A native and an invasive dune grass share similar, patchily distributed, root-associated fungal communities

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Abstract

Fungi are ubiquitous occupiers of plant roots, yet the impact of host identity on fungal community composition is not well understood. Invasive plants may benefit from reduced pathogen impact when competing with native plants, but suffer if mutualists are unavailable. Root samples of the invasive dune grass *Ammophila arenaria* and the native dune grass *Leymus mollis* were collected from a Californian foredune. We utilised the Illumina MiSeq platform to sequence the ITS and LSU gene regions, with the SSU region used to target arbuscular mycorrhizal fungi (AMF). The two plant species largely share a fungal community, which is dominated by widespread generalists. Fungi detected on only one species were rare, accounting for a small proportion of the data. The SSU region recovered AMF from more samples and from more Glomeromycota lineages than ITS or LSU. A high degree of turnover among samples was observed, but there was no evidence for strong distance-decay.

Keywords

Sand dune, arbuscular mycorrhizal fungi, invasive species, host specificity, endophytes, community composition, spatial variation, Illumina MiSeq, *Ammophila arenaria*, symbiosis

1. Introduction

Many different fungi associate with plant roots, including mycorrhizal fungi, endophytes and pathogens. Their co-occurrence in root systems has been demonstrated by laboratory experiments (Lace et al. 2014; Sun et al. 2014), greenhouse investigations (Zhou et al. 2016) and field surveys (Muller & Hilger 2015; Vandegrift et al. 2015). Arbuscular mycorrhizal fungi (AMF) are relatively well characterised. Their position on the mutualism – parasitism continuum can shift with the environment (Johnson & Graham 2013). Potential benefits that AMF confer to the host plant include the provision of nutrients (Smith et al. 2011; Thirkell et al. 2015) and protection from abiotic stresses such as drought (Boyer et al. 2015) or high salinity (Estrada et al. 2013). Endophytes, typically defined as non-mycorrhizal plant occupants causing no visible disease (Schulz & Boyle 2005), are less well studied (Mandyam & Jumpponen 2014) but can also benefit their host (Hubbard et al. 2014; Murphy et al. 2015; Rodriguez et al. 2008). Endophytes can be extremely flexible in their environmental niche and function. The capacity of some endophytes to occupy both roots and insects was recently reviewed by Barelli et al. (2015) and there is evidence that horticultural pathogens can live as benign endophytes in other crops or in natural ecosystems (Malcolm et al. 2013;

Martin & Dombrowski 2015). However, many fungal plant root occupants remain poorly understood (Sieber & Grunig 2013), despite their probable importance for plant community composition (Dostalek et al. 2013; Rillig et al. 2014; Shi et al. 2016).

The role of host identity in structuring root fungal communities is unclear (Dickie et al. 2015). Field investigations suggest the effect of host species is context-specific for fungal communities, with this found to be an important control in some cases (Becklin et al. 2012; Hazard et al. 2013; Kernaghan & Patriquin 2015; Tejesvi et al. 2013; Vályi et al. 2014), but not in others (Glynou et al. 2015; Li et al. 2015; Porras-Alfaro et al. 2014; Saks et al. 2013). The phylogenetic relationships of the hosts being compared may be important, with root fungal community similarity increasing with host relatedness in grasslands (Wehner et al. 2014). This trend has also been shown for plant pathogens in general (Barrett et al. 2009; Gilbert & Webb 2007). Closely related plants may tend to share fungal communities because the host traits that influence interactions with fungi tend to be phylogenetically conserved (Wehner et al. 2014) with plant functional group and plant life form known to be potentially important for AMF community composition (Chagnon et al. 2015; Varela-Cervero et al. 2015). But even here, the evidence is contradictory, with other studies reporting increasing differences between AMF communities as host relatedness increases in some environments (Reinhart & Anacker 2014; Veresoglou & Rillig 2014) and host species differences being detected even at the level of cultivar in agricultural systems (Corredor et al. 2014; Mao et al. 2014).

Understanding host-specific relationships in fungi is particularly important in the context of invasive plant ecology. Invasive and co-occurring native plants can share fungal communities (Knapp et al. 2012) and both may benefit from fungal root occupants (Molina-Montenegro et al. 2015). However invasive plants can also impact fungal communities to the detriment of native plants (Callaway et al. 2008; Ruckli et al. 2014; Yang et al. 2014). The 'enemy release hypothesis' suggests that invasive plants flourish because, for a time at least, local pathogens do not occupy them or have a minimal impact relative to pathogens in the host's native range and/or to impacts on co-occurring native competitors (Keane & Crawley 2002). This hypothesis is supported by evidence for decreased fungal pathogen loads on plants introduced to North America compared to those in the species' native ranges in Europe (Mitchell et al. 2010). *Pinus contorta* seedlings grew better in soils from Sweden, where the plant has been introduced, than in soils from *P. contorta's* native range in Canada, due to differing biotic interactions (Gundale et al. 2014). However, common garden experiments in California found no evidence that native clover species suffered greater negative impacts from fungal pathogens than do co-occurring exotic clover species (Parker & Gilbert 2007). The invasive plant *Vincetoxicum rossicum* associates with many potentially pathogenic fungal root

occupants in Canada. Roots of plants sampled from sites invaded less than 12 years previously had fungal communities as rich as those from sites invaded around 100 years ago, suggesting pathogens can accumulate quickly on non-native plants (Day et al. 2015). Focusing instead on fungi that can associate with new arrivals, the 'enhanced mutualism hypothesis' points to occasions where invasive plants appear to receive support from beneficial microbes (Baynes et al. 2012; Sun & He 2010). The forb *Solidago canadensis*, which is invasive in China, benefitted more from soil micro-organisms in greenhouse experiments than the native grass *Stipa bungeana* (Sun & He 2010) and *Triabica sebifera* seedlings had higher levels of AMF colonisation in their introduced range in the United States than in their native range in China (Yang et al. 2015). However a meta-analysis by Bunn et al. (2015) found that, in general, invasive plants do not experience greater benefits from mutualists than native plants.

Ammophila arenaria (L.) Link is an important sand dune stabiliser in its native Europe (Huiskes 1979), and was moved to the United States for erosion control more than a century ago (Lamson-Scribner 1895). The species has been established in Humboldt Bay, Northern California, where it has become invasive, since at least 1901 (Buell et al. 1995). Here, it co-occurs with the grass *Leymus mollis* (Trin.) Pilg., which is native to Asia and North America (Barkworth et al 2007). *L. mollis* is well adapted to coastal dunes being tolerant of burial, salt spray and high substrate salinity (Gagné & Houle 2002) but has been widely displaced by *A. arenaria* on the west coast of the United States (Pickart & Sawyer 1998). Soil feedback experiments in the greenhouse suggest that *A. arenaria* has not benefitted from release from soil enemies in this region (Beckstead & Parker 2003). Seedling growth was lower in non-sterilised than in sterilised soil, and multiple potential pathogens were isolated from them. Recent efforts using culturing indicate that *A. arenaria* shares a common root endophyte community with co-occurring dune grasses (David et al. 2015). However there are no studies examining the whole fungal community associated with *A. arenaria* roots and co-occurring dune plants. There are few studies of endophytes in non-agricultural herbaceous plants (Sieber & Grunig 2013) and little work to date using next generation sequencing to study the mycobiota of invasive plants (Coats & Rumpho 2014).

Next generation sequencing is becoming a vital tool in microbial community profiling, rapidly revealing the complexity of plant microbiomes (Schlaeppi & Bulgarelli 2015). It enables detection of non-culturable fungi and permits the simultaneous screening of large numbers of samples (Lindahl et al. 2013). The phylogenetic groups detected will, to some extent, be influenced by the gene region that is amplified (Tedersoo et al. 2015). Long favoured for its relatively accurate taxonomic resolution to species level, the internal transcribed spacer (ITS) is the official barcode region for fungi (Schoch et al. 2012) and the best

represented in public databases (Brown et al. 2014). The large sub unit (LSU) region, however, is useful for resolving deeper taxonomic relationships (Porter & Golding 2012), may detect a wider range of taxa, and can also be easier to amplify (Bonito et al. 2014, first author, pers. obs.). The small subunit (SSU) region is commonly used for AMF, and the majority of AMF sequences in public databases are generated with SSU primers (Öpik et al. 2014). Sequencing multiple gene regions may provide a more complete community picture than one region alone.

Here we use Ilumina MiSeq next generation sequencing of the ITS, LSU and SSU rDNA genes to examine the fungal communities in the roots of *A. arenaria* and *Leymus mollis* where the plants co-occur in an extensive foredune at Humboldt Bay, northern California. In addition to comparing the fungal communities in these two plant species and the performance of the alternative gene regions, this study examines distance-decay effects in compositional turnover across the site. Measurements of pH were also taken at each sample point as pH has previously been found to co-vary with fungal communities in dunes (David et al. 2015; Geml et al. 2014).

2. Materials and methods

2.1 Study site

Root samples were collected from an established foredune section immediately north of the Lanphere Dunes in the Humboldt Bay National Wildlife Refuge, California, during June 2013. While dune restoration activities have been carried out in adjacent areas since the 1980s, the section sampled had not been subject to such management. The seaward face of the foredune was dominated by *Ammophila arenaria*, but also included *Leymus mollis*. The native grass occurred in patches of '*Leymus mollis* Herbaceous Alliance' (for a description of this vegetation type see California Native Plant Society 2015), scattered among large blocks of dense *A. arenaria,* and with *A. arenaria* in small incipient foredunes below the main dune face. *Lupinus arboreus* Sims. and *Carpobrotus chilensis* (Molina) N. E. Br. were occasionally present. The mean annual precipitation for the area is 1,157 mm, the mean annual low is 7.9 ^{o}C and the mean annual high is 14.6 ^{o}C (PRISM Climate Group 2016).

2.2 Sample collection

Samples were collected along 2.26 km of the foredune face, between the dune foot and crest, with the maximum sampled vertical distance from the dune foot being 15 metres. We identified 19 locations, separated from each other by a minimum of 50 metres, where *A. arenaria* and *L. mollis* co-occurred. The

locations varied in size. A single sampling point was randomly selected within each of the 19 locations which extended less than two metres along the dune face, two sampling points were used for locations between two and ten metres long, and three sampling points were selected where locations extended for more than ten metres. Where multiple samples were taken at a location, the sampling points were at least two metres apart. There was at least one stem of each of the sampled plant species at each sampling point, with the stems of the two species being no more than one metre apart, and with no alternative plant species growing in the immediate vicinity. The position of each location was recorded at the first sampling point, using a Garmin GPS. In total we collected 72 samples (36 per species) from 36 sampling points. At each sample point, we removed sand with a trowel and collected the first roots revealed from each species, with roots being traced back to the parent stems to confirm species identity. The roots were located between 6 cm and 50 cm beneath the surface and a maximum of approximately 30 cm of root length was collected per sample. Samples were stored at 4^{o}C for a maximum of ten days. Approximately 15 cm of root length per sample was cleaned by shaking in 4% tween 80, before a sterile water rinse. We chopped the cleaned roots under sterile conditions and dried them at room temperature in a vacuum in 2ul centrifuge tubes. Sand samples were also collected from alongside each root sample. Measurements for pH were taken from 5g of sand in 10mls of KCL using a pH meter. All sand samples were combined and analysed for total C, total N (with the Leco® test), P, K, Ca, Mg, Mn and Zn (with the Mehlich 1 test) at the Center for Applied Isotope Studies, University of Georgia, USA.

2.3 DNA extraction and high throughput sequencing

Dried roots were ground in a SPEX CertiPrep 2000 Geno/Grinder. DNA extractions were based on the CTAB method (Gardes & Bruns 1993) and performed as follows. The samples were first soaked overnight in 850 µL CTAB buffer at 4 ^{o}C before being heated for an hour to 65 ^{o}C. An equal volume of 24:1 of chloroform:iso-Amyl was then added. DNA precipitation following centrifugation was performed with 1.5 volume of chilled isopropanol before incubation for one hour at -20 ^{o}C. The DNA pellet then obtained following further centrifugation was re-suspended in 500 µL of TE buffer, before 50 µL of 3M Na acetate and 500 µl of chilled isopropanol were added. Samples were chilled for 20 minutes at -80 ^{o}C before pellets were again obtained by centrifugation. These were washed twice in chilled 80% ethanol and the DNA was eluted in 50 µL of water.

A three step PCR modified from Lundberg et al. (2013) was used for DNA amplification. For the first step, the ITS region was first amplified with the primers ITS1f (Gardes & Bruns 1993) and ITS4 (White et al. 1990), and the LSU region with the primers LROR (Moncalvo et al. 1995) and LR3 (Vilgalys & Hester 1990).

