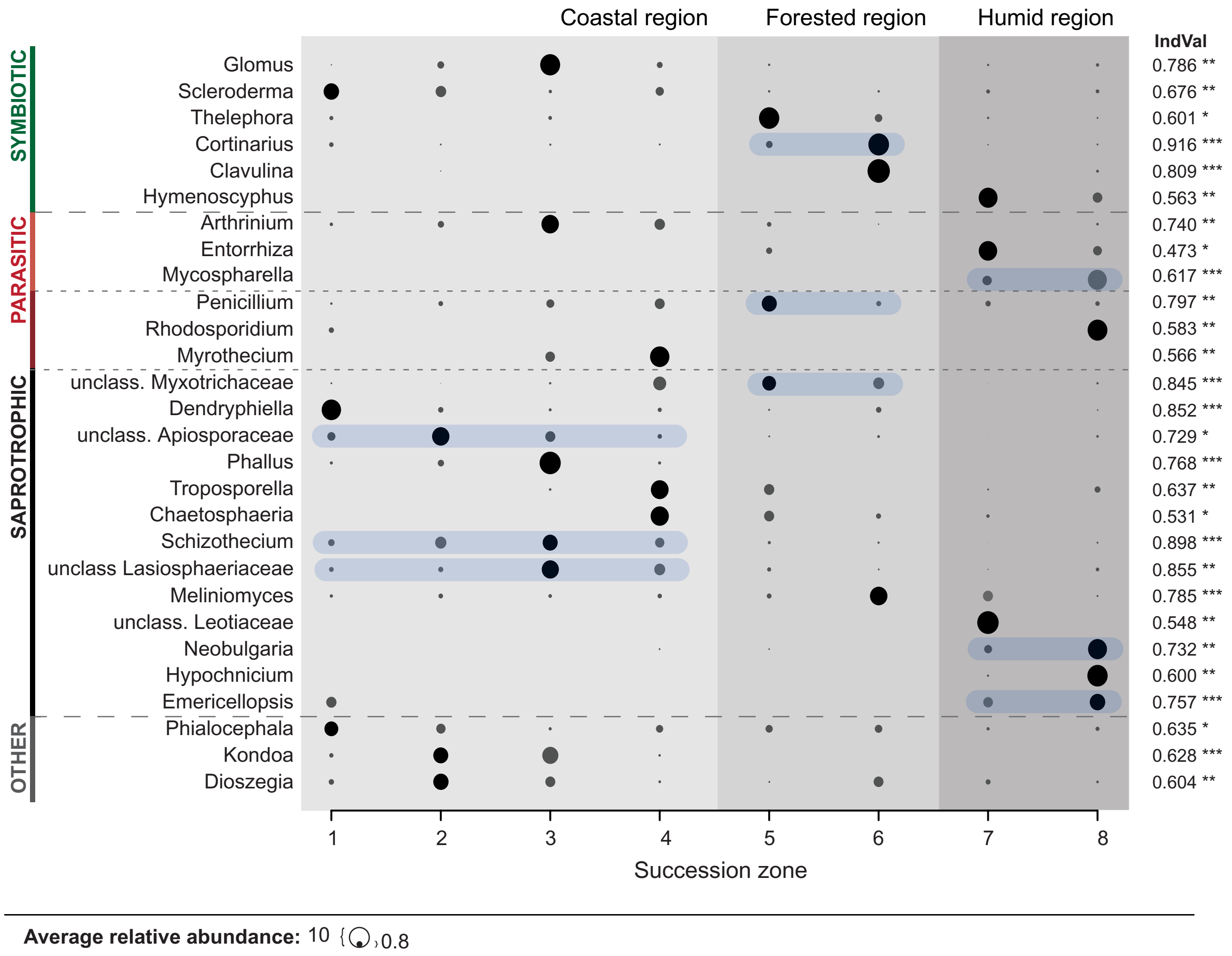


Fig. 4. Bubble plot of the top indicator genera grouped by lifestyle for each dune succession zone and region. The size of the bubble reflects the average relative abundance (%) of each genus in a given succession stage in terms of number of reads. Black circles indicate the zone for which a given genus is an indicator, and the blue rectangles mark indicators of a dune region. Indicator values and significance level (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$) are displayed on the right side of the plot.

