Scattered far and wide: a broadly distributed temperate dune grass finds familiar fungal root associates in its invasive range

Renee B Johansen[a,g], Peter Johnston[b], Piotr Mieczkowski[c], George L.W. Perry[d], Michael S. Robeson[e], Rytas Vilgalys[f], Bruce R Burns[g]

a: Corresponding author. Biosciences Division, Los Alamos National Laboratory, Mailstop M888, Los Alamos, NM87545, USA. rjohansen@lanl.gov

b: Landcare Research, Private Bag 92170, Auckland Mail Centre, Auckland 1142, New Zealand. JohnstonP@landcareresearch.co.nz

c: Department of Genetics, University of North Carolina, Chapel Hill, NC 27599-7264, U.S.A. Piotr_Mieczkowski@med.unc.edu

d: School of Environment, The University of Auckland, Private Bag 92019, Auckland, New Zealand. george.perry@auckland.ac.nz

e: Fish, Wildlife and Conservation Biology, Colorado State University, Fort Collins, CO 80523, USA. soilbdelloid@gmail.com

f: Department of Biology, Duke University, Durham, NC 27708, USA. fungi@duke.edu

g: School of Biological Sciences, University of Auckland, Private Bag 92019, Auckland Mail Centre, Auckland 1142, New Zealand. b.burns@auckland.ac.nz

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For supplementary material, email the corresponding author at reneejohansen@gmail.com

Abstract

Deterministic and stochastic factors interact to generate biogeographic patterns in fungal communities, challenging efforts to predict which fungal assemblages will develop in association with introduced plants. The coastal dune grass *Ammophila arenaria* has been moved around the world. We sampled *A. arenaria* roots in its native range in the United Kingdom, and in Australia and New Zealand where it is invasive. The co-occurring native dune grass *Leymus arenarius* was also sampled in the United Kingdom, and the native dune grass *Spinifex sericeus* was sampled in Australia and New Zealand. *A. arenaria* associates with root fungal communities in its invasive range that are richer than those in the United Kingdom, and richer than those in co-occurring native grasses, demonstrating that exotic plants are not necessarily depauperate in fungal associates. Most of the dominant OTUs associating with *A. arenaria* were shared among all three geographic regions. However, community composition varied among regions, with differences in temperature, nitrogen and pH correlating with community change. Within regions, community composition varied among dunes, although the majority of sequences obtained were in OTUs that were detected in multiple dunes. Host plant species identity influenced fungal community composition, although the majority of the data were in OTUs that were shared between hosts. While the roots sampled appeared healthy, many of the dominant fungi recovered are potentially pathogenic. This study demonstrates that many fungi are widely dispersed, and that introduced plants are likely to associate with functionally diverse fungal communities that include species from their native ranges. However root-associated fungal community composition is variable, even at regional and local scales in a common plant in a common environment.

1. Introduction

Root-associated fungi have fundamental impacts on plant health, and consequently can influence plant community structure (Aguilar-Trigueros and Rillig 2016; Dostalek et al. 2013; Rillig et al. 2014; Shi et al. 2016). Fungal associates may, therefore, influence the success of introduced plants (Vestergård et al. 2015). Different root fungal communities could result in differential plant performance in the native versus the introduced range, and even influence

whether a plant becomes invasive. For example, depauperate fungal communities can constrain the spread of introduced plants where mutualists are lacking (Nuñez et al. 2009; Pringle et al. 2009; Spence et al. 2011). On the other hand, the Enemy Escape hypothesis suggests that invasive plants flourish when released from pathogens encountered only in their native range (Keane and Crawley 2002). However, introduced plants can quickly acquire fungal associates from a range of functional groups after they arrive in a new location and some might counteract this effect (Day et al. 2015; Majewska et al. 2015). Altogether there is a lack of data comparing entire fungal communities in the roots of plants in their introduced versus their native ranges. High throughput sequencing, which allows the examination of fungal communities to a much greater extent than previous methods, has not yet been extensively adopted by invasive plant ecologists (Coats and Rumpho 2014).

Differences in fungal communities associating with a plant in its native and introduced range will depend in part on the dispersal abilities of the potential associates. The Baas Becking hypothesis assumes dispersal is unlimited, stating that 'everything is everywhere, but the environment selects'. While this hypothesis influenced expectations regarding fungal dispersal for decades (Baas Becking 1934; Hazard et al. 2013) in recent years it has been largely abandoned (Bahram et al. 2014). Although molecular studies indicate that some fungal taxa have high dispersal capacity (Fröhlich-Nowoisky et al. 2012; Kivlin et al. 2014) and broad ranges (Bonfim et al.; Cox et al. 2016; Davison et al. 2015; Timling et al. 2014) consistent with Baas Becking, fungi can also be dispersal limited, even at regional and local scales (Beck et al. 2015; David et al. 2015; Talbot et al. 2014).

Following dispersal, abiotic environmental conditions may determine establishment success (Peay et al. 2016). At regional scales, climatic conditions may be an important limiting factor (Glynou et al. 2016; Miyamoto et al. 2015; Tedersoo et al. 2014). At more local scales, soil conditions including levels of nitrogen, phosphorus (Leff et al. 2015), carbon (Wu et al. 2013) and pH (Geml et al. 2014) influence fungal communities.

The biotic soil environment is also important (Nemergut et al. 2013). The fungal communities associating with the roots of co-occurring established plants are likely to be a major source of fungal inoculum available to introduced plants. However, it is difficult to predict whether an identical or alternative community will develop in a new arrival to a plant community. Plant host species identity has been shown to impact fungal community composition in some

studies (Barrett et al. 2009; Becklin et al. 2012; Kernaghan and Patriquin 2015; Tedersoo et al. 2010; Tejesvi et al. 2013; Vályi et al. 2014), but in other cases, host plant identity has had limited or no influence on root endophytes (David et al. 2015; Glynou et al. 2016; Knapp et al. 2012) and communities of arbuscular mycorrhizal fungi (Davison et al. 2015; Li et al. 2015; Saks et al. 2013). The degree of relatedness between resident and introduced plant host species may impact the degree to which they share available fungal lineages (Bufford et al. 2016; Wehner et al. 2014). As well, both facilitation and competition among fungi render arrival order important in determining fungal community composition (Fukami et al. 2010; Werner and Kiers 2015). Stochastic factors further impact the assembly of fungal communities, compounding variation at small spatial scales and complicating efforts to predict fungal community composition at any given location (Beck et al. 2015; Powell et al. 2015).

Ammophila arenaria (L.) Link is a coastal dune grass native to Europe that was transported around the world for erosion control in the latter part of the 19th century (Bell 1987; Dixon et al. 2004; Wiedemann and Pickart 1996). It is known to share many fungi with native dune grasses in the United States (David et al. 2015; Johansen et al. 2015), but a direct comparison of fungal communities in its native and introduced ranges has not previously been undertaken. There have also not been previous efforts to examine fungal communities in co-occurring native grasses outside of the United States.

In this study, we used next generation sequencing to characterize the fungal communities associated with *A. arenaria* roots in three regions – its native range in the United Kingdom, and in Australia and New Zealand where the plant has been introduced. In addition, spatially explicit sampling allowed us to characterize heterogeneity in the fungal communities within regions and within dunes. Although a common biome was sampled, the regions encompassed were separated by vast distances across which abiotic factors differed, and we predicted that the fungal community richness would be similar but composition would differ among regions. Within regions, where dispersal limitation is likely to be less important and environmental conditions less variable, we predicted that fungal community richness and composition would be similar among dunes. Given the frequently disturbed nature of the dune environment, we hypothesised that there would be high spatial turnover in fungal community composition within dunes and this would be related to distance between samples. We also examined the

fungal communities in the roots of co-occurring native dune grasses in each of the three regions. These grasses provide baseline community composition data, and also enable us to examine whether host plant identity impacts fungal community composition in the dune environment. However, given the level of relatedness between the two grasses in each location, and the results from our previous study of the fungal communities in a dune in the United States (Johansen et al., 2015), we hypothesised that host plant identity impacts would not be significant. Finally, we examined the general community profile of fungi occupying the roots of dune grasses.