Primers NS1 and NS4 (White et al. 1990) were initially used to provide a template for later amplification of the SSU region. The first PCRs were carried out in 12.5 µL reactions and included 0.4 µL (10 µm) of each primer, 2 µL (1mM) of DNTP, 1.25 µL of BSA (10mg/ml), 0.0625 µL of Qiagen® *Taq* DNA polymerase, 1.25 µL of Qiagen® 10X buffer with 15mM MgCl2 and 2.5 µL of undiluted DNA. Thermocycler protocols were, for ITS, initial heating to 95 °C (10 mins), 10 cycles at 95 °C for one min, 50 °C for one min, 72 °C for one min; and 72 °C for ten mins. LSU protocols were initial heating for five mins at 95 °C, 10 cycles at 95 °C for one min 30 secs, 60 °C for one min 30secs, 72 °C for one min 30 secs; and 72 °C for ten mins. NS1/NS4 protocols were initial heating for three mins at 94 °C, 10 cycles at 94 °C for 30 secs, 50 °C for one min, 72 °C for one min 20 secs; and 72 °C for ten mins. In the second step, primer constructs were used which included a frameshift section (six per gene region), a linker section recognised in the third step, and gene specific sequences which continued to amplify the target regions. These sequences were as above apart from the SSU region which was amplified with NS31 (Simon et al. 1992) and AML2 (Lee et al. 2008). Each 25 µL reaction contained 5µl of DNA from step one, 0.8 µL of each primer, 4 µL of DNTP, 2.5 µL of BSA, 0.125 µL of Qiagen® *Taq* DNA polymerase, 2.5 µL of Qiagen® 10X buffer with 15mM MgCl2 (concentrations as above). The thermocycler protocols were as above for ITS and LSU, but 20 cycles were performed. For SSU, they were initial heating for five mins at 94 °C, 20 cycles at 94 °C for 30 secs, 58 °C for one min, 72 °C for one min; and 72 °C for ten mins. The third step ligated MID tags, incorporating a linker region recognising that used in round two and Illumina adaptor sequences. This time 25 µL reactions were used which contained 0.5 µL of each primer, 4 µL of DNTP, 1 µL of BSA, 0.125 µL of Qiagen® *Taq* DNA polymerase, 2.5 µL of Qiagen® 10X buffer with 15 mM MgCl2. Concentrations were as for step one apart from the primers which were diluted to 5 µm. The thermocycler protocol was initial heating to 95 °C (ten mins), 5 cycles at 95 °C for one min, 63 °C for one min, 72 °C for one min 20 secs; and 72 °C for 10 mins. Final operations were undertaken at the University of North Carolina's High-Throughput Sequencing Facility. Amplicon cleaning, size selection and purification were performed using magnetic beads. Quantification was done using a LabChip® GX. Normalised libraries were pooled on a Tecan robotic workstation. Paired-end 2 × 300 bp sequencing was performed with an Illumina® MiSeq instrument. Sequence data has been deposited in the NCBI Sequence Read Archive (SRA), the accession number is SRP080210.

2.4 Bioinformatics

Bioinformatics was undertaken with sequences from the forward direction only. We used the general approach of Andrei et al. (2015). Sequences were demultiplexed in QIIME v 1.8.0 (Caporaso et al. 2010)

using the split_libraries_fastq.py script with quality checking steps disabled. All python scripts are from QIIME unless otherwise stated. Sequences were sorted by gene region and primers were removed using cutadapt v1.7.1 (Martin 2011). The UPARSE pipeline introduced by Edgar (2013) was then largely followed; all options prepended with '-' are from USEARCH (Edgar, 2010). Sequences were trimmed to 220bp using `-fastq_filter`. This step included quality filtering, using `-maxee` 0.5. Sequences were dereplicated and singletons were removed. Operational taxonomic unit (OTU) clustering was performed with *de novo* chimera checking disabled (`-uparse_break -999`). Clustering, chimera checking and classification (`-assign_taxonomy.py`) were undertaken for each region as follows, with the minh value set following examination of alignments of sequences designated chimeric at more stringent minh values. Where classification was done using BLAST against GenBank, uncultured/environmental sample sequences were excluded from searches, and only results from published studies were used unless otherwise stated.

ITS sequences were clustered at 97% similarity and reference-based (`-uchime_ref`) chimera checking was then performed against the QIIME formatted UNITE database v.7 dataset 'uchime_sh_refs_whole_ITS.fasta' (Abarenkov et al. 2010), with '`-minh` 0.5'. OTUs were classified in QIIME (Caporaso et al. 2010) via `-assign_taxonomy.py` against the UNITE reference database v.7 dynamic data set (Abarenkov et al. 2010). OTUs classified as non-fungal (2) or with no BLAST hit (18) were checked with BLAST against GenBank. They were retained in the dataset as fungi where there were matches with sequences from fungi with coverage of at least 50%, and identity matches of at least 75% (and where there were no better matches with non-fungal organisms). OTUs with no class assigned (41) or classified to class by matches with uncultured sequences (9) were also checked against GenBank. This identified further OTUs that were determined to be non-fungal and were removed from the dataset. Classes were assigned where matches with coverage of at least 85% and identity matches of at least 90% were attained. Sequences identified as Glomeromycota were also checked against GenBank and retained in this class where coverage and identity matches of 70% and 80% were obtained; their top listed matches are reported. The number of OTUs per class, and classified as Zygomycota *incertae sedis*, was then calculated. The top 20 most frequently sequenced OTUs were queried against GenBank with BLAST. The top listed match named to species by the depositors (and where a better published match of an alternative genus was not available), with at least 50% query cover attained, was recorded. In two instances an unpublished match was selected as it was generated by one of the authors. The UNITE species hypothesis (Kõljalg et al. 2013) for OTU matches of 98% or better was recorded. The frequency of these OTUs as a

proportion of the total sequences was calculated. The top listed GenBank matches named to the level of genus or species for putative Glomeromycota were recorded.

LSU sequences were clustered at 98% similarity, because of the more conserved nature of this gene region (Porter & Golding 2012). Reference based chimera checking (with '`--minh` 1.28') was performed against an in-house developed QIIME formatted SILVA LSU v119 database (Pruesse et al. 2007), that contained sequences clustered at 99%. Classification was undertaken using RDP classifier (Wang et al. 2007) with the confidence threshold set at 50% as is recommended for short sequences, otherwise default settings were used. OTUs classified as non-fungi (290) were queried against GenBank with BLAST, and retained in the dataset with a class assigned, or removed, as per the ITS criteria. An additional 50 OTUs classified as fungi with a confidence level of less than 90%, were also checked against GenBank. Eight were removed as their best GenBank matches, in terms of coverage and identity, were with non-fungal organisms. The top 20 most frequently sequenced OTUs were identified as for the ITS region, but matches to genus were accepted. Where an equally good match could have been made with a sequence from a different genus or family, the lowest level classification shared between these different GenBank matches is given. Where this was incongruent with the order for the given name, N/A was recorded for order. The frequency of these OTUs as a proportion of the total sequences was calculated. The classification of the 21 OTUs which had been assigned a class by RDP classifier with a confidence level of 70% - 72% was checked against GenBank, and determined to be correct to the level of class. All OTUs classified by RDP as Glomeromycota were checked against GenBank and retained as such where the class was confirmed by GenBank as above. Otherwise OTUs with classifications with confidence levels below 70 for class were removed from the dataset used for determining richness per class, as their classification to this level was not found to be consistently correct. The total number of OTUs per class, or classified as Zygomycota incertae sedis, were calculated. OTUs in classes detected by LSU, but which were not recovered by ITS, were checked against GenBank and their class was confirmed or updated. The top listed match named to the level of genus or species for putative Glomeromycota on GenBank was recorded.

SSU sequences were clustered at 97% similarity. Chimera checking was performed against the SILVA SSU Ref NR v.123 database (Pruesse et al. 2007) in VSEARCH v.1.9.2 (Rognes et al. 2015) using '`--minh` 0.28'. Sequences deemed chimeric were checked against GenBank with BLAST. Most sequences were identified as non-AMF, those that were likely AMF were deemed so as they had 100% coverage with other AMF sequences and identity matches of at least 98%, this also suggests they are non-chimeric, and they were retained in the dataset. Initial classification of all OTUs was then performed with SINA v.1.2.11 (Pruesse

et al. 2012). Unclassified OTUs were checked by BLAST against GenBank, seven of these were deemed likely to be AMF, which was further confirmed by phylogenetic placement. This was performed using the sequences from Fig.2 in Krüger et al. (2012). These were aligned with our AMF OTUs using MAFFT v.7.017 (Katoh et al. 2002) in Geneious v.8.0.5 (Kearse et al. 2012), with *Mortierella verticillata* (GenBank #AF157145.1) and *Umbelopsis ramanniana* (GenBank #X89435.1) downloaded for use as outgroup species. These are in sister clades to the Glomeromycota (Lin et al. 2014; Tisserant et al. 2013). Ambiguous regions were manually edited. Geneious was used to build a maximum likelihood tree with PhyML (Guindon & Gascuel 2003) using the GTR substitution model, to select Krüger et al. (2012) sequences for the final tree. To simplify the final tree, the closest match to each OTU was retained, remaining sequences were randomly removed with, where possible, a minimum of two sequences per genus with no matching OTU retained. BLAST searches were conducted against the MaarjAM database (Öpik et al. 2010) for each of our AMF OTUs, the top listed match was added to the dataset. All sequences were aligned as above. A Bayesian inference tree was built using MrBayes v.3.2.6 (Ronquist & Huelsenbeck 2003) in the CIPRES portal (Miller et al. 2010); 10 million generations were run, otherwise default settings were used.

2.5 Statistical analyses

We examined whether sequencing depth was sufficient to capture fungal community richness by generating alpha rarefaction plots via QIIME. Sampling adequacy was calculated using the specaccum function in the R library vegan (v. 2.3-4; Oksanen et al. 2016) using R 3.2.0 (R-Development-Core-Team, 2015). The dataset was then rarefied to an even depth of 950 sequences per sample for the ITS region and 3000 sequences per sample for the LSU region. Insufficient sequencing depth meant that seven *A. arenaria* and two *L. mollis* samples were discarded for ITS, and two *A. arenaria* and four *L. mollis* samples for LSU. Because sequence numbers were highly divergent between samples for SSU with many samples having small numbers, a non-rarefied OTU table was converted to relative abundance values. We ordinated the fungal community data using non-metric multidimensional scaling (nMDS) on untransformed data, on data subject to a 4^{th}-root transformation, and on untransformed data containing only OTUs which occurred in more than five samples. We used the Bray-Curtis metric as our dissimilarity measure (Faith et al. 1987). To assess whether the fungal communities associated with each species differed in their location (polygon centroid) in the ordination space we used permutational multivariate ANOVA (PERMANOVA; Anderson 2001) via the `adonis` command in vegan. We also evaluated whether the fungal communities differed in their spread across ordination space via a multivariate analysis of homogeneity of dispersion (Anderson 2006) with the `betadisper` command in vegan. The influence of

pH on fungal community was visualised using a smoothed surface fit to the ordination using generalised additive models (`ordisurf` command in vegan using 10 knots). As the outcomes of the ordination-based and distance analyses were qualitatively the same irrespective of the transformation used, we report only the 4^{th}-root transformation in the results. For the ITS and LSU regions the similarity of host species pairs taken at each sample point was examined using a Monte Carlo permutation method in which the median dissimilarity between the 18 pairs was compared against a distribution of the median of pairs drawn at random from the total pool (based on 1×10^4 medians of 18 non-adjacent pairs selected at random without replacement). To assess the extent of fine-grained spatial turn-over using the LSU data-set we estimated a distance-decay curve (i.e. a curve showing the decline in community similarity as geographic distance between communities increases) using a log-linear binomial generalised linear model (GLM) following the approach described in Millar et al. (2011). For this analysis, we used only points where GPS co-ordinates had been taken and that had sufficient data to be retained after rarefying.

3. Results

3.1 Sampling, PCR and Illumina MiSeq sequencing

DNA was successfully amplified from 33 *A. arenaria* samples and 34 *L. mollis* samples for the ITS region. The ITS1f primer generated 154,804 sequences that passed quality control criteria. These sorted into 310 OTUs, 291 of which were classified as fungi. Collectively these contained 153,433 sequences, 56,275 generated by *A. arenaria* samples, which clustered into 222 fungal OTUs and 98,234 generated by *L. mollis* samples, which clustered into 252 fungal OTUs. The two species shared 183 OTUs. There were an average of 2,290 fungal ITS sequences (sd 1,388) and 30 OTUs (sd 12) per sample. On average, each OTU was obtained from 7 samples (sd 9.6).

A total of 34 *A. arenaria* and 35 *L. mollis* samples amplified for the LSU region. LROR returned 437,484 quality controlled sequences, which sorted into 1667 OTUs; of these 1,401 were classified as fungi and they contained 385,076 sequences. *A. arenaria* samples generated 183,328 fungal LSU sequences clustered into 1,135 OTUs and *L. mollis* 201,748 sequences clustered into 1,159 OTUs. The two host species shared 893 LSU fungal OTUs. On average each sample had 5,581 fungal LSU sequences (sd 2,521), 123 OTUs (sd 44) and each OTU was obtained from 6 samples (sd 8).

For SSU, a total of 34 *A. arenaria* and 35 *L. mollis* samples amplified. The NS31 primer generated 109,434 sequences which sorted into 166 OTUs. Of these, 73,770 sequences which grouped into 24 OTUs were classified as Glomeromycota (AMF). *A. arenaria* produced 24,416 AMF sequences, *L. mollis* 49,354

sequences, and each had 22 AMF OTUs, 20 of which were shared with the other host plant. There was substantial variation in the number of sequences per sample for AMF as detected by SSU, with an average of 1,069 and a standard deviation of 2,003. Each sample had an average of 4 AMF OTUs (sd 3), with 34 *A. arenaria* and 28 *L. mollis* samples found to have AMF by the SSU region. Each OTU was obtained from 13 samples on average (sd 10).

The curves on the alpha rarefaction plots were almost saturated for ITS and LSU for both plant species, despite there being far fewer sequences and OTUs for ITS than for LSU. The curves were saturated for SSU (Supplementary Fig. 1). However, species accumulation curves suggest that additional samples would have recovered additional OTUs across the dune using the ITS and LSU regions (Supplementary Fig.2). The species accumulation curve for SSU was starting to asymptote.