Glomus (syn. *Rhizophagus*)*,* a common genus of AMF, had a high relative abundance in the coastal zone, particularly in the foredune area. *Hymenoscyphus*, an indicator of the inter-ridge swale, is a diverse genus within the *Ascomycota* that contains species that are known ectomycorrhizal and ericoid symbionts, and others that are known plant pathogens and saprobes. *Entorrhiza*, a genus of *Basiodiomycota*, and a known parasite of plants in the *Juncaceae* and *Cyperaceae* families (Cannon and Kirk, 2007), was identified as an indicator taxa of the inter-ridge swale. Overall, most indicator genera were saprotrophs, but some, like the oleaginous red yeast *Rhodosporidium* (Li *et al*., 2007)*,* can also act as parasites. *Kondoa* and *Dioszega* are two genera of yeast within the *Basidiomycota*, and were indicators of the pioneer zone. *Dioszega* has previously been isolated from soils, plant leaves and roots, and may be interacting with AMF in soils (Takashima *et al*., 2001; Renker *et al*., 2004; Connell *et al*., 2010).

The fact that many EcM fungi are facultative saprotrophs could explain the presence of EcM genera (e.g. *Scleroderma*) in the coastal region, as well as the concomitant decline in saprotrophic fungi (Fig. 3 and Fig. 4) in the forested region (Tedersoo *et al*., 2003; Cullings and Courty, 2009). Through resource competition, EcM fungi may replace certain decomposers in the forested region (Leake *et al*., 2003). Interestingly, a co-dominance of saprotrophic and EcM fungi, as we observed in the dune forest, was described previously in other forested ecosystems (Leake *et al*., 2003). The decline in plant pathogens observed in the forested region can likely be explained by antagonistic interactions with EcM fungi (Fig. 3), as has been reported in other studies (e.g. Leake *et al*., 2003; Xu *et al*., 2012).

Conclusion

This study, as the first comprehensive ITS analysis of a dune ecosystem, revealed several interesting patterns in fungal community structure and the relative abundance of functional groups. We demonstrated the presence of a taxonomically and functionally diverse community across the dune sequence, including in the barren foredunes. The variations we observed in soil fungal community structure indicate the presence of fungal succession in both taxonomy and lifestyle in this sand dune ecosystem. As this system appears to be representative of other coastal sand dunes in terms of soil and environmental properties, it should provide reliable insights into the soil fungal dynamics in sand dune ecosystems. Direct testing of fungal metabolic potential and functional abilities, with measurements of enzymatic activities for example, will be required to demonstrate explicitly the functional importance of fungi.

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Supporting information

Additional Supporting Information may be found in the online version of this article at the publisher’s website:

Appendix S1. Experimental procedures.
Fig. S1. Typical profile of ecological succession in a dune ecosystem.
Fig. S2. Rarefaction curve of OTUs in each soil sample of the coastal (A), forested (B) and wetland (C) regions against the number of 454 reads excluding singletons. (D) Sampling effort as the number of OTUs against the total number of soil sample, the dashed line showing total richness extrapolation and the shaded areas representing the 95% confidence

interval. Both analyses are based on 1000 iterations of re-sampling without replacement.

Fig. S3. Mean relative abundance of the major classes of soil fungi in the different succession zones.

Fig. S4. Changes in fungal life strategies across dune succession stages. A–G show mean and standard error of the number of genera found in samples for each functional guild. Different lower case letters indicate significant differences across succession zones. H combines the abundance of all functional guilds.

Table S1. Main plant species of each succession zone based on indicator species analysis.

Table S2. Mean and standard error of topographical variables.

Table S3. Soil chemical properties with ANOVA testing.

Table S4. Putative functional assignment of fungal genera.

Table S5. Datasets used as explanatory variables in the redundancy analysis.