2. Materials and Methods

2.1. Sample collection

We collected *Ammophila arenaria* samples from three regions – the United Kingdom (UK, two sites in England, five sites in Wales), Australia (four sites) and New Zealand (four sites) (Fig.1). Root samples of the native dune grass *Spinifex sericeus* were collected from one of the Australian (Lakes Entrance) and one of the New Zealand (Waikawa Beach) sites. Root samples of the native grass *Leymus arenarius* were collected from one site (Newborough Warren) in England. *A. arenaria* was highly dominant at each of the sites where only this species was collected. At Lakes Entrance in Australia, *Spinifex sericeus* was slightly more prevalent than *A. arenaria.* At the other sites in the other regions where alternative grasses were collected, they were co-dominant with *A. arenaria*. All samples were collected during summer; UK samples were collected during June 2014, New Zealand samples during January 2015 and Australian samples during February 2015. Site location details, soil properties and climate data are provided in Table S1 (supplementary data). At each site, 24 root samples of *A. arenaria* were collected from the foredune face. Usually this was in a 100 m × 12 m belt transect, with the shorter side placed between the dune toe and first ridge, but at one site in Australia (Seven Mile) the belt transect was reduced to 100 m × 9.5 m to keep the transect within the foredune face. At the sites in England and Australia where two plant species were sampled, the species intermingled on the dune, and roots were collected from each where they grew within one metre of each other. These transects were expanded to 150 m, and 200 m, respectively. At the New Zealand site, the two species typically grew in monocultural blocks. Therefore 12 samples of each were collected in pairs along a transect where two such blocks were adjacent, and the other 12 were collected from 50 m × 12 m quadrats on either side.

Sampling points were selected via random-stratification, with the dunes divided vertically into thirds (lower, middle and upper dune sections), and eight sample points were taken in each. Roots were collected from the depth at which they first appeared, with the minimum depth sampled being approximately two cm and the maximum depth sampled being approximately 50 cm. Most samples included several separate root pieces, cumulatively consisting of about 30 cm of root length, while a single continuous root piece was used where no others were uncovered at the sample point. Where non-target species were present, or where two species were collected, roots were traced back to the stem so that the host could be determined. Sand adjacent to the root samples was collected from each sample point within each dune and then mixed before being sent to the Center for Applied Isotope Studies, University of Georgia, USA. For the combined samples from each dune, total C and N were measured using the micro-dumas combustion method, total P using acid persulfate digest, and pH in water with a pH meter. Samples were stored at 4 ^{o}C for a maximum of five days before processing. Approximately 15 cm of root length per sample was selected to represent the root sections available (i.e. different thicknesses/lateral root proliferation) and cleaned by shaking in 4% Tween 80. Following a sterile water rinse, roots were chopped in sterile conditions and dried at room temperature in a vacuum in 2 mL centrifuge tubes.

2.2. DNA extraction and high throughput sequencing

Samples were ground in either a SPEX CertiPrep 2000 Geno/Grinder or ball mill MM 301 (Retsch, Dusseldorf, Germany). DNA was extracted with the CTAB method (Gardes and Bruns 1993) as follows. An overnight soak in 850 µL CTAB buffer at 4 ^{o}C preceded heating for an hour to 65 ^{o}C. An equal volume of chloroform:isoamyl alcohol (24:1) was then added. DNA was precipitated following centrifugation with 1.5 volume of chilled isopropanol before incubation for one hour at -20 ^{o}C. The DNA pellet 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 then pellets were again obtained by centrifugation. The pellets were washed twice in chilled 80% ethanol before the DNA was eluted in 50 µL of water.

DNA amplification used three rounds of nested PCR, in a process modified from Lundberg et al. (2013). In round one, the ITS region was amplified with the primers ITS1f (Gardes and Bruns 1993) and ITS4 (White et al. 1990). The PCRs were carried out in 12.5 µL reactions

and included 0.4 µL (10 µM) of each primer, 2 µL (1 mM) of DNTP, 1.25 µL of BSA (10 mg/ml), 0.0625 µL of Qiagen® *Taq* DNA polymerase, 1.25 µL of Qiagen® 10X buffer with 15 mM $MgCl_2$ and 2.5 µL of undiluted DNA. Thermocycler protocols were initial heating to 95 ^oC (10 min), 10 cycles at 95 ^oC for one min, 50 ^oC for one min, 72 ^oC for one min; and 72 ^oC for ten mins. In the second PCR round, primer constructs including one of six frameshifting nucleotide sequences (used to increase sequence diversity at each position to improve sequencing results), a linker sequence recognized at the third step, and the ITS sequences as above were used. Each 25 µL reaction contained 5 µL of PCR product 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 15 mM $MgCl_2$ (concentrations as above). Thermocycler protocols were as above but 20 cycles were performed. The third PCR round ligated MID tags (barcodes), incorporating a linker region recognizing that used in round two and Illumina adaptor sequences. The 25 µL reactions used contained 10 µL of PCR product from round 2, 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 15mM $MgCl_2$. Concentrations were as for step one apart from the primers which were diluted to 5 µM. The thermocycler protocol was initial heating to 95 ^oC (10 min), 5 cycles at 95 ^oC for one min, 63 ^oC for one min, 72 ^oC for one min 20 secs; and 72 ^oC for 10 mins. Further sequencing preparations were undertaken at the University of North Carolina's High-Throughput Sequencing Facility. Magnetic beads were used for amplicon cleaning, size selection and purification. Quantification was done using a LabChip GX and prepared libraries were pooled on a Tecan robotic workstation. An Illumina MiSeq instrument was used to perform paired-end 2 x 300 bp sequencing. The majority of samples from the UK were sequenced together, without samples from the other regions, but 16 were sequenced in a run with the Australian samples. New Zealand samples were not sequenced with those from the other regions.

2.3. Bioinformatics

Bioinformatics was undertaken with sequences from the forward direction only, using the general approach of Andrei et al. (2015). Demultiplexing was done in QIIME v1.8.0 (Caporaso et al. 2010) using the `split_libraries.py` script with quality checking steps disabled. Cutadapt v1.7.1 (Martin 2011) was used to select the ITS1f reads and remove the primer sequence. The UPARSE pipeline (Edgar 2013) was then largely followed. The

`fastq_filter` command was used to trim sequences to 220bp, and to perform quality filtering, with the maxee value set to 0.5. Following de-replication and singleton removal, OTU picking was undertaken with *de novo* chimera checking disabled (uparse break -999), otherwise default settings were used. Reference-based chimera checking was performed using the USEARCH (Edgar 2010) `uchime_ref` command against the Unite database QIIME release v.7 dataset 'uchime_sh_refs_whole_ITS.fasta' (Abarenkov et al. 2010). The `minh` value was set to 1.65 following examination of alignments of sequences designated chimeric at more stringent `minh` values. Classification was done in QIIME v1.8.0 (Caporaso et al. 2010) using the UNITE reference database v.7 dynamic data set (Abarenkov et al. 2010). OTUs classified as non-fungal or with no BLAST hit were checked with BLAST against GenBank, with uncultured/environmental sample sequences excluded. These OTUs were retained in the dataset where there were matches with fungal sequences with coverage of at least 80 bp, and identity matches of at least 80% (and where there were no better matches with non-fungal organisms). No attempt was made to classify them. The number of OTUs per class (where this had been assigned by the reference database), and classified as Zygomycota *incertae sedis*, was calculated. The top 20 most frequently sequenced OTUs for the *A. arenaria* samples within each region were determined. To moderate the influence of dunes with high sequence numbers, we rarefied the data so that each sample within a region had the same number of sequences (see below). The rarefied data was used to determine the frequency of these OTUs within regions, while unrarefied data was used to ascertain their presence within the three regions. The top listed GenBank match from a published study for these OTUs, named to species by the depositors (and where a better published match of an alternative genus was not available) was recorded. Where query cover of that match was at least 50% and the identity match was at least 97%, the UNITE species hypothesis (Kõljalg et al. 2013) was recorded if available. Match names were updated, and classes and orders were assigned, using Mycobank (www.mycobank.org).

2.4. Statistics

Alpha rarefaction plots were generated in QIIME to examine whether sequencing depth was sufficient to adequately capture fungal community richness. These plots also allow richness among sites and regions to be compared. To visualise shared and unique OTU numbers per region, a Venn diagram was constructed using data from the *A. arenaria* samples only.

The dataset was then rarefied such that each retained sample had an even sampling depth (see Table S2 for details on final sample numbers per dune and sequencing depths where rarefied data were used for within-dune analyses). For among-region comparisons, where rarefied data were used, each dune in each region was represented by 146,000 sequences. We omitted Broadhaven South from the UK for the among-region analysis due to poor sequencing performance. For the within-region ordination and between-host comparisons, UK samples were represented by 2,800 sequences each, Australian samples by 7,000 sequences each, and New Zealand samples by 14,000 sequences each. For the within-region distance decay analyses, the UK sites were represented by 103,479 sequences, the NZ sites by 561,227 sequences and the Australian sites by 312,430 sequences.