3.2 Host species influence

The nMDS ordinations show no community separation by plant host for either the whole fungal community or for AMF (Fig.1). The lack of plant host influence is also supported by PERMANOVA analyses (for all three gene regions $p > 0.05$); there was also no evidence for the communities associated with each plant having heterogeneous variances ($p > 0.2$ for all three gene regions). Combined, these analyses suggest that the fungal communities associated with the two plant species are not statistically distinguishable. Comparing the occurrence of the top 20 most frequently sequenced OTUs across both plants combined, for ITS and LSU, by host plant also reveals that plant species has a minimal influence over fungal community composition (Supplementary Table 1). Each of these OTUs is found in both species, and the number of samples they are detected in is often similar for each plant, although the frequency of these (as a proportion of total sequences) is more variable between the hosts. While each host has unique OTUs for each gene region, these contain only a small fraction of the sequences. The 39 ITS OTUs unique to *A. arenaria* are found in, on average, only 1.2 samples (sd 0.61) and account for only 2% of the data. There are 242 unique LSU OTUs in this plant occurring, on average, in 1.6 samples (sd 0.98) and accounting for 1.7% of the data. *L. mollis* has 69 unique ITS OTUs in an average of 1.7 samples (sd 0.97), accounting for 5% of the data. For LSU, it has 266 unique OTUs in an average of 1.4 samples (sd 0.74) accounting for 3.5% of the data.

The most frequently sequenced SSU OTUs show much greater differences between the plant species both in terms of the number of samples each OTU is detected in and the frequency of these. While OTU 1 has 34% of the SSU AMF sequences for *A. arenaria* and 41% for *L. mollis*, the frequency for OTU 2 is 1.5% and

35% for these plants respectively. However, the latter number is largely due to only one *L. mollis* sample where this OTU has 10,998 sequences and it is, in fact, detected in fewer samples for this species (11 vs. 21, Supplementary Table 2). A similar trend is seen for OTU 9, with 40% and 2% of the sequences for the two plants, although this OTU is both frequently and evenly detected in *A. arenaria* samples (34 vs 6). The majority of the SSU OTUs are shared.

3.3 Spatial heterogeneity

The Monte Carlo permutation analysis shows that, on average, fungal assemblages in immediately adjacent samples (maximum distance < 1 m) are less dissimilar to each other than they are to non-adjacent samples (ITS: 0.616 vs. 0.602-**0.783**-0.939 (5-**50**-95 percentile) and LSU: 0.695 vs. 0.671-**0.835**-0.929). Likewise, the median dissimilarity between adjacent samples is much lower than the median of randomly drawn non-adjacent pairs (ITS: 0.616 vs. 0.735-**0.784**-0.828 and LSU: 0.695 vs. 0.798-**0.835**-0.866) (Fig.2)., However, there is no relationship between the compositional similarity of a pair of samples and the geographic distance separating them (i.e. no evidence for any distance-decay effect), with β (the slope of the log-linear binomial regression) not significantly different from zero for either host species (Fig. 3).

3.4 Soil measurements and pH influence

Based on the fit of a spline surface to the nMDS ordination the association between fungal community composition and pH is not significant for the ITS data (the pH term in the smoother has an approximate *p*-value of 0.061) but is significant for LSU (approximate *p*-value < 0.001, Fig.4). There was little variation in pH and only six samples had pH values of less than 6.4 but a trend for samples to separate by pH value is apparent, particularly for LSU. Soil nutrient levels are low (Table 1).

3.5 Community diversity and structure

The ITS region together with our classification approach detected 13 taxonomic classes (including 'Zygomycota *incertae sedis*') while the LSU region detected 18 classes (Fig. 5). Our ITS methods detected only one class missing from the LSU dataset, Orbiliomycetes. This omission is likely to be a result of misclassification by RDP. At least two OTUs classified as Lecanoromycetes, removed from the 'class' dataset due to that classification having a confidence level of only 14%, were found by GenBank matches to be Orbiliomycetes. The Sordariomycetes was the richest class for both ITS and LSU, containing 92 and 760 OTUs respectively, representing 36% and 65% of the total richness by class. The richness of the Dothideomycetes was similar for ITS with 67 OTUs (26% of the total) but heavily reduced for LSU with 149

OTUs (13% of the total). Glomeromycete richness was reported as similar by ITS and LSU, with 26 and 25 OTUs respectively (Supplementary Table 3).

The two most frequently sequenced ITS OTUs, each with around 12% of the data, found 100% matches for both query cover and identity with *Plectosphaerella cucumerina* and *Microdochium bolleyi* respectively (Table 2). The 20 most frequently sequenced ITS OTUs account for 76% of the data. The majority of these OTUs are ascomycetes and there are three basidiomycetes. Three OTUs have identity matches poorer than 90%. The most dominant class is Sordariomycetes, with at least 11 OTUs. There are at least ten orders.

The 20 most frequently sequenced OTUs for LSU account for 64% of the data, again the ascomycetes dominate and there are four Basidiomycetes (Supplementary Table 4). The closest match for OTU 7 is with a *Glomus* sequence, although it is poorly matched with query cover and identity match scores of 80% and 81%. This is the only 'frequently sequenced' identity match found below 94% for LSU. The Sordariomycetes again dominate with 11 OTUs, and there are at least 12 orders. There are 11 OTUs which found alternative, equally good matches as those given and ten of these matches found agreement with those listed only at the level of family or higher. There is less dominance by the two most frequently sequenced OTUs, these contain 7% and 6.4% of the data, and belong to the Helotiaceae and Hypocreales.

The ITS region detected Glomeromycota sequences in 25 samples while the LSU detected AMF in 46 samples. SSU detected AMF in 62 samples. The GenBank matches suggested all the AMF detected by ITS were from the Glomeraceae family and represent only two genera. Eighteen of these had identity matches of 95% or better (Supplementary Table 3). The LSU recovered greater richness, with an additional two families and 5 genera, 21 identity matches of 95% or better were found. In addition to recovering AMF from the largest number of samples, SSU recovered the greatest richness, with six families and nine genera identified for this region (Fig.6). These have broad phylogenetic spread. The Glomeraceae family was again the most diverse for SSU, with 10 OTUs, and also contained the top two most frequently sequenced OTUs (Supplementary Table 2). One of these was closely matched with *Rhizophagus fasciculatus* on the phylogenetic tree and had 39% of the SSU AMF sequences; the other was closely matched with *Glomus macrocarpum* and had 24% of the sequences. The top five most frequently sequenced OTUs accounted for 94% of the data. The phylogenetic tree, with 100% statistical support, places five OTUs alongside their MaarjAM matches in a clade with no reference sequences from Kruger et al. (2012). This clade is named to order, as it places with other Archaeosporales. The GenBank names would indicate these OTUs belong

to the genus *Archaeospora*, but the tree suggests they are more closely related to *Ambispora* and *Geosiphon*. Most OTUs are more closely placed with GenBank sequences than with reference sequences.

4. Discussion

4.1 Host species influence

The composition of the fungal communities observed in the roots of the co-occurring invasive grass *A. arenaria* and the native grass *L. mollis* was similar. While there were OTUs unique to each host species, they accounted for a small fraction of the data and occurred in few samples. Given the variability in sample composition across the dune, these OTUs may have been missed in the alternative plant by chance. Sequence frequency cannot be taken as a direct representation of fungal abundance, particularly within samples but can be meaningfully compared across samples (Amend et al. 2010; Nguyen et al. 2015). There are differences in the sequence frequency of OTUs between the two plant species, but sequence frequency also differs hugely among samples within host species. We cannot rule out a potential impact of host species on individual fungal lineages and the nMDS stress values are high but high turnover among samples within plant species, as well as the isolation of many OTUs from both plant species, resulted in no indication, at the community level, of host species influencing community composition. Minimal plant host influence on endophyte communities has been observed for gypsophilous plants in New Mexico (Porras-Alfaro et al. 2014), rainforest plants in Borneo (Sato et al. 2015) and within semi-arid Hungarian grasslands supporting both native and invasive plants (Knapp et al. 2012). Our study compares a native and invasive plant, but both are members of the Poaceae. Other work has suggested close relatives are more likely to share fungi (Gilbert & Webb 2007; Wehner et al. 2014), although a thorough investigation of this hypothesis in this setting would have required additional sampling, from more distantly related plants on the dune. With both plant species sharing a common fungal community, it is unlikely an escape from pathogens explains the success of *A. arenaria* on the dune surveyed. Both this plant and *L. mollis* also associate with a range of AMF, at least some of which are likely to be mutualistic. It is possible, however, that the fungal communities may interact differently within the two hosts, with *A. arenaria* benefitting more from mutualists and/or suffering less disease. Inoculation experiments with endophytes on a range of grasses and forbs demonstrated that even though a wide range of hosts could be occupied, the impact of occupation differed by host species (Mandyam et al. 2012). AMF can also have different impacts on different host plants (Johnson et al. 2005) and fungi may act as pathogens or endophytes depending on the plant host (Malcolm et al. 2013). Previous work demonstrated that *A. arenaria* suffers negative soil feedback in California but did not investigate the soil relationships of *L. mollis* (Beckstead &

Parker 2003). Further investigation is needed to determine whether fungal community functions differ between the two plants. In addition, the dune surveyed was dominated by *A. arenaria*, and this plant could be influencing the fungal community in *L. mollis*. Root fungal communities can be impacted by neighbouring plants (Kohout et al. 2015; Radić et al. 2012; Toju et al. 2014). The tendency of paired samples on the dune to be more similar to each other than to those not adjacent suggests that neighbouring root sections have the potential to influence each other's fungal occupants in this environment.

4.2 Spatial heterogeneity

The composition of the fungal community varies considerably across the dune. While examining paired samples provides evidence for spatial structure (as indicated by community similarity) at distances of less than one metre, there is no statistical evidence that similarity between two samples is explained by distance beyond this threshold. The two closest GPS points are separated by approximately 25m and while we may have found evidence for distance-decay if we had measured points closer to each other, the results suggest the community is not dispersal limited at the scale of the dune. Changes in community composition occur alongside pH changes, as was seen by David et al. (2015) in their study of endophytes cultured from dune grass roots. There may be other unmeasured influential abiotic variables that explain compositional pattern on the dune, and biotic interactions can also influence fungal community composition (Werner & Kiers 2015). In addition, stochastic processes are likely to be important. Dunes are naturally disturbed environments and ephemeral habitats may preclude niche-based assembly (Powell & Bennett, 2015).

4.3 Community composition: ITS and LSU

A diverse fungal community is present in the Humboldt Bay dune. The OTU accumulation plots by sample suggest greater sampling would have uncovered even greater richness. The OTU accumulation plots by sequence depth are more difficult to interpret, given ITS produced far fewer OTUs than LSU but the curves for both regions are nearly saturated. The low sequencing depth for ITS may be due to either poor PCR or sequencing performance. At any rate there is a link between sequencing depth and OTU number, as has been directly demonstrated previously (Smith & Peay 2014). It is likely greater sequencing depth explains the greater richness, in terms of total OTU numbers and taxonomic breadth, recovered by LSU. Previous work utilising both ITS and LSU generated similar numbers of OTUs from the two regions (Bonito et al.

2014). Adequate sequencing depth is necessary to capture subtle environmental signals and this is demonstrated here, with changes in pH and fungal community composition being more apparent for LSU.

The LSU region suggests high richness in individual roots, with an average of 123 OTUs per root sample. OTU numbers must be interpreted with caution as they may not accurately represent species numbers, due to difficulties separating erroneous sequences created in the sequencing process from rare OTUs (Nguyen et al. 2015; Porter & Golding 2012). There are also differences in intraspecific genetic variation among fungi, which are not catered for by the use of a single OTU clustering value and can inflate OTU numbers (Ryberg 2015). Additionally, the use of different gene regions, laboratory practises and bioinformatics approaches make meaningful comparisons of richness between studies difficult (Lindahl et al. 2013; Meiser et al. 2014; Tedersoo et al. 2015). Nevertheless, it is notable that high richness in fungal communities in dunes has been detected previously. Using the ITS region, Geml et al. (2014) detected 1,211 non-singleton fungal OTUs from 10 composite soil samples from dunes in the Netherlands. Soil and Pinaceae root samples from a relic foredune plain in Canada generated 1,613 non-singleton fungal OTUs (Roy-Bolduc et al. 2015). High richness specifically within roots has also been reported by studies using next generation sequencing. Between 65 and 225 fungal OTUs per plant were generated by subsampled root systems of the perennial herb *Onosma echioides* (Muller & Hilger 2015). The detection of DNA, however, does not necessarily signal the presence of an active, root-inhabiting fungus. In our study, root samples were thoroughly cleaned but some particles of sand remained attached to roots, fungal material may have accompanied these. The number of fungi that associate with only outer root layers may be far greater than those which penetrate deeper (Bazin et al. 1990). The use of RNA might have suggested the active fungal community is different from that detected here (Baldrian et al. 2012).

Our study clearly demonstrates the utility of the ITS region for classification. For example, while over half the frequently sequenced OTUs for LSU found GenBank identity matches of 98% or better, many of these matched equally well with alternative, differently named sequences. This could have been due to the conserved nature of this region or to inaccurate naming. Although the ITS region does not accurately separate all species (Ryberg 2015), OTUs can be identified with more confidence. Even so, incomplete records of fungal diversity, commonly revealed by unidentifiable OTUs in next generation sequencing studies (Peršoh 2015; Ramirez et al. 2014), challenge the identification of some OTUs here. The species of even 'identified' OTUs can remain uncertain. For example, two frequently sequenced ITS OTUs, 1 and 17, are identified as *Plectosphaerella cucumerina* by different GenBank sequences with 100% identity matches. These OTUs may represent either genuinely different species within this genus, or reflect intra-

specific genetic and morphological variability (Carlucci et al. 2012). Adding further complication, most sequences in the relevant UNITE species hypothesis for the match for OTU 17 are named *Acremonium nepalense*. Culture-based work continues to be valuable in validating next generation sequencing results and improving knowledge of fungal biology. Three frequently sequenced ITS OTUs with named identity matches of 88% - 92%, and one with no named match, are 100% identity matches with sequences from dune grass endophytes cultured by David et al. (2015).

The Sordariomycete and the Dothideomycete classes contain the most OTUs. This is not surprising, given these are the third most speciose, and the most speciose, ascomycete classes respectively (Kirk et al. 2008). The UNITE species hypothesis records indicate several of the dominant ITS OTUs are geographically widespread and associate with a range of phylogenetically diverse plants, including gymnosperms as well as angiosperms, in both natural and managed ecosystems, as saprobes or pathogens. This pattern supports the possibility that many root endophytes are widespread generalists (Knapp et al. 2012). An exception among this group is the *Paneolus* species; members of this basidiomycete genus are not known to directly associate with plant roots and are saprotrophic. Also notable is OTU 14, the only member of this group belonging to the order Helotiales, which contains some of the best studied dark septate endophytes (Knapp et al. 2015). The UNITE species hypothesis associated with this OTU has rarely been detected and the only plant association listed is *A. arenaria*. This OTU may represent a dune specialist, although it has a 98% match to a sequence from an environmental sample from roots of an alpine grass (GenBank accession **#KR063522.1**).

4.4 Community composition: AMF

The SSU region recovered AMF OTUs from most root samples and reveals wide phylogenetic diversity among this group. However ITS and LSU suggested that this group has low phylogenetic diversity and is not prevalent in this environment, with only one putative AMF OTU, detected by LSU, placing among the most frequently sequenced OTUs for these regions. That the ITS region recovered similar numbers of AMF OTUs as LSU and SSU, despite failing to recover the same phylogenetic diversity, may reflect the tendency for high intraspecific variability in this gene region for some AMF (Stockinger et al. 2010; Stockinger et al. 2009). Altogether, our results suggest that the SSU region is required for accurate recovery of AMF, although deeper sequencing may have improved the AMF results for ITS and LSU. The ITS region may also have been more useful for AMF recovery if an initial step using AMF specific primers had been utilised (Kohout et al. 2015).

The high diversity and recovery rate of the Glomeraceae family seen here is typical for AMF studies, both in dunes (Johansen et al. 2015; Rodríguez-Echeverría & Freitas 2006; Yamato et al. 2012) and in other environments (Sun et al. 2015; Van Geel et al. 2015; Varela-Cervero et al. 2015). This outcome suggests this family is highly diverse, competitive in a range of environments, and/or easily recoverable. The phylogenetic tree includes both closely paired Maarjam matches, and OTUs without close matches on long branches. This is not surprising given that while the Glomeromycota are environmentally flexible and geographically widespread (Davison et al. 2015), the phylum likely contains substantial undescribed diversity (Cheeke et al. 2015; Johansen et al. 2015; Senes-Guerrero & Schubler 2015). AMF identification is further challenged by a paucity of sequences from taxonomically described species (Öpik et al. 2014). Of particular interest is the Archaeosporales *incertae sedis* clade at the base of the tree, containing five of our OTUs and their Maarjam matches but no reference sequences. This clade includes a match (GenBank #FJ194498) previously placed in an 'unknown' clade by Opik et al. (2014), which they suggest demonstrates unknown diversity above the level of genus. The extreme dominance of the AMF community by a small number of OTUs is also not unusual for studies of AMF (Dumbrell et al. 2010) but again could reflect the easy recovery of these entities, rather than their ecological dominance.

4.5 Conclusions

Our results suggest that host plant species does not have a significant influence over fungal communities in the roots of dune grasses. We also report the ability of an invasive grass to associate with a phylogenetically, and probably functionally, diverse fungal community in its new range. This community is dominated by widespread generalists but also includes diversity not represented in public databases. While pH was shown to have some correlation with community composition, there is unexplained spatial variation in community composition across the dune with high turn-over between samples not explained by distance. The results suggest that extensive sampling is needed to capture the majority of the community, and to identify determinants of community structure in this environment. Direct comparisons between the gene regions surveyed are difficult due to the poor sequencing results for ITS. However this region was the most useful for classification, while the SSU region is recommended for phylogenetically accurate and extensive recovery of AMF. Future work will examine the fungal community in *Ammophila arenaria* across a broader geographic range.

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Captions

(Figures are in caption order, tables one and two are shown last in the document)

Fig.1: Plots of fungal communities in Californian *Ammophila arenaria* and *Leymus mollis* roots, based on 4^{th}-root transformed abundance data from the ITS (A) and LSU (B) regions, and arbuscular mycorrhizal fungal communities revealed by the SSU (C) regions, using non metric multidimensional scaling. Note in all cases stress is reasonably high so caution needs to be taken in interpreting the ordination plots (Clarke, 1993)

Fig.2: Dissimilarity of communities in roots of *Ammophila arenaria* and *Leymus mollis* based on 4^{th}-root transformed abundance data from the ITS (A, B) and LSU (C, D) regions. Histograms on the left (A, C) show the distribution of dissimilarities for all non-adjacent pairs with the dots being the dissimilarities for the immediate neighbours (n = 18 pairs) with their median depicted by the vertical line. Sub-plots B and D show the median dissimilarity in pairs of immediate neighbours (vertical line) as opposed to the median dissimilarity of 18 pairs of randomly selected non-adjacent sites based on 1×10^4 resamplings.

Fig.3: Relationship between geographic distance and compositional similarity of fungal communities in Californian *Ammophila arenaria* (A) and *Leymus mollis* roots (B). The spatial structure in the fungal communities is not explained by geographic distance. The dashed lines show a fitted log-linear binomial model with β its slope (mean ± 1 SEM) estimated using a leave-one-out jackknife approach as per Millar et al. (2011)

Fig.4: Relationship between soil pH and fungal community for the ITS (A) and LSU (B) gene regions. The nMDS ordinations (Figure 1) are overlain on a smoothed surface showing variation in pH.

Fig.5: Histogram showing the proportion of fungal operational taxonomic units (OTUs) assigned to each class detected by the ITS (A) and LSU (B) regions. OTUs were derived from DNA sequences, obtained from roots of *Ammophila arenaria* and *Leymus mollis* plants.

Fig.6: BI tree based on SSU sequences, showing the phylogenetic diversity of the arbuscular mycorrhizal fungi detected in *Ammophila arenaria* and *Leymus mollis* roots. Posterior probability values greater than 90 are indicated above the branches. Sequences labelled with only an operational taxonomic unit (OTU) number and marked with a ● were obtained from the dune, sequences with GenBank accession numbers were downloaded from GenBank and represent the closest matches to our OTUs according to the Maarjam database (Opik et al. 2010), the remaining sequences are from Krüger et al. (2012). The GenBank

record names are used for the Maarjam matches, the majority of names used for the Krüger et al. (2012) sequences are as given by the authors but some have been changed as per Redecker et al. (2013), which was also referred to for the designation of families.

Supplementary Figure 1 : Rarefaction curves for fungal ITS (A), LSU (B) and SSU (C) sequences by host plant species, showing operational taxonomic unit (OTU) accumulation with sequencing depth, for *Ammophila arenaria* and *Leymus mollis* roots. Initial resampling was performed at a depth of 2000 sequences for ITS and LSU, and 250 for SSU, with sequences also accumulated at these rates for subsequent iterations.

Supplementary Figure 2: Species accumulation curves for fungal ITS (A) and LSU (B) sequences, and arbuscular mycorrhizal fungal SSU (C) sequences, showing operational taxonomic unit (OTU) accumulation with sampling depth, for combined samples from *Ammophila arenaria* and *Leymus mollis* roots. The shading indicates 95% confidence intervals around the mean, the Mau Tau estimator was used.

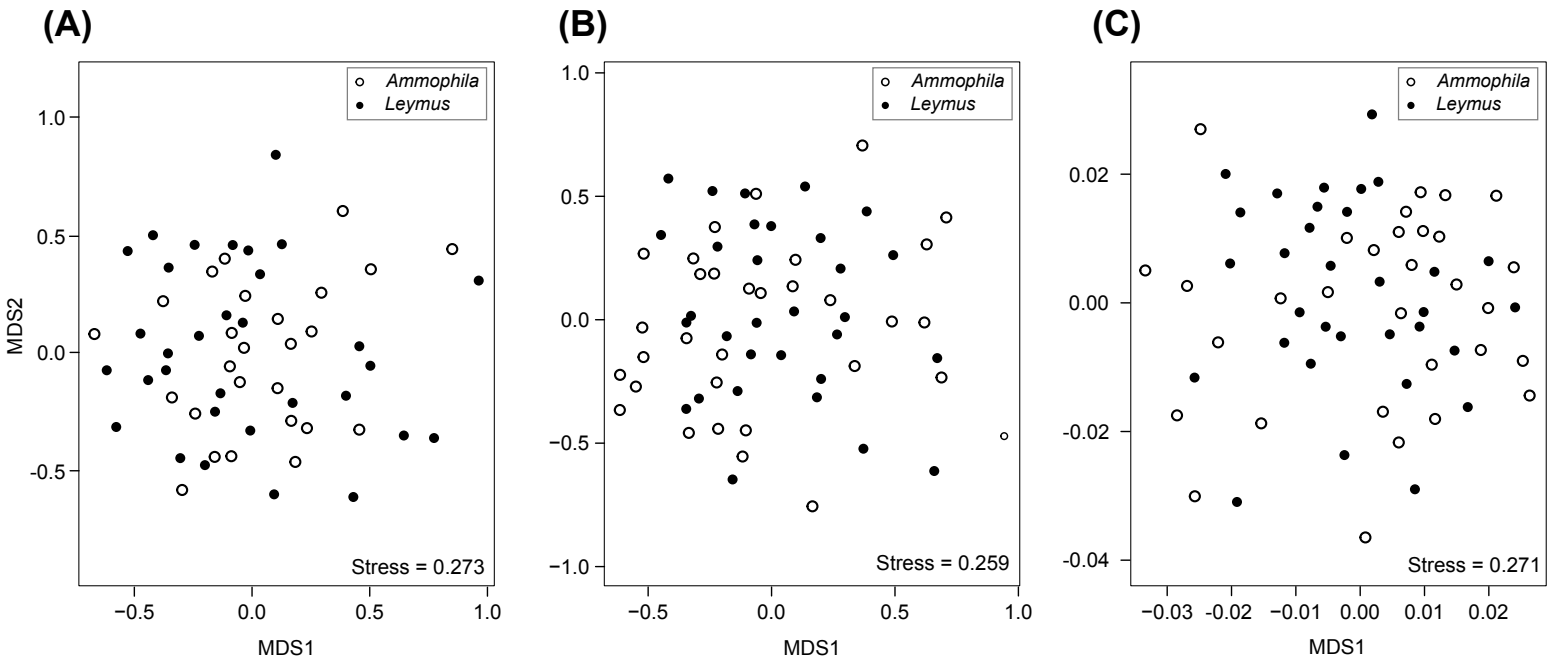
Supplementary Table 1: The top 20 most frequently sequenced operational taxonomic units (OTUs) for the ITS and LSU regions, and all arbuscular mycorrhizal fungal (AMF) OTUs for the SSU region. For each OTU the total sequences, the frequency of occurrence as a proportion of all fungal (or, for SSU, AMF) sequences, the number of *Ammophila arenaria* and *Leymus mollis* root samples it was obtained from and the frequency of occurrence in each host as a proportion of all fungal or AMF sequences in that host, is given.

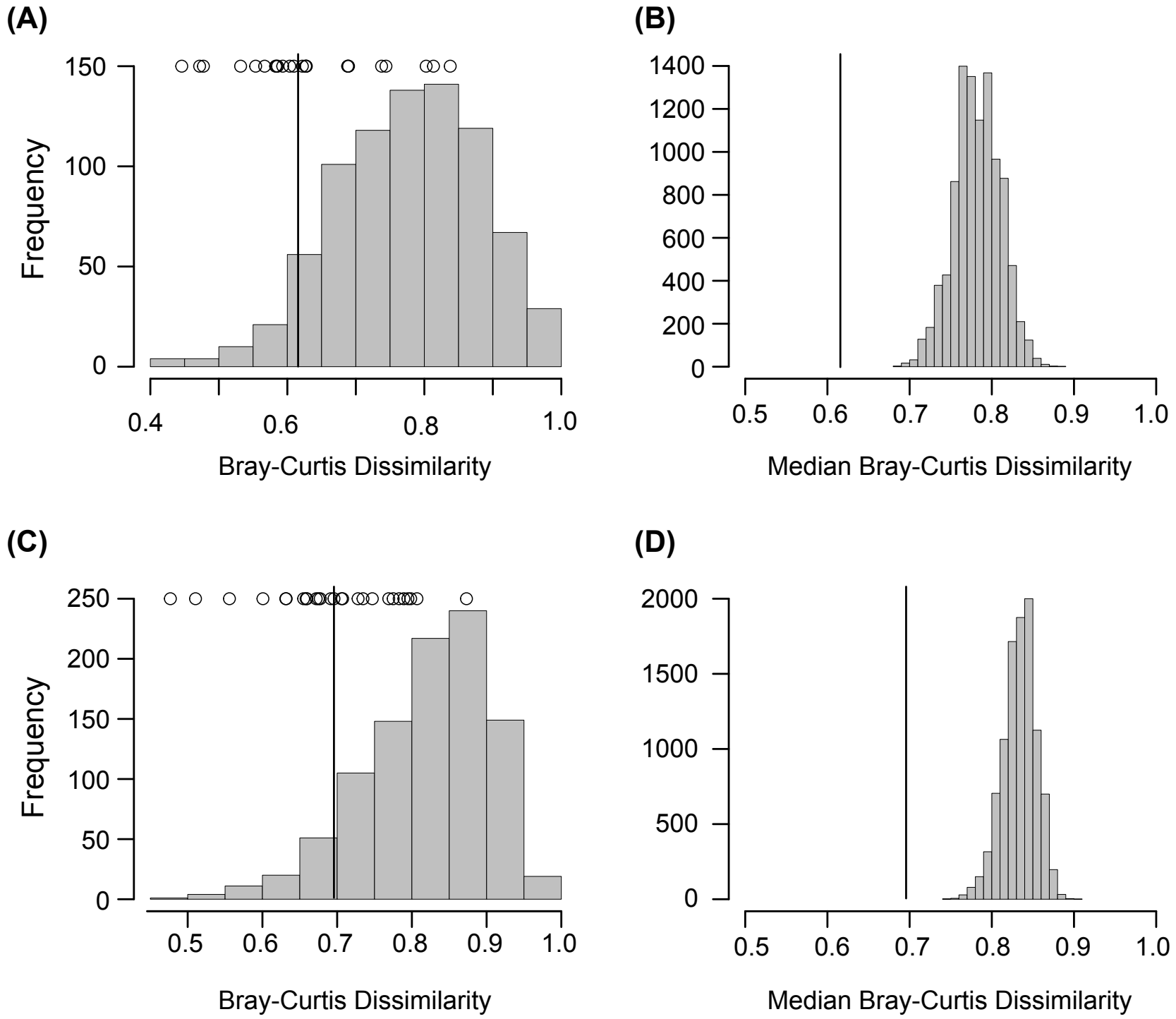
Supplementary Table 2: The number of sequences for each operational taxonomic unit (OTU) representing arbuscular mycorrhizal fungi, as detected by the SSU region, in *Ammophila arenaria* (A) and *Leymus mollis* (L) root samples. The OTUs are classified as per Fig. 6.