We ordinated the fungal community data, for within-region, among-region, and between-host comparisons, using non-metric multidimensional scaling (nMDS) on untransformed data, 4^{th}-root transformed data, presence-absence data, and on untransformed data containing only OTUs which were in more than 10% of samples being compared. Ordinations of these site-species matrix transformations were qualitatively similar and showed similar stress values, so in the following we present only the 4^{th}-root transformations. We also evaluated similarity in fungal communities from the lower, middle and upper sections across the dune faces. Rarefied data were used for all these analyses and the Bray-Curtis metric was used as a dissimilarity measure (Faith et al. 1987). For within-region and among-region comparisons, we used only *A. arenaria* data. Within-dune analyses were performed only on those dunes where alternative hosts were not collected. To assess whether the fungal communities being compared differed in their location (polygon centroid) in the ordination space we used permutational multivariate ANOVA (PERMANOVA; Anderson 2001) via the `adonis` command in the R library vegan (Oksanen et al. 2017; R Development Core Team, 2016) with 999 permutations in all cases. We evaluated whether the fungal communities differed in their dispersion (spread) across ordination space via a multivariate analysis of homogeneity of dispersion (Anderson 2006) with the `betadisper` command in vegan. The relationship between abiotic conditions and community composition among regions was assessed by fitting vectors of the regionally averaged abiotic variables across the ordination space (using the `envfit` command in vegan) with significance assessed via permutation (n = 999).

We used the indicator ('diagnostic') species approach described by De Cáceres & Legendre (2009) and De Cáceres et al. (2010) to identify (suites of) OTU(s) that characterised specific site groups (e.g. locations, host species). This method isolates 'faithful' taxa – that is taxa that are consistently present/absent from groups or sites and so whose presence/absence has predictive power with respect to site group identification. We used the indicspecies package in R for these analyses and assessed significance with $n = 9999$ permutations. Because of the very large number of taxa we considered we controlled for the familywise error rate in these tests using the false discovery rate correction of Benjamini & Hochberg (1995).

The impact of distance on turnover in community composition among dunes within regions, and spatial turnover across these dunes, was assessed with rarefied data, with distance-decay relationships modelled with log-linear binomial generalized linear models (GLM) following Millar et al. (2011). We also investigated whether species richness was higher towards the back of dunes where only *A. arenaria* was collected by comparing richness in samples collected from the lower, middle and upper sections of the dunes.

2.5. Accession number

Demultiplexed sequence data has been deposited in the NCBI Sequence Read Archive (SRA), the accession number is PRJNA322225.

3. Results

3.1. Sampling, PCR and Illumina MiSeq sequencing

We successfully extracted and amplified DNA from all 24 samples collected from each dune, apart from Broadhaven South in the UK, from which sequences were obtained from only 21 samples. The ITS1f primer generated 10,291,415 sequences that passed quality control, and these clustered into 4,756 OTUs. Of these, 10,267,286 sequences which clustered into 4,536 OTUs were classified as fungi. Blasting the 297 OTUs against GenBank which were initially unclassified, or classified as non-fungi by the UNITE database, resulted in 78 of these being retained as fungi. New Zealand had 6,001,199 sequences, Australia 2,803,901 and the United Kingdom 1,462,186. For dunes from which only *A. arenaria* was sampled, sequence totals ranged between 103,479 for Broadhaven South in the United Kingdom and 1,773,284 for Kawhia Ocean Beach in New Zealand. These sites also had the lowest and highest OTU

numbers at 274 and 1,476 respectively (Table 1). The alternative plants sampled always had more sequences than *A. arenaria* but did not always have more OTUs (Table 2). The curves on the alpha-rarefaction plots are beginning to asymptote for some sites but still suggested that deeper sequencing would have generated more OTUs (Fig. 2). Considering the regions as a whole, the curves were nearly saturated.

3.2. Fungal community profile

OTUs were recovered from 22 classes. The Sordariomycetes class contained 1,568 OTUs, nearly half of those which we were able to classify (Fig. 3). The next most diverse classes were the Glomeromycetes, Dothideomycetes, and Agaricomycetes, with several hundred OTUs each. In the UK, Australia and New Zealand, the 20 most frequently sequenced OTUs accounted for 52%, 60% and 58% of the data respectively, with the most frequently sequenced OTU having between 7% and 9% of the sequences (Table S3). The majority of the 47 *A. arenaria* OTUs comprising the most frequently sequenced list were ascomycetes, (35 OTUs), the next most dominant phylum was the Basidiomycota (nine OTUs). Only two of the most frequently sequenced OTUs were in the Glomeromycota, one in the UK and one in Australia. There was only one frequently sequenced Chytridiomycota but this OTU, with a 100% match to *Olpidium brassicae*, was in the top 20 lists for all three regions. The four OTUs placed in the top 20 lists for all three regions have matches of at least 98% with sequences from species previously recorded as pathogens. According to the UNITE species hypotheses, three of these (*Plectosphaerella cucumerina, Microdochium bolleyi*, and *Lewia infectoria*) have broad global ranges and associate with a wide variety of hosts. The dunes surveyed hosted OTUs that may be undescribed species; while only two of the most frequently sequenced OTUs from the UK had matches with identity values below 95%, there were five such OTUs from Australia and 11 from New Zealand. The two most frequently sequenced OTUs in New Zealand had identity values with matches of only 90% and 85%, although allowing matches from environmental sequences found identity values of 97% (HQ010683.1) and 95% (GQ512112.1, query cover values were 96% and 92%).

3.3. Spatial heterogeneity in A. arenaria fungal communities

Despite *A. arenaria* being in its native range in the UK, at equivalent sequencing depths the fungal communities on UK dunes were less rich than those in Australasia (Australia and New Zealand, Fig. 2). New Zealand had the most *A. arenaria* OTUs (Fig. 4). Many *A. arenaria* OTUs were widely distributed, with 609 (14%) occurring in all three regions (Fig. 4). Australia and New Zealand shared more OTUs with each other than with the UK, but each had OTUs found in the UK but not in the other Australasian location. While most OTUs (62%) were recovered from only one region, the OTUs found in two or all three regions contained the majority of the sequence data – between 89% and 96% of sequences per region. In addition, many of the top 20 most frequently sequenced OTUs for each region were geographically widespread (Table S3). Of the 47 most frequently sequenced OTUs listed, 33 were recovered from all three regions and nine were in more than one region's most frequently sequenced list.

The nMDS ordination, however, showed strong separation by region, with New Zealand and Australian *A. arenaria* communities being more similar to each other than to those in the United Kingdom (Fig. 4; PERMANOVA: $F_{2,11} = 3.333$, $r^2 = 0.377$, $p = 0.001$). There was no statistically significant difference in dispersion across the ordination space among the regions ($F_{2,11} = 0.3873$, $p = 0.688$). Regional differences in fungal communities correlated with changes in pH and nitrogen levels among the regions, which were strongly co-linear, and with maximum and minimum temperatures, which were also correlated (Fig. 5). No significant correlation was found between fungal community composition and either annual rainfall or levels of phosphorous or carbon.

Richness among dunes varied within regions, even when sequencing depths were the same (Fig. 2d). The dune with the highest richness of fungi associated with *A. arenaria* roots in the UK, Braunton Burrows, had more than twice as many OTUs as the most depauperate dune, Broadhaven South (Table 1). There was less variation in richness in Australia and New Zealand. While the proportion of *A. arenaria* OTUs unique to any one site in a region was between 14% and 30%, the proportion of sequence data in these OTUs was small, being between 0.06% and 0.68%. However the nMDS ordinations again showed differences in community structure across dunes (Fig. 6). In Australia, the fungal communities at Dennington and Seven Mile Beach were similar to each other, but they separated from Rosebud and Lakes Entrance ($F_{3,85} = 8.1511$, $r^2 = 0.223$, $p = 0.001$). There was no difference

in scatter among the sites ($F_{3,85} = 1.083$, $p = 0.361$). In the New Zealand plots, the majority of samples separated by site ($F_{3,86}$, = 8.217, $r^2 = 0.223$, $p = 0.001$). There was less variation among samples at Oreti and Pegasus than at Kawhia or Waikawa (median distance of sites to centroid: 0.507 (Kawhia), 0.450 (Oreti), 0.454 (Pegasus) and 0.483 (Waikawa)), and there was a significant difference in dispersion between the sites ($F_{3,86} = 6.615$, $p = 0.0004$). For the UK sites, PERMANOVA analysis revealed community differences among the dunes ($F_{6,145} = 8.1184$, $r^2 = 0.251$, $p = 0.001$), but there were also significant differences in dispersion between sites ($F_{6,145} = 3.832$, $p = 0.001$). Distance was correlated with community difference among dunes in New Zealand, but not in the UK or Australia (Fig. S1). Soil nutrient levels were low (Table S1).