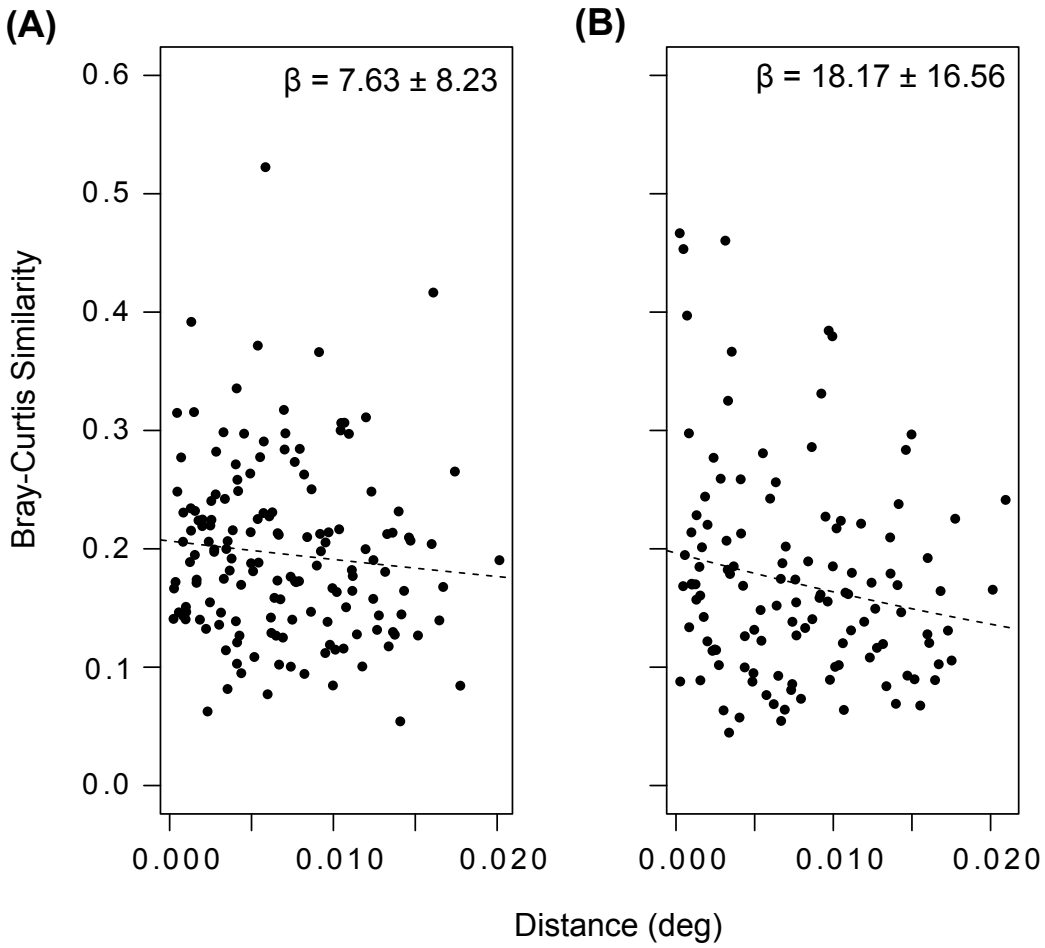
Supplementary Table 3: Operational taxonomic units (OTUs) classified as arbuscular mycorrhizal fungi which were detected by the ITS and LSU regions, from DNA sequences obtained from *Ammophila arenaria* and *Leymus mollis* root samples. The top listed GenBank match for each OTU from a published study which is named to the level of genus or species, and the query cover and identity values for that match, are given.

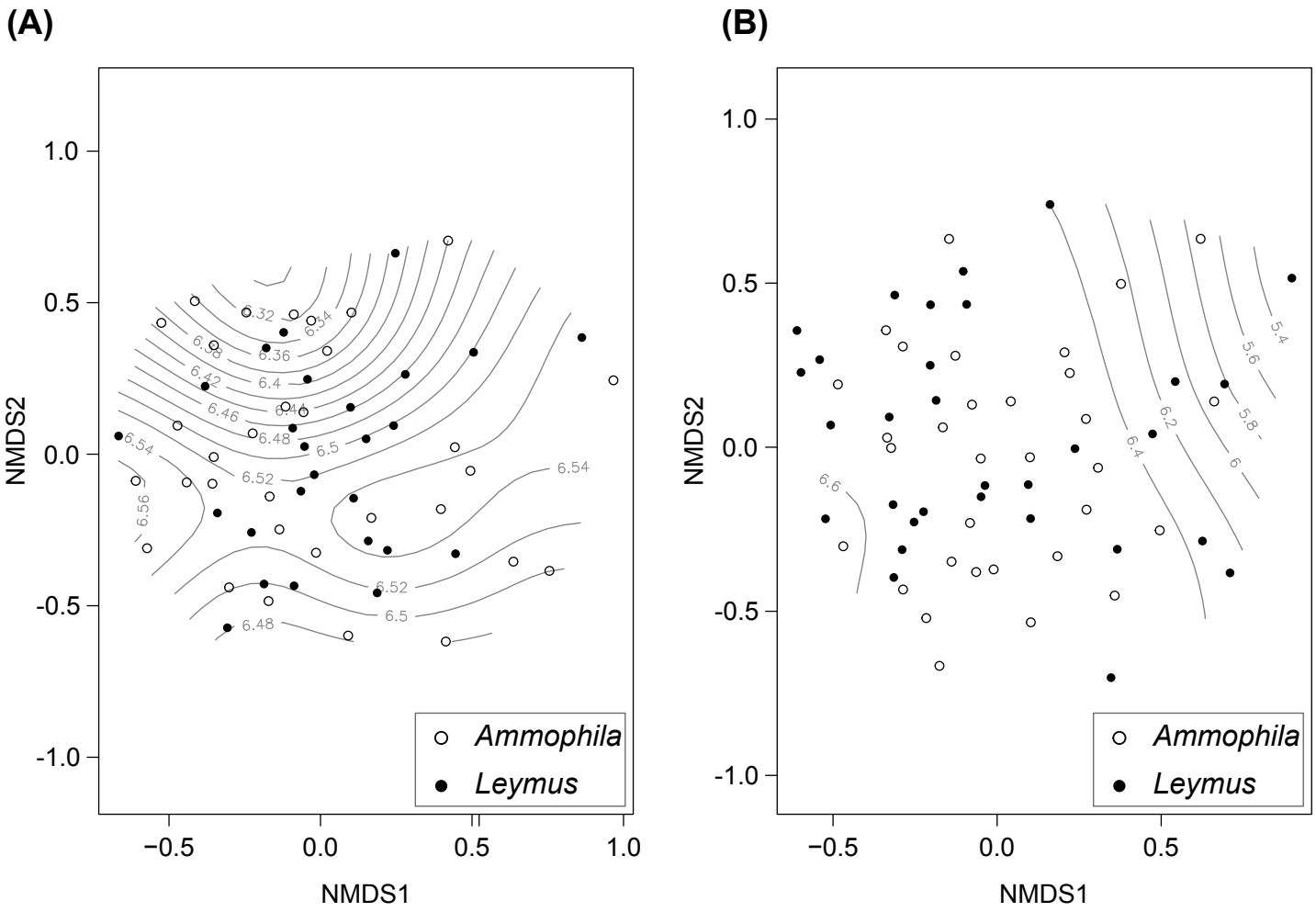
Supplementary Table 4: The top listed GenBank matches from a published study and named to at least the level of genus for the 20 most frequently sequenced operational taxonomic units (OTUs) for the LSU region. The query cover and identity values for that match are given. Where an alternative, equally good match was generated, the lowest taxonomic level at which that match agreed with the listed match is

given. Names are as given on GenBank, Class and Order (where this could be assigned and where top listed matches are in agreement) are as given on Mycobank (www.mycobank.org). The OTUs were obtained from root samples from *Ammophila arenaria* and *Leymus mollis* plants.

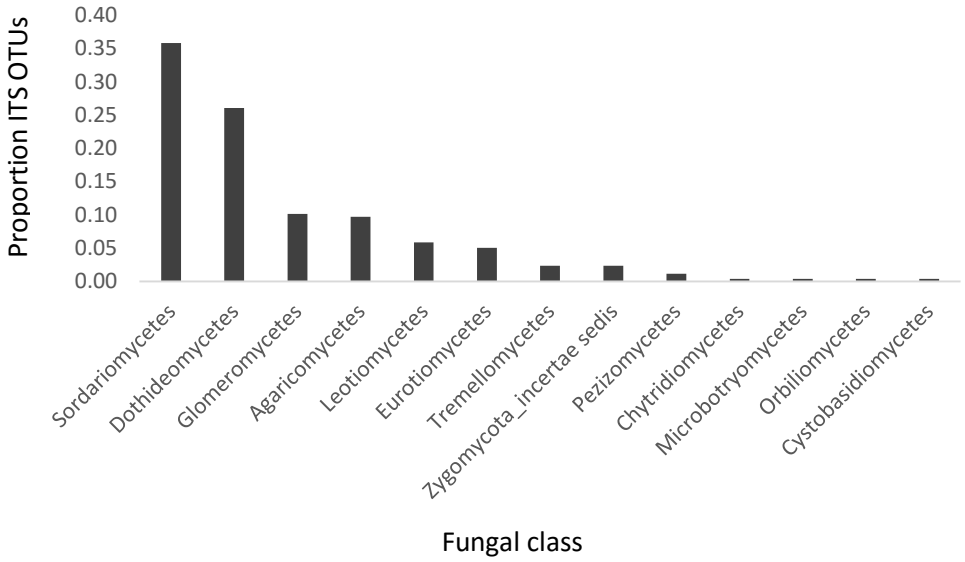

(A)
(B)
(C)
Ammophila
Leymus
MDS1
MDS2
Stress = 0.273
Stress = 0.259
Stress = 0.271


(A)
Frequency
150
100
50
0
0.4
0.6
0.8
1.0
Bray-Curtis Dissimilarity
(B)
1400
1200
1000
800
600
400
200
0
0.5
0.6
0.7
0.8
0.9
1.0
Median Bray-Curtis Dissimilarity
(C)
Frequency
250
200
150
100
50
0
0.5
0.6
0.7
0.8
0.9
1.0
Bray-Curtis Dissimilarity
(D)
2000
1500
1000
500
0
0.5
0.6
0.7
0.8
0.9
1.0
Median Bray-Curtis Dissimilarity

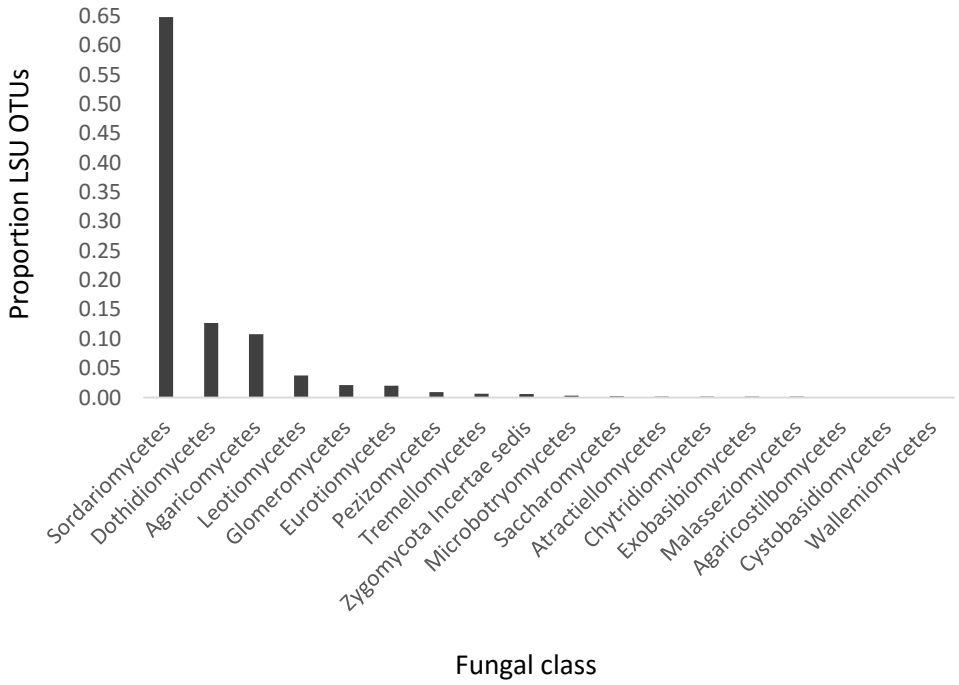

(A)
(B)
β = 7.63 ± 8.23
β = 18.17 ± 16.56
Bray-Curtis Similarity
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.000
0.010
0.020
Distance (deg)