There was also high beta-diversity among samples within dunes, demonstrating that high spatial turnover occurred over small extents. Some sites showed a trend toward decreasing similarity with increasing distance, with seven of the twelve sites sampled generating slopes that were different from zero based on bootstrap resampling (Fig. S2). Relationships between distance and community similarity were statistically significant at Seven Mile Beach in Australia, Kawhia Ocean Beach and Pegasus Bay Beach in New Zealand, and at Braunton Burrows and Pembrey Beach in the UK (Fig. S2). There was no consistent evidence that fungal community composition was influenced by vertical position on the dune although there were significant differences in community by position at Morfa Harlech in the UK ($F_{2,20} = 1.914$, $p = 0.001$) and Seven Mile Beach in Australia ($F_{2,22} = 1.684$, $p = 0.001$, data not shown). There was no difference in the richness of root samples collected from the lower, middle and upper sections of the dunes (data not shown).

We did not identify small and consistent groups of indicator OTUs; rather, we found many hundreds of potential indicator taxa for any given site group. At the regional level, of the 1229 OTUs we found 110 (9.0%) to be significant indicators at $\alpha = 0.01$ (with familywise correction) and within the UK, Australia and NZ we found 163 / 1470 (11.1 %), 267 / 2399 (11.1%) and 301 / 1963 (15.3%) statistically significant indicator OTUs, respectively (with correction applied). These OTUs are not discussed further.

3.4. Host plant species influence over fungal community composition

Fungal community richness was similar between *A. arenaria* and *S. sericeus* at Waikawa Beach in New Zealand. In contrast, *A. arenaria* associated with a richer community than *S. sericeus* at Lakes Entrance in Australia and *L. arenarius* was considerably richer than *A. arenaria* at Newborough Warren in the UK (Fig. 2, Table 2). Again, the majority of sequences were in shared OTUs (Table 2), with the most frequently sequenced OTUs found in both host plants in each dune (and often in similar numbers of samples, Table S4). There was more overlap in fungal communities when comparing hosts than when comparing regions (Fig. S3), but PERMANOVA suggests separation by host species. However, r^2 values were very low (Australia: $F_{1,44} = 2.799$, $r^2 = 0.060$, $p = 0.001$; NZ: $F_{1,40} = 0.9474$, $r^2 = 0.053$, $p = 0.001$; UK: $F_{1,43} = 2.067$, $r^2 = 0.046$, $p = 0.002$).

4. Discussion

4.1. Spatial heterogeneity in A. arenaria fungal communities among regions

We predicted that *A.arenaria* would associate with fungal communities of similar richness in its native and introduced ranges. However the increase in richness in the fungal communities associating with *A. arenaria* roots outside its native range was dramatic. A global comparison of richness in soil fungi (Tedersoo et al. 2014) describes similar richness among the regions we sampled. That study also identified high precipitation as driving high soil fungal richness although the opposite effect has been described for root endophytes in Europe (Glynou et al. 2016). Our results do not demonstrate a consistent precipitation-richness relationship across regions. The UK sites are uniformly wetter than those in Australia, which had a richer fungal community, while the New Zealand sites are both slightly wetter and richer in species than those in Australia. Similar inconsistencies were seen with temperature; slightly cooler New Zealand is slightly richer than warmer Australia although the coolest region on average – the UK – is the least rich. Even though more sites were sampled in the UK, they cover a smaller geographic area than do the sites sampled in Australia and New Zealand. Sampling across a smaller range could have resulted in lower richness being captured across dunes in the UK, due to dispersal limitation or to reduced variation in abiotic factors, which could influence fungi, among sites. For example, the greater distance covered in Australia and New Zealand correlates with greater temperature differences between the hottest and coldest sites in these regions compared to the UK. Temperature change has previously been shown to correlate with change in fungal community composition (Goldmann et al. 2016; Tedersoo et al. 2014).

In addition, we acknowledge that despite rarefying our data to accommodate for differences in sequencing depths among regions, the reduced richness observed in the UK could have resulted from a difference in performance of the run that sequenced the majority of the UK samples. Nevertheless, our results demonstrate that an invasive plant outside its native range can associate with rich fungal communities. Comparisons of fungal richness in the roots of plants in their native and non-native ranges are rarely undertaken, but AMF community richness has been shown to be roughly equivalent in clover species sampled in their native UK and in New Zealand where they have been introduced (Mcginn et al. 2016). On the other hand, fungal communities associating with *Pinus contorta* were less rich in that species introduced range than in its native range (Gundale et al. 2016)

A. arenaria encounters many of the same soil fungi in both its native and invasive ranges. While each of the three regions we sampled harboured OTUs not recovered elsewhere, the majority of OTUs in each region were shared with at least one other region. These shared OTUs comprised the vast majority of the sequence data. New Zealand and Australia shared more OTUs with each other than with the more distant UK, which could result either from more frequent fungal dispersal between the two closer regions, or from those regions sharing more similar environments and/or geological history. Despite its greater isolation, however, the UK had almost the same amount of data in shared OTUs as New Zealand. We recognize that the ITS region fails to separate some fungal lineages (Ryberg 2015), hence our dataset may include lineages with restricted ranges that have been grouped into single OTUs. It is also possible that contamination could have resulted in some shared OTUs, which the use of negative controls might have ruled out. However all of the New Zealand samples were sequenced separately from those obtained in the UK and Australia, negating the opportunity for tag switching among these samples and those from the other regions during sequencing (Carlsen et al. 2012). OTU sharing among regions as a result of errors in MID tag production was eliminated by the use of unique MID tags for every sample. Our bioinformatics procedures ensured any sequences assigned to the wrong region by incorrect barcodes were discarded. While there are few global level studies of fungal ranges, next generation sequencing provides evidence for some soil fungi (Cox et al. 2016; Tedersoo et al. 2014) and for AMF (Davison et al. 2015) having wide ranges spanning multiple land masses.

Although our sampling took place in a common plant in a common environment, and even though the majority of sequences were in shared OTUs, the ordination analysis shows clear differences in total fungal community composition by region, as we hypothesised. The robustness of this pattern across data treatments shows that differences in relative abundances of sequences occurred alongside the presence of unique OTUs. Our results suggest that differences in temperature extremes among regions might explain this trend. As discussed above, correlations between fungal community change and temperature have been described previously, although corresponding changes in other environmental variables such as precipitation (Goldmann et al. 2016, Glynou et al. 2016) and plant community variation (Timling et al. 2014) challenge efforts to determine the relative importance of temperature in general. Soil resource levels also influence dune fungi communities. Compositional differences corresponded with changes in nitrogen and pH among regions. However, the nitrogen gradient was heavily influenced by the Australian sites, with nitrogen levels so low in Australian sands that it was effectively undetectable. Potential influences of these soil qualities over fungal community composition have been reported previously (David et al. 2016; Geml et al. 2014; Leff et al. 2015; Walker et al. 2014). We also acknowledge that we sampled at different relative time points during summer in the three regions and this could account for some of the differences seen in fungal community composition.

4.2. Spatial heterogeneity in A. arenaria fungal communities within regions

Differences in fungal richness and community composition were also seen among dunes within regions. Again, differences in the climatic variables we considered do not appear to be driving differences in richness. For example, where sequencing depths were the same, the wettest site in the UK, Pembrey Beach, had the highest richness while in Australia, the driest site, Seven Mile, was the second richest. Community composition also differed within regions, despite the vast majority of sequences being in OTUs that were shared among sites. Variable community composition could be due to environmental variation or differences in invasion history (i.e. time since invasion, which is not known for most sites), but stochastic factors are also likely to be significant. For example, soil quality did not explain variation in soil fungi composition across Scotland (Powell et al. 2015) or variation in root and soil associates of an Australian heath species collected along a 500 km long transect (Beck et al. 2015). In both of these cases it was concluded that stochastic processes were likely to be

important in driving community assembly. Mueller et al. (2016) suggested stochastic factors were behind the differential development of fungal communities in two secondary forests in Brazil. Stochastic processes may be particularly significant on dunes, as highly disturbed ephemeral environments may preclude niche-driven community assembly (Krüger et al. 2015; Martínez-García et al. 2015). While geographic distance was not consistently related to differences in community composition, fungal dispersal can be sporadic and is unlikely to be either constant in time or equal across species (Kivlin et al. 2014; Peay and Bruns 2014). Sand deposition promotes growth in *A. arenaria* (Huiskes 1979; Konlechner et al. 2013). Sand erosion and deposition could result in fungal community turnover, with new roots becoming available and community assembly depending on the fungi arriving at the time. While some dispersal will occur continuously, and later arrivals could still be detected by sequencing, their dominance may be reduced by already established fungi (Fukami et al. 2010; Werner and Kiers 2015) resulting in the development of different communities in different dunes.

4.3. Spatial heterogeneity in A. arenaria fungal communities within dunes

There was a high degree of community turnover within dunes, which, in contrast to our hypothesis, was not consistently related to geographic distance between samples, suggesting that fungi are highly dispersed within dunes. High beta-diversity for fungal communities over small extents is commonly reported (Cheeke et al. 2015; Kjøller and Rosendahl 2014; Queloz et al. 2011; Ramirez et al. 2014; Talbot et al. 2014). The fine-grained heterogeneity we observed indicates that extensive sampling is necessary to capture the composition of fungal communities fully, and likely reflects the frequently disturbed nature of the dune environment. We found no evidence that vertical dune position influenced fungal community richness and no consistent evidence for dune position influencing fungal community composition. This lack of effect may be because the distances surveyed between the lower and upper dune regions were short. The vertical environmental gradient seen on dunes has been found to influence endophytic fungal communities in dune grass roots in the United States (David et al. 2016) and AMF in a dune in Japan (Yamato et al. 2012). However, in these instances the vertical distance surveyed was much greater and so may have spanned a greater environmental gradient, which will in turn have a consistently greater effect on fungal communities.

4.4. Host plant species influence over fungal community composition

The fungal communities associated with *A. arenaria* roots in its invasive range were richer than those in the native grasses sampled, further confirming that a diverse array of fungi can associate with non-native plants. Again, the majority of data were in OTUs shared between host species, and all of the most frequently sequenced OTUs were found in both host species. This level of sharing is unsurprising given that the host plant species are in the same family, and closely related hosts are more likely to share fungi (Gilbert and Webb 2007; Gundale et al. 2016; Wehner et al. 2014). Our hypothesis that plant host species would not impact fungal community composition was not supported, as composition differed between *A. arenaria* and the alternative host plants surveyed at each of the three dunes. While the r^2 values obtained for each comparison are low, the host species impacts on fungal community composition shown in this study contrast with results from a previous study we conducted, using the same methodology, that found no statistical evidence for differences in fungal communities between *A. arenaria* and an alternative native grass in California (Johansen et al. 2016.) Previous studies have demonstrated that host plant identity impacts fungal community assembly. AMF differed among Poaceae species in grasslands in Germany (Vályi et al. 2014) and host identity influenced both AMF and non-AMF fungal communities in herbaceous alpine perennials in Colorado, U.S.A (Becklin et al. 2012). More research is needed to understand the circumstances under which host identity influences fungal community composition (Dickie et al. 2015), and to examine differences in fungal functions between hosts. Differing fungal relationships between native and exotic plants can influence competitive relationships between them (Yang et al. 2014) and could therefore influence invasion trajectories (Pringle et al. 2009).

4.5. Fungal community profile

Most OTUs could be identified to class, with the Sordariomycetes dominant in the dune environments sampled. This class, the third most speciose of the ascomycete classes (Kirk et al. 2008), has also been found to be dominant in Amazonian tropical forest soils (Mueller et al. 2016; Peay et al. 2013). The UNITE species hypotheses reveal the extent to which some of the most common fungi are wide-ranging and flexible in terms of environment and host. However classifying OTUs by matching them to sequences in public databases remains problematic. For example two of our OTUs matched the same sequence named as *Lewia infectoria*, with 97% and 98% identity values. These OTUs may represent either different

species or intraspecific variability. The UNITE species hypothesis associated with the match has a different name from the match, and includes sequences with 28 different names. In other cases, we could not identify even frequently recovered OTUs with any confidence. While this inability may reflect a poor knowledge of the diversity of the fungal kingdom (Kõljalg et al. 2013) these OTUs do not necessarily represent unknown species. Many formally described species do not have sequences in public databases and rectifying this shortcoming would improve efforts to classify OTUs (Brock et al. 2009; Öpik et al. 2014).

Where we obtained named matches with high identity values, they reflect the high functional and phylogenetic diversity represented by the fungal community on the dunes. These matches were commonly with sequences from pathogenic fungi (e.g. *O. brassicae, P. cucumerina, M. bolleyi, L. infectoria, Sarocladium strictum, Fusarium oxysporum, Fusarium graminearum*), some of which have been recovered from a wide range of environments and hosts. However, they may not be pathogenic on the dune grasses sampled. Fungi that are pathogenic on some plants can exist as apparently harmless endophytes on others (Bonfim et al. 2016; Malcolm et al. 2013). There were also matches with the saprotrophic dune specialists *Psathyrella ammophila* and *Peziza ammophila*.

Only two Glomeromycota matches, which may be AMF, were recorded as frequently sequenced. This fungal group is considered to be functionally important in dunes and many morphologically described AMF have been found in this environment (Koske et al. 2004). Molecular techniques have previously recovered Glomeromycota extensively associating with dune grass roots, including those of the plant host species used in this study (Johansen et al. 2015; Johansen et al. 2016; Rodríguez-Echeverría and Freitas 2006; Yamato et al. 2012). Primer choice can heavily influence the AMF recovered from environmental surveys (Johansen et al. 2016, Kohout et al. 2014) and the general fungal primer we used may not be optimal for recovering the group. Studies targeting this group typically use the small sub unit (SSU) rDNA region. While we have recovered more AMF OTUs in the dune environment than 'virtual taxa' recognized via the SSU region, and more OTUs than morphospecies currently described (Öpik et al. 2014; Öpik et al. 2016), this may reflect the high variability within the ITS region that can occur within AMF taxa (Stockinger et al. 2010; Stockinger et al. 2009).

5. Conclusion

We demonstrate that many of the same root-associated fungi can be detected in coastal dunes, associated with dune grasses, in both the Northern and Southern Hemispheres. Consequently, invasive plants may encounter familiar fungi as they expand their ranges even if they cross vast geographic distances. In addition, there is evidence that invasive species can associate with local fungi whose distribution may be more restricted so that altogether their root-associated fungal communities are not necessarily depauperate in comparison to those in their native range. With the vast majority of our data in shared OTUs, both among and within regions, dispersal limitation does not seem to be prevalent among dominant members of the fungal communities associating with *A. arenaria* roots. However dispersal may be sufficiently inconsistent in time and space to explain some of the multi-scale heterogeneity we observed. Differences in temperature and soil resource levels correlated with differences in community composition at large extents, suggesting that they influence dune root associates. While native dune grasses co-occurring with *A. arenaria* shared most of this plant's fungal associates, host plant identity consistently influenced fungal community composition, showing this can be important for even closely related plants. The predominance of potential pathogens in apparently healthy roots, and the regular recovery of OTUs without matches to sequences from described organisms, highlights a lack of knowledge of fungal community function and composition in natural ecosystems.

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References

Abarenkov, K., Nilsson, R.H., Larsson, K.H., Alexander, I.J., Eberhardt, U., Erland, S., Høiland, K., Kjøller, R., Larsson, E., Pennanen, T., Sen, R., Taylor, A.F.S., Tedersoo, L., Ursing, B.M., Vrålstad, T., Liimatainen, K., Peintner, U., Kõljalg, U., 2010. The UNITE database for molecular identification of fungi - recent updates and future perspectives. New Phytologist 186, 281-285.

Aguilar-Trigueros, C.A., Rillig, M.C., 2016. Effect of different root endophytic fungi on plant community structure in experimental microcosms. Ecology and Evolution 6, 8149-8158.

Anderson, M.J., 2001. A new method for non-parametric multivariate analysis of variance. Austral Ecology 26, 32-46.

Anderson, M.J., 2006. Distance-based tests for homogeneity of multivariate dispersions. Biometrics 62, 245-253.

Andrei, A.S., Robeson, M.S., Baricz, A. Coman, C., Muntean, V., Ionescu, A., Etiope, G. Alexe, M., Sicora, C.I., Podar, M., Banciu, H.L., 2015. Contrasting taxonomic stratification of microbial communities in two hypersaline meromictic lakes. ISME Journal 9, 2642-2656.

Baas Becking, L.G.M., 1934. Geobiologie of Inleiding tot de Milieukunde. W.P. van Stockum & Zoon, The Hague, The Netherlands.

Bahram, M., Peay, K.G., Tedersoo, L., 2014. Local-scale biogeography and spatiotemporal variability in communities of mycorrhizal fungi. New Phytologist 205, 1454-63.

Barrett, L.G., Kniskern, J.M., Bodenhausen, N., Zhang, W., Bergelson, J., 2009. Continua of specificity and virulence in plant host-pathogen interactions: Causes and consequences. New Phytologist 183**,** 513-529.

Beck, S., Powell, J.R., Drigo, B., Cairney, J.W.G., Anderson, I.C., 2015. The role of stochasticity differs in the assembly of soil- and root-associated fungal communities. Soil Biology and Biochemistry 80, 18-25.

Becklin, K.M., Hertweck, K.L., Jumpponen, A., 2012. Host identity impacts rhizosphere fungal communities associated with three alpine plant species. Microbial Ecology 63, 682-693.

Bell, A., 1987. Alien dune plants reshape our beaches. ECOS 54, 3-6.

Benjamini, Y., Hochberg,Y., 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. Journal of the Royal Statistical Society Series B 57, 289-300.