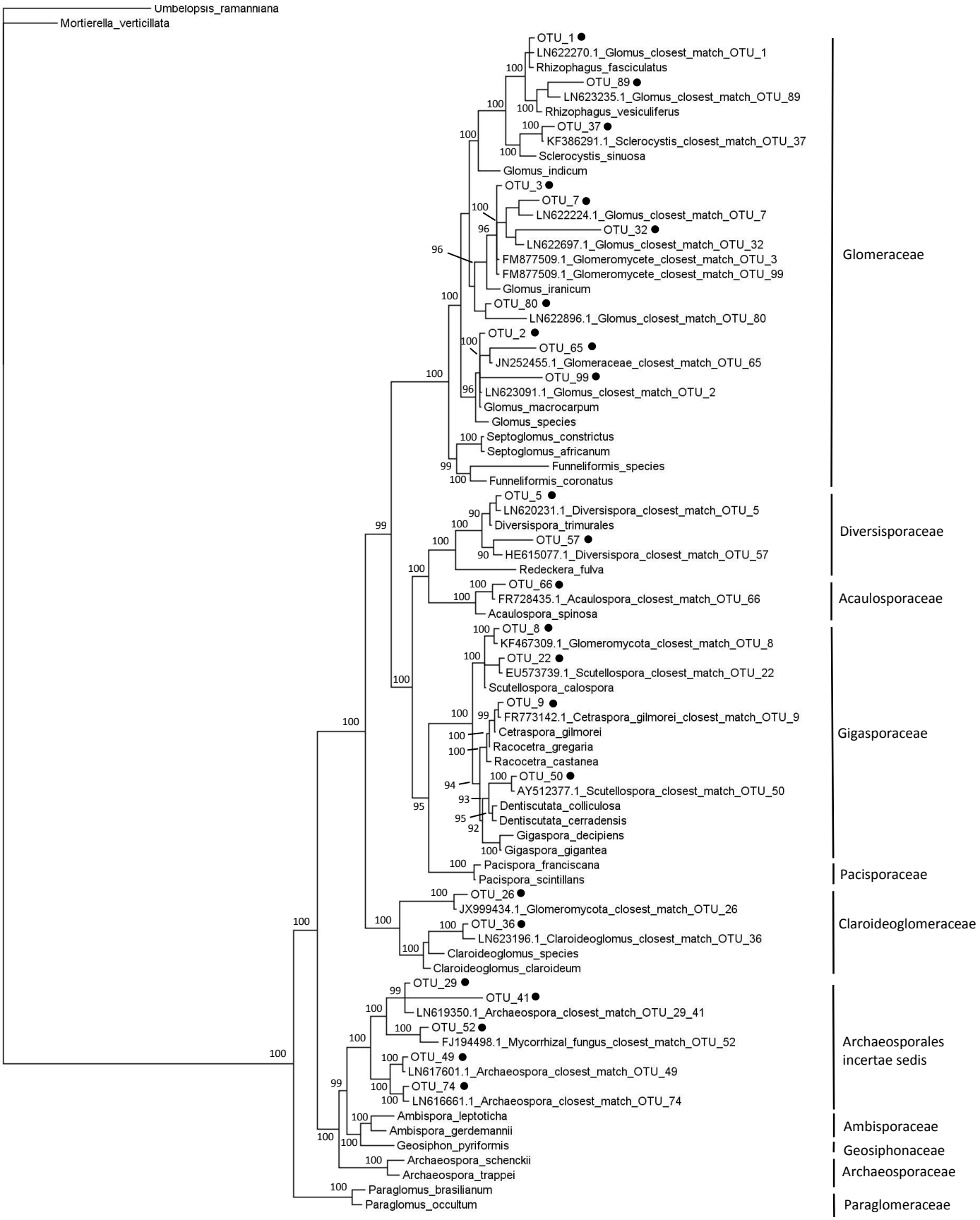

(A)
(B)
NMDS1
NMDS2
Ammophila
Leymus
6.32
6.34
6.36
6.38
6.4
6.42
6.44
6.46
6.48
6.5
6.52
6.54
6.56
5.4
5.6
5.8
6
6.2
6.4
6.6

(A)

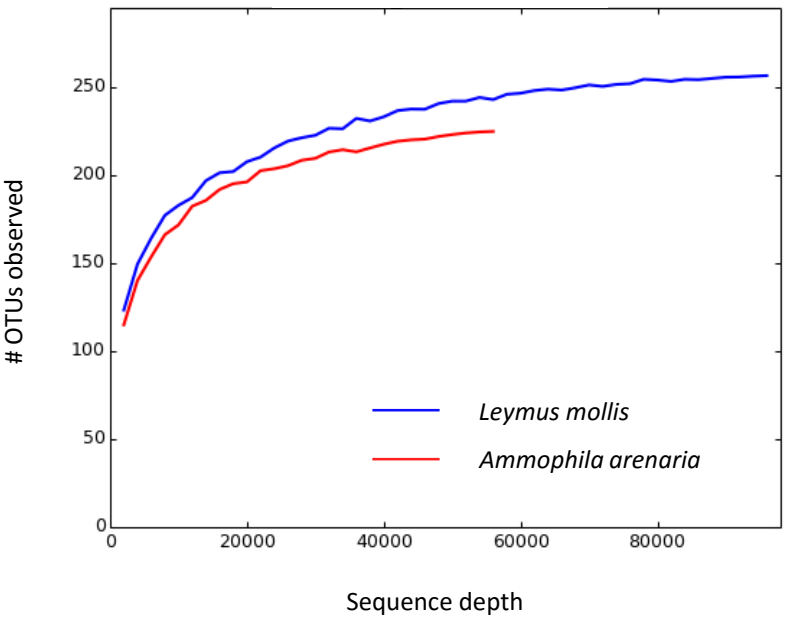

Proportion ITS OTUs
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
Sordariomycetes
Dothideomycetes
Glomeromycetes
Agaricomycetes
Leotiomycetes
Eurotiomycetes
Tremellomycetes
Zygomycota_incertae sedis
Pezizomycetes
Chytridiomycetes
Microbotryomycetes
Orbiliomycetes
Cystobasidiomycetes
Fungal class

(B)

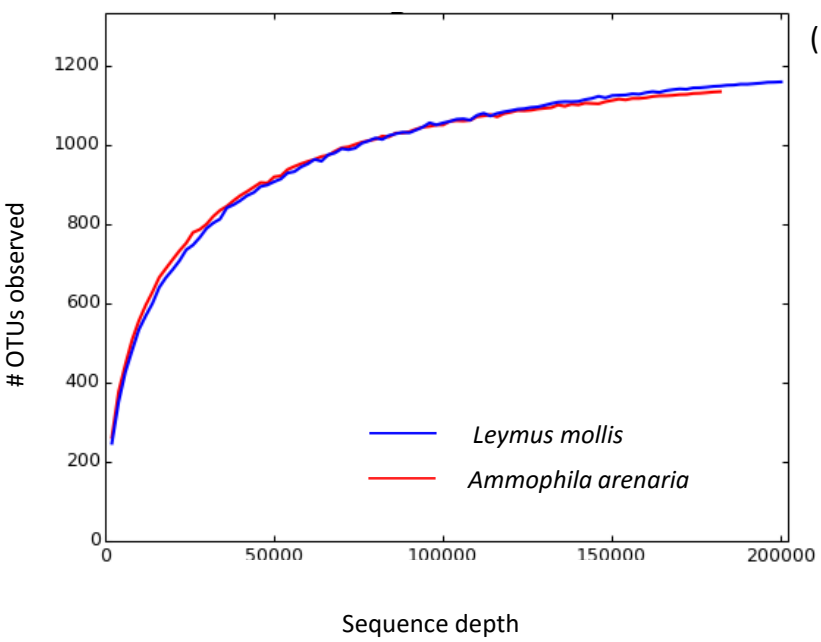

Proportion LSU OTUs
0.65
0.60
0.55
0.50
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
Sordariomycetes
Dothidiomycetes
Agaricomycetes
Leotiomycetes
Glomeromycetes
Eurotiomycetes
Pezizomycetes
Tremellomycetes
Zygomycota Incertae sedis
Microbotryomycetes
Saccharomycetes
Atractiellomycetes
Chytridiomycetes
Exobasibiomycetes
Malasseziomycetes
Agaricostilbomycetes
Cystobasidiomycetes
Wallemiomycetes
Fungal class


Umbelopsis_ramanniana
Mortierella_verticillata
OTU_1
LN622270.1_Glomus_closest_match_OTU_1
Rhizophagus_fasciculatus
OTU_89
LN623235.1_Glomus_closest_match_OTU_89
Rhizophagus_vesiculiferus
OTU_37
KF386291.1_Sclerocystis_closest_match_OTU_37
Sclerocystis_sinuosa
Glomus_indicum
OTU_3
OTU_7
LN622224.1_Glomus_closest_match_OTU_7
OTU_32
LN622697.1_Glomus_closest_match_OTU_32
FM877509.1_Glomeromycete_closest_match_OTU_3
FM877509.1_Glomeromycete_closest_match_OTU_99
Glomus_iranicum
OTU_80
LN622896.1_Glomus_closest_match_OTU_80
OTU_2
OTU_65
JN252455.1_Glomeraceae_closest_match_OTU_65
OTU_99
LN623091.1_Glomus_closest_match_OTU_2
Glomus_macrocarpum
Glomus_species
Septoglomus_constrictus
Septoglomus_africanum
Funneliformis_species
Funneliformis_coronatus
Glomeraceae
OTU_5
LN620231.1_Diversispora_closest_match_OTU_5
Diversispora_trimurales
OTU_57
HE615077.1_Diversispora_closest_match_OTU_57
Redeckera_fulva
Diversisporaceae
OTU_66
FR728435.1_Acaulospora_closest_match_OTU_66
Acaulospora_spinosa
Acaulosporaceae
OTU_8
KF467309.1_Glomeromycota_closest_match_OTU_8
OTU_22
EU573739.1_Scutellospora_closest_match_OTU_22
Scutellospora_calospora
OTU_9
FR773142.1_Cetraspora_gilmorei_closest_match_OTU_9
Cetraspora_gilmorei
Racocetra_gregaria
Racocetra_castanea
OTU_50
AY512377.1_Scutellospora_closest_match_OTU_50
Dentiscutata_colliculosa
Dentiscutata_cerradensis
Gigaspora_decipiens
Gigaspora_gigantea
Gigasporaceae
Pacispora_franciscana
Pacispora_scintillans
Pacisporaceae
OTU_26
JX999434.1_Glomeromycota_closest_match_OTU_26
OTU_36
LN623196.1_Claroideoglomus_closest_match_OTU_36
Claroideoglomus_species
Claroideoglomus_claroideum
Claroideoglomeraceae
OTU_29
OTU_41
LN619350.1_Archaeospora_closest_match_OTU_29_41
OTU_52
FJ194498.1_Mycorrhizal_fungus_closest_match_OTU_52
OTU_49
LN617601.1_Archaeospora_closest_match_OTU_49
OTU_74
LN616661.1_Archaeospora_closest_match_OTU_74
Archaeosporales incertae sedis
Ambispora_leptoticha
Ambispora_gerdemannii
Ambispora
Geosiphon_pyriformis
Geosiphonaceae
Archaeospora_schenckii
Archaeospora_trappei
Archaeosporaceae
Paraglomus_brasilianum
Paraglomus_occultum
Paraglomeraceae