Bonfim, J.A., Vasconcellos, R.L.F., Baldesin, L.F., Sieber, T.N., Cardoso, E.J.B.N., 2016. Dark septate endophytic fungi of native plants along an altitudinal gradient in the Brazilian Atlantic forest. Fungal Ecology 20, 202-210.

Brock, P.M., Döring, H., Bidartondo, M.I., 2009. How to know unknown fungi: the role of a herbarium. New Phytologist 181, 719-724.

Bufford, J. L., Hulme, P. E., Sikes, B. A., Cooper, J. A., Johnston, P. R., Duncan, R. P., 2016. Taxonomic similarity, more than contact opportunity, explains novel plant–pathogen associations between native and alien taxa. New Phytologist 212, 657-667.

Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., Fierer, N., Pẽa, A.G., Goodrich, J.K., Gordon, J.I., Huttley, G.A., Kelley, S.T., Knights, D., Koenig, J.E., Ley, R.E., Lozupone, C.A., McDonald, D., Muegge, B.D., Pirrung, M., Reeder, J., Sevinsky, J.R., Turnbaugh, P.J., Walters, W.A., Widmann, J.,

Yatsunenko, T., Zaneveld, J., Knight, R., 2010. QIIME allows analysis of high-throughput community sequencing data. Nature Methods 7, 335-336.
Carlsen, T., Aas, A.B., Lindner, D., Vrålstad, T., Schumacher, T., Kauserud, H., 2012. Don't make a mista(g)ke: is tag switching an overlooked source of error in amplicon pyrosequencing studies? Fungal Ecology 5, 747-749.
Cheeke, T.E., Schütte, U.M., Hemmerich, C.M., Cruzan, M.B., Rosenstiel, T.N., Bever, J.D., 2015. Spatial soil heterogeneity has a greater effect on symbiotic arbuscular mycorrhizal fungal communities and plant growth than genetic modification with *Bacillus thuringiensis* toxin genes. Molecular Ecology 24, 2580-2593.
Coats, V. C., Rumpho, M. E., 2014. The rhizosphere microbiota of plant invaders: An overview of recent advances in the microbiomics of invasive plants. Frontiers in Microbiology, 5, 368.
Cox, F., Newsham, K.K., Bol, R., Dungait, J.A.J., Robinson, C.H., 2016. Not poles apart: Antarctic soil fungal communities show similarities to those of the distant Arctic. Ecology Letters, 19, 528-536.
David, A.S., Seabloom, E.W., May, G., 2016. Plant host species and geographic distance affect the structure of aboveground fungal symbiont communities and environmental filtering affects belowground communities in a coastal dune ecosystem. Microbial Ecology 71, 912-926.
Davison, J., Moora, M., Öpik, M. Adholeya, A., Ainsaar, L., Bâ, A., Burla, S., Diedhiou, A. G., Hiiesalu, I., Jairus, T., Johnson, N.C., Kane, A., Koorem, K., Kochar, M., Ndiaye, C., Pärtel, M., Reier, Ü, Saks, Ü, Singh, R., Vasar, M., Zobel, M., 2015. Global assessment of arbuscular mycorrhizal fungus diversity reveals very low endemism. Science 349, 970-973.
Day, N.J., Dunfield, K.E., Antunes, P.M., 2015. Temporal dynamics of plant–soil feedback and root-associated fungal communities over 100 years of invasion by a non-native plant. Journal of Ecology 103**,** 1557-1569.
De Cáceres, M.D., Legendre, P., 2009. Associations between species and groups of sites: indices and statistical inference. Ecology 90, 3566-3574.
De Cáceres, M.D., Legendre, P., Moretti, M., 2010. Improving indicator species analysis by combining groups of sites. Oikos 119, 1674-1684.
Dickie, I.A., Alexander, I., Lennon, S., Dickie, I.A., Alexander, I., Lennon, S., Öpik, M., Selosse, M.A., van der Heijden, M.G.A., Martin, F.M., 2015. Evolving insights to understanding mycorrhizas. New Phytologist 205, 1369-1374.
Dixon, P., Hilton, M., Bannister, P., 2004. *Desmoschoenus spiralis* displacement by *Ammophila arenaria*: The role of drought. New Zealand Journal of Ecology 28, 207-213.
Dostalek, T., Pankova, H., Munzbergova, Z., Rydlova, J., 2013. The effect of AMF suppression on plant species composition in a nutrient-poor dry grassland. PLoS ONE 8, 1-11.
Edgar, R.C., 2010. Search and clustering orders of magnitude faster than BLAST. Bioinformatics 26, 2460-2461.
Edgar, R.C., 2013. UPARSE: highly accurate OTU sequences from microbial amplicon reads. Nature 10, 996-1000.
Faith, D.P., Minchin, P.R., Belbin, L., 1987. Compositional dissimilarity as a robust measure of ecological distance. Vegetatio 69, 57-68.
Fröhlich-Nowoisky, J., Burrows, S.M., Xie, Z., Engling, G., Solomon, P.A., Fraser, M.P., Mayol-Bracero, O.L., Artaxo, P., Begerow, D. Conrad, R., Andreae, M.O., Després,

V.R. and Pöschl, U., 2012. Biogeography in the air: Fungal diversity over land and oceans. Biogeosciences 9, 1125-1136.
Fukami, T., Dickie, I.A., Wilkie, P.J., Paulus, B.C., Park, D., Roberts, A., Buchanan, P.K., Allen, R.B., 2010. Assembly history dictates ecosystem functioning: Evidence from wood decomposer communities. Ecology Letters 13, 675-684.
Gardes, M., Bruns, T.D., 1993. ITS primers with enhanced specificity for basidiomycetes-application to the identification of mycorrhizae and rusts. Molecular Ecology 2, 113-118.
Geml, J., Gravendeel, B., van der Gaag, K.J., Neilen, M., Lammers, Y., Raes, N., Semenova, T.A., Knijff, P.d., Noordeloos, M.E., 2014. The contribution of DNA metabarcoding to fungal conservation: diversity assessment, habitat partitioning and mapping red-listed fungi in protected coastal *Salix repens* communities in the Netherlands. PLoS ONE 9, e99852.
Gilbert, G.S., Webb, C.O., 2007. Phylogenetic signal in plant pathogen-host range. Proceedings of the National Academy of Sciences of the United States of America 104, 4979-4983.
Glynou, K., Ali T., Buch, A.K., Haghi, Kia, S., Ploch, S., Xia, X., Çelik, A., Thines, M., Maciá-Vicente, J.G., 2016. The local environment determines the assembly of root endophytic fungi at a continental scale. Environmental Microbiology 18, 2418-2434.
Goldmann, K., Schröter, K., Pena, R., Schöning, I., Schrumpf, M., Buscot, F., Polle, A., Wubet.,T., 2016. Divergent habitat filtering of root and soil fungal communities in temperature beech forests. Scientific Reports 6, 31439.
Gundale, M.J., Almeida, J.P., Wallander, H., 2016. Differences in endophyte communities of introduced trees depend on the phylogenetic relatedness of the receiving forest. Journal of Ecology 104, 1219-1232.
Hazard, C., Gosling, P., Van Der Gast, C.J., Mitchell, D.T., Doohan, F.M., Bending, G.D., 2013. The role of local environment and geographical distance in determining community composition of arbuscular mycorrhizal fungi at the landscape scale. ISME Journal 7, 498-508.
Huiskes, A.H.L., 1979. Biological Flora of the British Isles. Journal of Ecology 67, 363-382.
Johansen, R.B., Vestberg, M., Burns, B.R., Park, D., Hooker, J.E., Johnston, P.R., 2015. A coastal sand dune in New Zealand reveals high arbuscular mycorrhizal fungal diversity. Symbiosis 66, 111-121.
Johansen, R.B., Johnston, P., Mieczkowski, P., Perry, G.L.W., Robeson, M.S., Burns, B.R., Vilgalys, R., 2016. A native and an invasive dune grass share similar, patchily distributed, root-associated fungal communities. Fungal Ecology 23, 151-155.
Keane, R. M., Crawley, M. J., 2002. Exotic plant invasions and the enemy release hypothesis. Trends in Ecology and Evolution 17, 164-170.
Kernaghan, G., Patriquin, G., 2015. Diversity and host preference of fungi co-inhabiting *Cenococcum* mycorrhizae. Fungal Ecology, 17, 84-95.
Kirk, P.M., Cannon, P.F., Minter, D.W., Stalpers, J.A., 2008. Aisnworth & Bisby's Dictionary of the Fungi, tenth ed. CABI Europe-UK, UK.
Kivlin, S.N., Winston, G.C., Goulden, M.L., Treseder, K.K., 2014. Environmental filtering affects soil fungal community composition more than dispersal limitation at regional scales. Fungal Ecology 12, 14-25.
Kjøller, R., Rosendahl, S., 2014. Cultivated and fallow fields harbor distinct communities of Basidiomycota. Fungal Ecology 9, 43-51.
Kohout, P., Sudová, R., Janoušková, M., Ctvrtlíková, M., Hejda, M., Pánková, H., Slavíková,

R., Stajerová, K., Vosátka, M., Sýkorová Z., 2014. Comparison of commonly used primer sets for evaluating arbuscular mycorrhizal fungal communities: Is there a universal solution? Soil Biology and Biochemistry 68, 482-493.