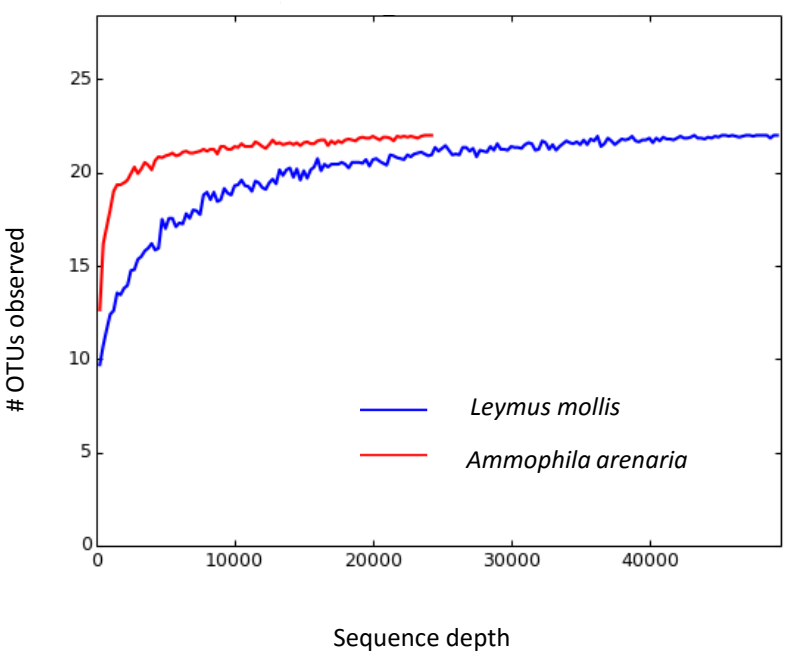
(A) ITS region

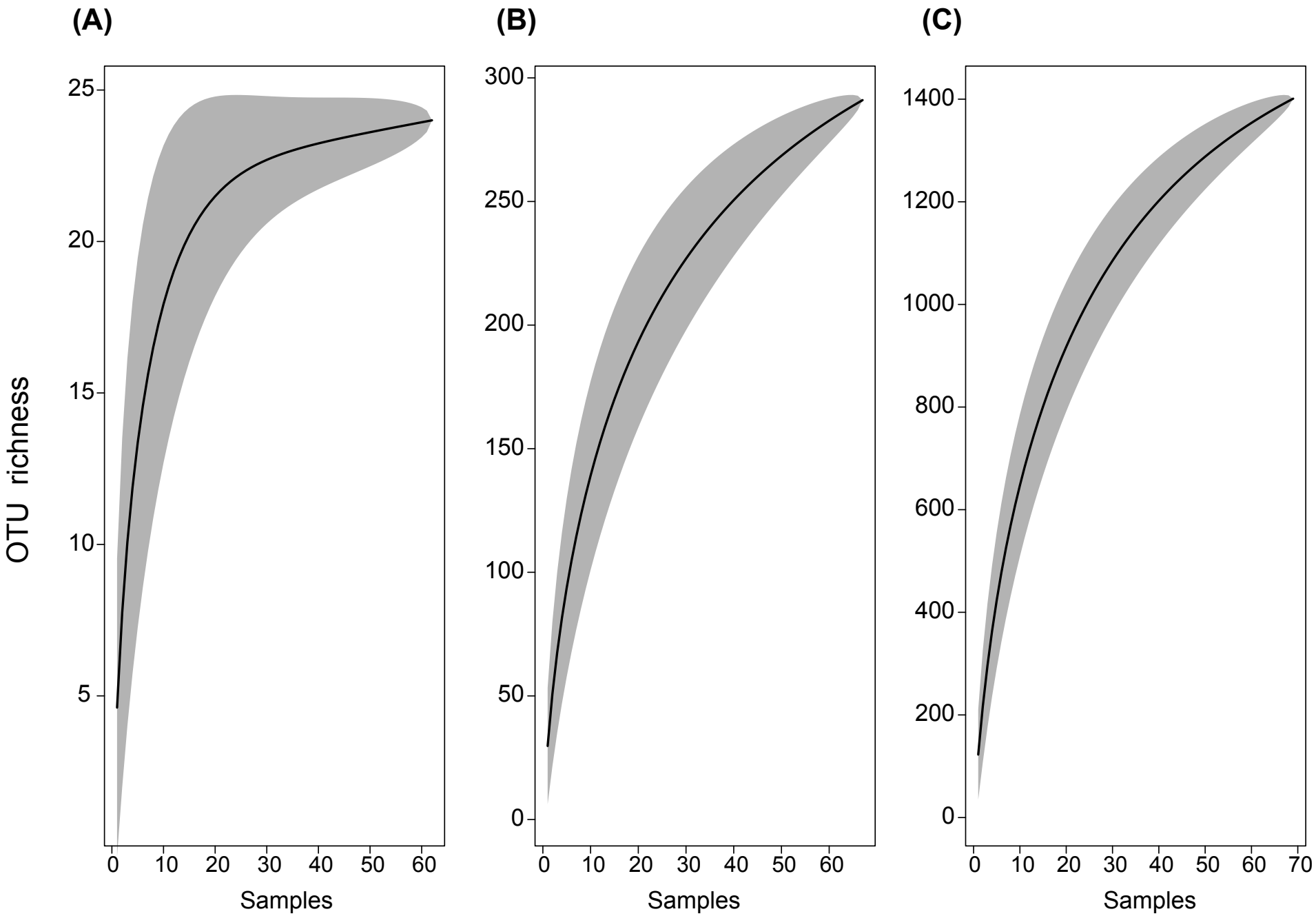


(B) LSU region



(C) SSU region




(A)
(B)
(C)
OTU richness
25
20
15
10
5
300
250
200
150
100
50
0
1400
1200
1000
800
600
400
200
0
0 10 20 30 40 50 60
0 10 20 30 40 50 60
0 10 20 30 40 50 60 70
Samples
Samples
Samples

Supplementary Table 1: The top 20 most frequently sequenced operational taxonomic units (OTUs) for the ITS and LSU regions, and all arbuscular mycorrhizal fungal (AMF) OTUs for the SSU region. For each OTU the total sequences, the frequency of occurrence as a proportion of all fungal (or, for SSU, AMF) sequences, the number of *Ammophila arenaria* and *Leymus mollis* root samples it was obtained from and the frequency of occurrence in each host as a proportion of all fungal or AMF sequences in that host, is given.

OTU ID #	Total seqs	Frequency (%)	Total *A. arenaria* samples	*A. arenaria* frequency (%)	Total *L. mollis* samples	*L. mollis* frequency (%)
ITS						
1	19118	12.46	21	11.83	26	12.67
2	18150	11.83	23	14.01	19	10.45
3	13689	8.92	28	7.77	30	9.49
4	8670	5.65	28	11.03	27	2.51
9	7322	4.77	12	2.88	18	5.80
5	7259	4.73	23	2.67	27	5.86
6	6502	4.24	7	3.57	9	4.57
7	6425	4.19	9	8.89	9	1.45
8	5896	3.84	4	1.88	9	4.93
10	3612	2.35	5	1.16	8	3.01
11	2909	1.90	13	2.98	17	1.25
12	2625	1.71	20	2.30	20	1.35
13	2261	1.47	9	0.12	13	2.24
14	2192	1.43	2	0.08	6	2.18
16	2122	1.38	6	1.03	5	1.57
23	1987	1.30	3	0.41	8	1.79
15	1613	1.05	1	0.005	2	1.64
17	1517	0.99	3	0.51	6	1.25
25	1495	0.97	7	0.61	10	1.17
43	1482	0.97	13	0.54	14	1.20
LSU						
1527	26867	6.98	31	9.34	23	4.84
1494	24682	6.41	32	4.82	35	7.87
1096	22953	5.96	33	8.85	32	3.34
4	21207	5.51	25	5.60	28	5.20
1627	18945	4.92	15	4.68	22	5.14
6	16087	4.18	14	3.41	18	4.88
7	13284	3.45	5	0.08	11	6.51
243	13230	3.44	31	1.84	32	4.89
9	13117	3.41	13	2.01	12	4.67
12	13085	3.40	10	5.47	6	1.51
10	11424	2.97	13	2.04	15	3.81
11	9305	2.42	26	2.78	24	2.09

OTU ID #	Total seqs	Frequency (%)	Total *A. arenaria* samples	*A. arenaria* frequency (%)	Total *L. mollis* samples	*L. mollis* frequency (%)
14	8022	2.08	7	4.33	7	0.04
13	7247	1.88	27	2.23	31	1.56
16	6108	1.59	11	0.80	15	2.30
1344	6069	1.58	12	1.65	16	1.51
19	5101	1.32	10	0.30	12	2.25
17	4061	1.05	9	2.21	1	0.0009
22	3473	0.90	13	1.36	16	0.49
20	3434	0.89	7	1.36	6	0.49
SSU						
1	28694	38.90	6	33.65	11	41.49
2	17674	23.96	21	1.49	11	35.07
9	10793	14.63	34	40.08	6	2.04
3	6181	8.38	11	0.33	8	12.36
5	6104	8.27	18	16.05	16	4.43
8	788	1.07	0	0.00	7	1.60
7	766	1.04	1	0.00	5	1.55
37	488	0.66	5	1.87	1	0.06
26	361	0.49	4	1.38	2	0.05
52	304	0.41	14	1.24	1	0.00
22	289	0.39	5	0.58	6	0.30
57	285	0.39	4	0.05	12	0.55
89	173	0.23	1	0.02	6	0.34
65	170	0.23	5	0.62	4	0.04
41	158	0.21	10	0.63	1	0.01
29	135	0.18	5	0.51	1	0.02
66	87	0.12	6	0.34	1	0.01
32	78	0.11	5	0.32	1	0.00
49	77	0.10	3	0.32	0	0.00
50	53	0.07	3	0.16	3	0.03
36	42	0.06	14	0.14	1	0.02
74	42	0.06	1	0.17	0	0.00
80	20	0.03	4	0.05	1	0.02
99	8	0.01	0	0.00	1	0.02

Supplementary Table 2: The number of sequences for each operational taxonomic unit (OTU) representing arbuscular mycorrhizal fungi, as detected by the SSU region, in *Ammophila arenaria* (A) *and Leymus mollis* (L) root samples. The OTUs are classified as per Fig. 6.

OTU Id#	L1	L2	L3	L4	L5	L6	L7	L8	L9	L10	L11	L12	L13	L14	L15	L16	L17	L18	L19	L20	L21	L22	L23	L24	L25	L26	L27	L28	L29
1	0	0	0	0	845	0	0	46	0	0	0	0	0	0	0	0	0	3309	0	1	0	0	1	522	274	0	1726	9582	0
2	0	0	559	1774	0	0	2519	10998	0	0	104	0	0	0	0	586	0	60	111	0	365	0	0	0	0	0	0	0	0
9	0	0	125	81	0	0	107	0	0	0	0	0	41	0	634	18	0	0	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	2901	0	0	0	323	0	0	0	0	0	0	0	672	1	0	0	0	231	0	0	0	0	0
5	0	0	0	0	13	0	0	0	13	0	0	9	261	344	0	48	0	0	0	0	1	222	2	18	1	0	290	23	0
8	0	0	0	0	2	0	4	0	0	0	8	0	654	0	0	0	0	1	2	0	117	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	361	0	0	0	54	0	0	0	0	0	0	0	81	0	0	0	0	25	0	0	0	0	0
37	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	32	0	0	0	0	0	0	0	0	0	0
26	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	23	0	0	0	0	0	0	0	0	0	0
52	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0
22	0	0	0	0	0	0	6	0	0	0	3	0	115	0	0	0	0	1	0	0	21	0	0	0	0	0	0	0	0
57	0	0	0	1	0	0	0	0	3	0	0	0	34	51	0	8	0	0	0	0	0	21	0	6	0	0	26	2	0
89	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	139	0	0	0	0	0	9	8	0	6	0	0
65	0	0	5	1	0	0	5	7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
41	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0
29	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10	0	0	0	0	0	0	0	0	0	0
66	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
32	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
49	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
50	0	0	0	0	0	0	0	0	0	0	13	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0
36	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8	0	0	0	0	0	0	0	0	0	0
74	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
80	0	0	0	0	0	0	9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
99	0	0	0	0	0	0	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total seqs	0	0	689	1857	862	0	5920	11051	16	0	511	9	1105	395	634	660	0	3511	945	2	504	243	3	811	283	0	2048	9607	0

L30	L31	L32	L34	L35	L36	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12	A13	A15	A16	A17	A18	A19	A20	A21	A22	A23	A24	A25	A26
4128	0	0	0	44	0	0	0	0	0	0	0	185	0	0	0	1116	0	0	0	20	0	0	397	0	0	0	0	0	0	0
0	0	0	1	0	232	0	1	0	26	2	24	10	9	32	83	0	0	18	32	7	0	0	1	0	1	1	2	0	0	36
0	0	0	0	0	0	36	430	291	298	386	39	201	127	25	341	401	141	181	31	268	423	495	559	14	679	92	217	162	214	33
1	0	0	0	1969	3	0	0	0	1	0	3	0	0	0	0	40	1	0	0	0	3	0	0	0	0	3	10	0	7	0
0	331	189	421	0	0	0	140	1	0	0	0	0	1	0	329	0	19	1386	0	24	0	26	0	0	0	260	2	363	0	5
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	244	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	31	1	0	0	0	0	2	0	0	0	0	0	0	421	0	1	0	0	0
0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	19	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	15	28	1	1	1	11	0	0	64	4	6	0	0	0
0	0	0	1	0	0	0	0	0	0	12	0	0	0	0	0	0	0	0	0	0	0	0	47	0	0	0	0	0	18	0
0	33	31	56	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	4	0	0
3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6	0	0	0	0	0
0	0	0	0	0	0	0	0	7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	3	1	1	21	0	0	1	0	0	0	0	123	0	2	0	0	0	0	1	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	13	0	9	97	0	5	0	0	0	0	0	0	0	1	0	0
0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	5	0	0	0	4	6	0	61	0	6	0	0	0	0
0	0	0	0	1	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	21	0	0	0	0	0	0	0	0	50	0	0	0	0	6	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	30	0	0	0	2	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	4	0	0	0	0	1	1	0	1	6	0	0	0	1	0	1	1	0	1	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	42	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	6	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4132	364	220	479	2258	235	36	581	302	326	421	66	428	162	58	767	1583	196	1907	64	332	431	591	1005	75	1173	366	244	530	239	74