Kõljalg, U., Nilsson, R.H., Abarenkov, K., Tedersoo, L., Taylor, A.F.S., Bahram, M., Bates, S.T., Bruns, T.D., Bengtsson-Palme, J., Callaghan, T.M., Douglas, B., Drenkhan, T., Eberhardt, U., Dueñas, M., Grebenc, T., Griffith, G.W., Hartmann, M., Kirk, P.M., Kohout, P., Larsson, E., Lindahl, B.D., Lücking, R., Martín, M.P., Matheny, P.B., Nguyen, N.H., Niskanen, T., Oja, J., Peay, K.G., Peintner, U., Peterson, M., Põldmaa, K., Saag, L., Saar, I., Schüßler, A., Scott, J.A., Senés, C., Smith, M.E., Suija, A., Taylor, D.L., Telleria, M.T., Weiss, M., Larsson, K.- H., 2013. Towards a unified paradigm for sequence-based identification of fungi. Molecular Ecology 22, 5271-5277.

Konlechner, T.M., Hilton, M.J., Orlovich, D.A., 2013. Accommodation space limits plant invasion: *Ammophila arenaria* survival on New Zealand beaches. Journal of Coastal Conservation, 17, 463-472.

Koske, R.E., Gemma, J.N., Corkidi, L., Sigüenza, C., Rincón, E., 2004. Arbuscular mycorrhizas in coastal dunes. In: Martínez, M.L., Psuty, N. (Eds.), Coastal Dunes, vol 171. Springer, Berlin Heidelberg, pp. 173-187.

Krüger, M., Teste, F.P., Laliberté, E., Lambers, H., Coghlan, M., Zemunik, G., Bunce, M., 2015. The rise and fall of arbuscular mycorrhizal fungal diversity during ecosystem retrogression. Molecular Ecology 24, 4912-4930.

Leff, J.W., Jones, S.E., Prober, S.M., Barberán, A., Borer, E.T., Firn, J.L., Harpole, W.S., Hobbie, S.E., Hofmockel, K.S., Knops, J.M.H., McCulley, R.L., La Pierre, K.L., Risch, A.C., Seabloom, E.W., Schützn, M., Steenbock, C., Stevens, C.J., Fierer, N., 2015. Consistent responses of soil microbial communities to elevated nutrient inputs in grasslands across the globe. Proceedings of the National Academy of Sciences of the United States of America 112, 10967-10972.

Li, X., Zhang, J., Gai, J., Cai, X., Christie, P., Li, X., 2015. Contribution of arbuscular mycorrhizal fungi of sedges to soil aggregation along an altitudinal alpine grassland gradient on the Tibetan Plateau. Environmental Microbiology, 17, 2841-2857.

Lundberg, D.S., Yourstone, S., Mieczkowski, P., Jones, C.D., Dangl, J.L., 2013. Practical innovations for high-throughput amplicon sequencing. Nature Methods 10, 999-1002.

Majewska, M.L, Błaszkowski, J., Nobis, M., Rola, K., Nobis, A., Łakomiec, D., Czachura, P., Zubek, S., 2015. Root-inhabiting fungi in alien plant species in relation to invasion status and soil chemical properties. Symbiosis 65, 101-115.

Malcolm, G.M., Kuldau, G.A., Gugino, B.K., Jiménez-Gasco, M.D.M., 2013. Hidden host plant associations of soilborne fungal pathogens: An ecological perspective. Phytopathology 103, 538-544.

Martin, M., 2011. Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet 17, 10-12.

Martínez-García, L.B., Richardson, S.J., Tylianakis, J.M., Peltzer, D.A., Dickie, I.A., 2015. Host identity is a dominant driver of mycorrhizal fungal community composition during ecosystem development. New Phytologist 205, 1565-1576.

Mcginn, K.J., Van der putten, W.H., Duncan, R.P., Shelby, N., Weser, C., Hulme, P.E. (2016) *Trifolium* species associate with a similar richness of soil-borne mutualists in their introduced and native ranges. Journal of Biogeography 43, 944-954.

Millar, R.B., Anderson, M.J., Tolimieri, N., 2011. Much ado about nothings: using zero similarity points in distance-decay curves. Ecology 92, 1717-1722.

Miyamoto, Y., Sakai, A., Hattori, M., Nara, K., 2015. Strong effect of climate on ectomycorrhizal fungal composition: Evidence from range overlap between two mountains. ISME Journal 9, 1870-1879.
Mueller, R.C., Rodrigues, J.L.M., Nüsslein, K., Bohannan, B.J.M., 2016. Land use change in the Amazon rainforest favors generalist fungi. Functional Ecology 30, 1845-1853.
Nemergut, D.R., Schmidt, S.K., Fukami, T., O'Neill, S.P., Bilinski, T.M., Stanish, L.F., Knelman, J.E., Darcy, J.L., Lynch, R.C., Wickey, P., Ferrenberg, S., 2013. Patterns and processes of microbial community assembly. Microbiology and Molecular Biology Reviews 77, 342-356.
Nuñez, M. A., Horton, T. R., Simberloff, D., 2009. Lack of belowground mutualisms hinders Pinaceae invasions. Ecology 90, 2352-2359.
Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., O'Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H. H., Wagner, H., 2017. Vegan: community ecology package. R package version 2.4.2. Retrieved from: http://CRAN.R-project.org/package=vegan.
Öpik, M., Davison, J., Moora, M., Zobel, M., 2014. DNA-based detection and identification of glomeromycota: the virtual taxonomy of environmental sequences. Botany 92, 135-147.
Öpik, M., Davison, J., 2016. Uniting species- and community-oriented approaches to understand arbuscular mycorrhizal fungal diversity. Fungal Ecology 24B, 106-113.
Peay, K.G., Baraloto, C., Fine, P.V.A., 2013. Strong coupling of plant and fungal community structure across western Amazonian rainforests. ISME Journal 7, 1852-1861.
Peay, K.G., Bruns, T.D., 2014. Spore dispersal of basidiomycete fungi at the landscape scale is driven by stochastic and deterministic processes and generates variability in plant–fungal interactions. New Phytologist 204, 180-191.
Peay, K.G., Kennedy, P.G., Talbot, J.M., 2016. Dimensions of biodiversity in the earth mycobiome. Nature Reviews Microbiology 14, 434-447.
Powell, J.R., Karunaratne, S., Campbell, C.D., Yao, H., Robinson, L., Singh, B.K., 2015. Deterministic processes vary during community assembly for ecologically dissimilar taxa. Nature Communications 6, 8444.
Pringle, A., Bever, J.D., Gardes, M., Parrent, J.L., Rillig, M.C., Klironomos, J.N., 2009. Mycorrhizal symbioses and plant invasions. Annual Review of Ecology, Evolution and Systematics 40, 699-715.
Queloz, V., Sieber, T.N., Holdenrieder, O., McDonald, B.A., Grünig, C.R., 2011. No biogeographical pattern for a root-associated fungal species complex. Global Ecology and Biogeography 20, 160-169.
R-Development-Core-Team., 2016. R: A Language and Environment for Statistical Computing, Version 3.2.0. R Foundation for Statistical Computing. In Vienna, Austria.
Ramirez, K.S., Leff, J.W., Barberán, A., Bates, S.T., Betley, J., Crowther, T.W., Kelly, E.F., Oldfield, E.E., Ashley Shaw, E., Steenbock, C., Bradford, M.A., Wall, D.H., Fierer, N., 2014. Biogeographic patterns in below-ground diversity in New York City's Central Park are similar to those observed globally. Proceedings of the Royal Society B: Biological Sciences 281, 1795.
Rillig, M. C., Wendt, S., Antonovics, J., Hempel, S., Kohler, J., Wehner, J., Caruso, T., 2014. Interactive effects of root endophytes and arbuscular mycorrhizal fungi on an experimental plant community. Oecologia 174, 263-270.