A27	A28	A29	A30	A31	A32	A33	A35	A36	Total seqs	Frequency (%)	Order	Family	Genus
0	0	0	0	2012	0	0	4486	0	28694	38.90	Glomerales	Glomeraceae	*Rhizophagus*
0	0	0	1	0	2	1	74	2	17674	23.96	Glomerales	Glomeraceae	*Glomus*
80	944	27	1	480	401	643	736	391	10793	14.63	Diversisporales	Gigasporaceae	*Cetraspora*
7	0	0	0	0	0	2	3	0	6181	8.38	Glomerales	Glomeraceae	*Glomus*
0	127	0	0	118	901	1	211	4	6104	8.27	Diversisporales	Diversisporaceae	*Diversispora*
0	0	0	0	0	0	0	0	0	788	1.07	Diversisporales	Gigasporaceae	*Scutellospora*
0	0	0	0	0	0	0	0	0	766	1.04	Glomerales	Glomeraceae	*Glomus*
0	0	0	0	0	0	0	0	0	488	0.66	Glomerales	Glomeraceae	*Sclerocystis*
0	0	0	0	0	0	309	6	0	361	0.49	Glomerales	Claroideoglomeraceae	*Claroideoglomus*
0	0	0	2	6	20	141	0	0	304	0.41	Archaeosporales	Unassignable	Unassignable
0	62	0	3	0	0	0	0	0	289	0.39	Diversisporales	Gigasporaceae	*Scutellospoa*
0	0	0	0	0	0	4	0	2	285	0.39	Diversisporales	Diversisporaceae	*Diversispora*
0	0	0	0	0	0	0	0	0	173	0.23	Glomerales	Glomeraceae	*Rhizophagus*
0	45	0	0	50	31	0	19	0	170	0.23	Glomerales	Glomeraceae	*Glomus*
0	0	0	1	0	1	0	0	0	158	0.21	Archaeosporales	Unassignable	Unassignable
0	0	0	0	0	0	0	0	0	135	0.18	Archaeosporales	Unassignable	Unassignable
0	0	0	0	0	0	0	0	0	87	0.12	Diversisporales	Acaulosporaceae	*Acaulospora*
0	52	0	9	0	0	0	0	14	78	0.11	Glomerales	Glomeraceae	*Glomus*
0	0	0	0	0	0	0	0	0	77	0.10	Archaeosporales	Unassignable	Unassignable
0	0	0	0	0	0	6	0	0	53	0.07	Diversisporales	Gigasporaceae	*Dentiscutata*
0	4	0	0	3	1	1	8	0	42	0.06	Glomerales	Claroideoglomeraceae	*Claroideoglomus*
0	0	0	0	0	0	0	0	0	42	0.06	Archaeosporales	Unassignable	Unassignable
0	0	0	0	0	3	0	1	0	20	0.03	Glomerales	Glomeraceae	*Glomus*
0	0	0	0	0	0	0	0	0	8	0.01	Glomerales	Glomeraceae	*Glomus*
87	1234	27	17	2669	1360	1108	5544	413	73770				

Supplementary Table 3: Operational taxonomic units (OTUs) classified as arbuscular mycorrhizal fungi which were detected by the ITS and LSU regions, from DNA sequences obtained from *Ammophila arenaria* and *Leymus mollis* root samples. The top listed GenBank match for each OTU from a published study which is named to the level of genus or species, and the query cover and identity values for that match, are given.

OTU ID#	GenBank match name	Match accession #	Query cover (%)	Identity (%)
ITS				
39	*Glomus* sp	FM253380.1	100	99
52	*Glomus macrocarpum*	NR_121448.1	100	98
54	*Glomus intraradices*	FJ769312.1	100	97
69	*Glomus intraradices*	AM980842.1	100	99
70	*Rhizophagus* cf *irregularis*	FR750117.1	100	96
74	*Glomus* sp	FR828471.1	74	84
88	*Glomus intraradices*	AJ567771.1	100	97
102	*Glomus* sp	KC182045.1	75	83
122	*Glomus* sp	FM253379.1	100	97
138	*Glomus intraradices*	AJ567739.1	100	99
150	*Glomus macrocarpum*	KC182043.1	100	88
154	*Rhizophagus irregularis*	FR750200.1	100	99
162	*Glomus macrocarpum*	FR750527.1	100	99
173	*Glomus* sp	KM056656.1	100	87
174	*Rhizophagus irregularis*	FR750197.1	100	100
198	*Glomus* sp	HE794039.1	100	86
212	*Glomus macrocarpum*	FR750544.1	100	96
226	*Rhizophagus intraradices*	FJ769312.1	100	96
253	*Glomus macrocarpum*	KC182043.1	75	93
256	*Glomus* sp	KM056656.1	100	86
270	*Glomus intraradices*	AJ567772.1	100	97
273	*Glomus macrocarpum*	FR750544.1	100	97
282	*Glomus intraradices*	AJ567766.1	100	97
287	*Glomus macrocarpum*	NR_121448.1	84	95
306	*Rhizophagus irregularis*	FR750196.1	100	100
192	*Glomus* sp	KC182047.1	74	85
LSU				
7	*Glomus macrocarpum*	FR750526.1	80	81
30	*Glomus* sp	KC182046.1	82	81
51	*Rhizophagus* cf *irregularis*	FR750090.1	100	99
91	*Dominikia duoreactiva*	KR105643.1	100	97
132	*Rhizophagus irregularis*	FR750198.1	100	97
153	*Glomus macrocarpum*	KC182042.1	100	99

OTU ID#	GenBank match name	Match accession #	Query cover (%)	Identity (%)
179	*Rhizophagus irregularis*	FR750087.1	100	98
226	*Dominikia duoreactiva*	KR105643.1	100	95
251	*Rhizophagus irregularis*	FR750196.1	100	100
275	*Diversispora gibbosa*	KJ850204.1	100	99
310	*Dominikia duoreactiva*	KR105643.1	100	96
324	*Glomus macrocarpum*	FR750371.1	100	98
385	*Rhizophagus irregularis*	FR750199.1	100	99
812	*Glomus macrocarpum*	FR750526.1	96	98
887	*Glomus macrocarpum*	KC182041.1	100	97
1149	*Racocetra alborosea*	JN689226.1	100	100
1158	*Dominikia duoreactiva*	KR105643.1	100	93
1221	*Glomus* sp	FR773851.1	95	100
1226	*Glomus macrocarpum*	FR750376.1	95	100
1242	*Glomus* sp	FN429106.1	96	98
1252	*Glomus intraradices*	HM625898.1	98	98
1326	*Dominikia duoreactiva*	KR105643.1	100	94
1329	*Rhizophagus irregularis*	FR750198.1	100	98
1539	*Rhizophagus irregularis*	FR750190.1	99	100
1654	*Glomus macrocarpum*	KC182042.1	86	99

Supplementary Table 4: The top listed GenBank matches from a published study and named to at least the level of genus for the 20 most frequently sequenced operational taxonomic units (OTUs) for the LSU region. The query cover and identity values for that match are given. Where an alternative, equally good match was generated, the lowest taxonomic level at which that match agreed with the listed match is given. Names are as given on GenBank, Class and Order (where this could be assigned and where top listed matches are in agreement) are as given on Mycobank (www.mycobank.org). The OTUs were obtained from root samples from *Ammophila arenaria* and *Leymus mollis* plants.

OTU ID #	GenBank match name	Phylum	Class	Order	Match accession #	Query cover (%)	Identity (%)	Consensus classification	Total seqs	Frequency (%)
1527	*Idriella lunata*	Ascomycota	Sordariomycetes	Xylariales	KP858980.1	100	99	N/A	26867	7.0
1494	*Sarocladium strictum*	Ascomycota	Sordariomycetes	Hypocreales	HG965074.1	100	99	Hypocreales	24682	6.4
1096	*Alternaria* sp	Ascomycota	Sordariomycetes	N/A	KR906715.1	100	99	Hypocreomycetidae	22953	6.0
4	*Plectosphaerella* sp	Ascomycota	Sordariomycetes	N/A	FJ430715.1	100	100	Glomerellales	21207	5.5
1627	*Ophiocordyceps arborescens*	Ascomycota	Sordariomycetes	Hypocreales	AB968414.1	100	96	Hypocreales	18945	4.9
6	*Panaeolus foenisecii*	Basidiomycota	Agaricomycetes	Agaricales	KC176293.1	100	100	*Panaeolus*	16087	4.2
7	*Glomus macrocarpum*	Glomeromycota	Glomeromycetes	Glomerales	FR750526.1	80	81	N/A	13284	3.4
243	*Pyrenophora tetrarrhenae*	Ascomycota	Dothidiomycete	Pleosporales	JN940090.1	100	98	Pleosporaceae	13230	3.4
9	*Eichleriella leveilleana*	Basidiomycota	N/A	N/A	AY509553.1	100	97	Basidiomycota	13117	3.4
12	*Cercophora newfieldiana*	Ascomycota	Sordariomycetes	Sordariales	AF064642.1	100	100	N/A	13085	3.4
10	*Chaetomidium subfimeti*	Ascomycota	Sordariomycetes	Sordariales	FJ666357.1	100	99	N/A	11424	3.0
11	*Halosarpheia japonica*	Ascomycota	Sordariomycetes	N/A	HQ009886.1	99	96	Hypocreomycetidae	9305	2.4
14	*Morakotiella salina*	Ascomycota	Sordariomycetes	Microascales	AY864843.1	100	97	N/A	8022	2.1
13	*Cladosporium macrocarpum*	Ascomycota	Dothidiomycete	Capnodiales	KC311478.1	100	100	Capnodiales	7247	1.9
16	*Microscypha* sp.	Ascomycota	Leotiomycetes	Helotiales	JN086728.1	100	99	N/A	6108	1.6
1344	*Corollospora* sp.	Ascomycota	Sordariomycetes	Microascales	KJ406565.1	100	98	N/A	6069	1.6
19	*Pisorisporium cymbiforme*	Ascomycota	Sordariomycetes	Pisorisporiales	KM588904.1	100	94	N/A	5101	1.3
17	*Tomentellopsis bresadoliana*	Basidiomycota	Agaricomycetes	Atheliales	EU118674.1	100	98	N/A	4061	1.1
22	*Microdochium phragmitis*	Ascomycota	Sordariomycetes	Xylariales	KP858948.1	100	100	N/A	3473	0.9
20	*Exidiopsis* sp.	Basidiomycota	Tremellomycetes	Tremellales	AY509549.1	100	98	Exidiaceae	3434	0.9

Table 1: Substrate chemical characteristics (% total C and % total N via the Leco test, Mehlich 1 Ca, K, Mg, Mn, P, Zn) of 72 combined sand samples taken from a 2.26 km long area of foredune in Northern California.

Mehlich 1 mg/kg (ppm)						%	
Ca	K	Mg	Mn	P	Zn	C	N
625	83.5	113	7.57	63.86	1.1	0.124	0.011

Table 2: The top listed GenBank matches from a published study and named to the level of species (unless a better match to an alternative sequence named only to genus was available) for the 20 most frequently sequenced operational taxonomic units (OTUs) for the ITS region. The query cover, identity values and UNITE Species Hypothesis (Kõljalg et al., 2013) for that match are given. The names and taxonomy given are as per Mycobank (www.mycobank.org). The OTUs were obtained from root samples from *Ammophila arenaria* and *Leymus mollis* plants.

OTU ID #	GenBank match name	Phylum	Class	Order	Match accession #	Match UNITE S.H.	Query cover (%)	Identity (%)	Totals seqs	Frequency (%)
1	*Plectosphaerella cucumerina*	Ascomycota	Sordariomycetes	Phyllachorales	HM216207.1	SH190975.07FU	100	100	19118	12.46
2	*Microdochium bolleyi*	Ascomycota	Sordariomycetes	Xylariales	AM502265.1	SH213512.07FU	100	100	18150	11.83
3	*Sarocladium strictum*	Ascomycota	Sordariomycetes	Hypocreales	JQ676174.1	SH210203.07FU	100	100	13689	8.92
4	*Alternaria infectoria*	Ascomycota	Dothideomycetes	Pleosporales	JF340283.1	SH216783.07FU	100	98	8670	5.65
9	*Corollospora maritima*	Ascomycota	Sordariomycetes	Microascales	JN943387.1	N/A	72	84	7322	4.77
5	*Alternaria infectoria*	Ascomycota	Dothideomycetes	Pleosporales	JX421701.1	N/A	100	91	7259	4.73
6	*Panaeolus acuminatus*	Basidiomycota	Agaricomycetes	Agaricales	JF908518.1	SH178705.07FU	100	100	6502	4.24
7	*Apodus deciduus*	Ascomycota	Sordariomycetes	Sordariales	AY681199.1	SH193385.07FU	100	99	6425	4.19
8	*Heterochaete spinulosa*	Basidiomycota	Agaricomycetes	Auriculariales	JQ694111.1	N/A	100	88	5896	3.84
10	*Chaetomium globosum*	Ascomycota	Sordariomycetes	Sordariales	KP174693.1	N/A	100	96	3612	2.35
11	*Microdochium phragmitis*	Ascomycota	Sordariomycetes	Xylariales	AM502263.1	SH203161.07FU	100	99	2909	1.90
12	*Davidiella allicina*	Ascomycota	Dothideomycetes	Capnodiales	LN834354.1	None	100	100	2625	1.71
13	*Stemphylium solani*	Ascomycota	Dothideomycetes	Pleosporales	JQ781776.1	SH199527.07FU	100	99	2261	1.47
14	Helotiales sp.	Ascomycota	Leotiomycetes	Helotiales	HQ649858.1	SH198121.07FU	100	98	2192	1.43
16	Sordariomycetidae sp.	Ascomycota	Sordariomycetes	N/A	KP689127.1	N/A	99	92	2122	1.38
23	*Oliveonia pauxilla*	Basidiomycota	Agaricomycetes	Auriculariales	HQ441577.1	N/A	100	82	1987	1.30
15	*Trichoderma stromaticum*	Ascomycota	Sordariomycetes	Hypocreales	NR_077128.1	SH196035.07FU	100	99	1613	1.05
17	*Plectosphaerella cucumerina*	Ascomycota	Sordariomycetes	Incertae sedis	KF285996.1	SH190981.07FU	100	100	1517	0.99
25	*Fusarium pseudograminearum*	Ascomycota	Sordariomycetes	Hypocreales	DQ459871.1	SH220700.07FU	98	99	1495	0.97
43	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	1482	0.97