Rodríguez-Echeverría, S., Freitas, H., 2006. Diversity of AMF associated with *Ammophila arenaria* ssp. *arundinacea* in Portuguese sand dunes. Mycorrhiza 16, 543-552.
Ryberg, M., 2015. Molecular operational taxonomic units as approximations of species in the light of evolutionary models and empirical data from Fungi. Molecular Ecology 24, 5770-5777.
Saks, Ü., Davison, J., Öpik, M., Vasar, M., Moora, M., Zobel, M., 2013. Root-colonizing and soil-borne communities of arbuscular mycorrhizal fungi in a temperate forest understorey. Botany 92, 277-285.
Shi, N.-N., Gao, C., Zheng, Y., Guo, L.-D., 2016. Arbuscular mycorrhizal fungus identity and diversity influence subtropical tree competition. Fungal Ecology 20, 115-123.
Spence, L. A., Dickie, I. A., Coomes, D. A., 2011. Arbuscular mycorrhizal inoculum potential: A mechanism promoting positive diversity-invasibility relationships in mountain beech forests in New Zealand? Mycorrhiza 21, 309-314.
Stockinger, H., Krüger, M., Schüßler, A., 2010. DNA barcoding of arbuscular mycorrhizal fungi. New Phytologist 187, 461-474.
Stockinger, H., Walker, C., Schüßler, A., 2009. *'Glomus intraradices* DAOM197198', a model fungus in arbuscular mycorrhiza research, is not *Glomus intraradices*. New Phytologist 183, 1176-1187.
Talbot, J.M., Bruns, T.D., Taylor, J.W., Smith, D.P., Branco, S., Glassman, S.I., Erlandson, S., Vilgalys, R., Liao, H.L., Smith, M.E., Peay, K.G., 2014. Endemism and functional convergence across the North American soil mycobiome. Proceedings of the National Academy of Sciences of the United States of America 111, 6341-6346.
Tedersoo, L., Bahram, M., Põlme, S. Kõljalg, U., Yorou, N.S., Wijesundera, R., Villarreal Ruiz, L., Vasco-Palacios, A.M. et al., 2014. Global diversity and geography of soil fungi. Science 346, 1256688.
Tedersoo, L., Sadam, A., Zambrano, M., Valencia, R., Bahram, M., 2010. Low diversity and high host preference of ectomycorrhizal fungi in Western Amazonia, a neotropical biodiversity hotspot. ISME Journal 4, 465-471.
Tejesvi, M.V., Sauvola, T., Pirttilä, A.M., Ruotsalainen, A.L., 2013. Neighboring *Deschampsia flexuosa* and *Trientalis europaea* harbor contrasting root fungal endophytic communities. Mycorrhiza 23, 1-10.
Timling, I., Walker, D.A., Nusbaum, C., Lennon, N.J., Taylor, D.L., 2014. Rich and cold: Diversity, distribution and drivers of fungal communities in patterned-ground ecosystems of the North American Arctic. Molecular Ecology 23, 3258-3272.
Vályi, K., Rillig, M.C., Hempel, S., 2014. Land-use intensity and host plant identity interactively shape communities of arbuscular mycorrhizal fungi in roots of grassland plants. New Phytologist 205, 1577-1586.
Vestergård, M., Rønn, R., Ekelund, F., 2015. Above–belowground interactions govern the course and impact of biological invasions. AoB Plants 7, plv025-plv025.
Walker, J.K.M., Phillips, L.A., Jones, M.D., 2014. Ectomycorrhizal fungal hyphae communities vary more along a pH and nitrogen gradient than between decayed wood and mineral soil microsites. Botany 92, 453-463.
Wehner, J., Powell, J.R., Muller, L.A.H., Caruso, T., Veresoglou, S.D., Hempel, S., Rillig, M. C., 2014. Determinants of root-associated fungal communities within Asteraceae in a semi-arid grassland. Journal of Ecology 102, 425-436.
Werner, G.D.A., Kiers, E.T., 2015. Order of arrival structures arbuscular mycorrhizal colonization of plants. New Phytologist 205, 1515-1524.

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

Wiedemann, A.M., Pickart, A., 1996. The *Ammophila* problem on the Northwest Coast of North America. Landscape and Urban Planning 34, 287-299.

Wu, Y.T., Wubet, T., Trogisch, S., Both, S., Scholten, T., Bruelheide, H., Buscot, F., 2013. Forest age and plant species composition determine the soil fungal community composition in a Chinese subtropical forest. PLoS ONE 8, e66829.

Yamato, M., Yagame, T., Yoshimura, Y., Iwase, K., 2012. Effect of environmental gradient in coastal vegetation on communities of arbuscular mycorrhizal fungi associated with *Ixeris repens* (Asteraceae). Mycorrhiza 22, 623-630.

Yang, R., Zhou, G., Zan, S., Guo, F., Su, N., Li, J., 2014. Arbuscular mycorrhizal fungi facilitate the invasion of *Solidago canadensis* L. in southeastern China. Acta Oecologica 61, 71-77.

Tables

Table 1: Total sequences and OTUs per site derived from *A. arenaria* roots collected from dunes in three regions. The number of OTUs that are unique, within the region, to each site and the percentage of total OTUs at each site that they represent, as well as the percentage of sequences in these OTUs, of all sequences from each site, is given.

Region/Site	Total sequences	Total OTUs	OTUs unique within region (% of total OTUs)	% of seqs in unique OTUs
UK				
Braunton Burrows	209,430	746	155 (20.78%)	0.17%
Pembrey Beach	148,016	704	151 (21.45%)	0.17%
Broadhaven South	103,479	274	40 (14.6%)	0.07%
Ynyslas Sand Dunes	242,605	652	127 (19.48%)	0.07%
Morfa Harlech	226,738	651	107 (16.44%)	0.06%
Newborough Warren	146,318	318	49 (15.41%)	0.06%
Cabin Hill	182,047	623	129 (20.71%)	0.10%
Australia				
Dennington	312,430	1,004	300 (28%)	0.15%
Rosebud	492,429	999	303 (30%)	0.12%
Lakes Entrance	772,187	1,196	331 (28%)	0.07%
Seven Mile Beach	398,979	1,027	237 (23%)	0.12%
NZ				
Kawhia Ocean Beach	1,773,284	1,476	406 (28%)	0.35%
Waikawa Beach	561,227	1,220	184 (15%)	0.21%
Pegasus Bay Beach	1,677,560	1,364	235 (14%)	0.24%
Oreti Beach	937,452	1,327	394 (30%)	0.68%

Table 2: Total sequences and OTUs per site derived from *A. arenaria* and alternative host plant roots collected from dunes in three regions. The number of OTUs unique to each host and the proportion of sequences in these OTUs, of all sequences from each host, is given.

Site/Host	Total sequences	Total OTUs	OTUs unique to host at site	Proportion total seqs in unique OTUs
UK				
Newborough Warren *A. arenaria*	146,318	318	118	0.91%
Newborough Warren *L. arenarius*	203,553	511	311	6.31%
Australia				
Lakes Entrance *A. arenaria*	772,187	1,196	489	1.61%
Lakes Entrance *S. sericeus*	827,876	957	250	0.24%
NZ				
Waikawa Beach *A. arenaria*	561,227	1,220	341	0.48%
Waikawa Beach *S. sericeus*	1,051,676	1,345	466	0.76%

Figure captions

(*Figures follow in this order)*

Fig. 1: Location of sites where dune grass roots were collected.

Fig. 2: Rarefaction curves for sites in the UK (a), Australia (b), NZ (c), and for the three regions sampled (d), showing OTU accumulation with sequencing depth. Samples are from *A. arenaria* roots and *L. arenarius* roots in (a), from *A. arenaria* and *S. sericeus* roots in (b) and (c), and from *A. arenaria* roots in (d).

Fig. 3: Number of OTUs per class recovered from roots of *A. arenaria* sampled in the UK and Australasia, from *L. arenarius* roots sampled from the UK and from *S. sericeus* roots sampled from Australasia.

Fig. 4**:** Unique and shared OTUs recovered from *A. arenaria* sampled in New Zealand, Australia and the UK.

Fig. 5: Distribution of fungal communities in *A. arenaria* roots in three regions based on 4^{th}-root transformed data using non metric multidimensional scaling. The significant predictors minimum and maximum annual temperature are correlated with each other, as are total nitrogen and pH. Stress = 0.106, $F_{2,11} = 3.333$, $r^2 = 0.377$, $p = 0.001$.

Fig. 6: nMDS ordinations of fungal communities in *A. arenaria* roots in dunes in the UK (a), Australia (b), and New Zealand (c) based on 4^{th}-root transformed data using non metric multidimensional scaling. For the UK, stress = 0.242, $F_{6,145} = 8.1184$, $r^2 = 0.251$, $p = 0.001$, for Australia stress = 0.221, $F_{3,85} = 8.1511$, $r^2 = 0.223$, $p = 0.001$, for New Zealand, stress = 0.247, $F_{3,86}$, = 8.217, $r^2 = 0.223$, $p = 0.001$. Grey lines indicate distance from each location to the polygon centroid.

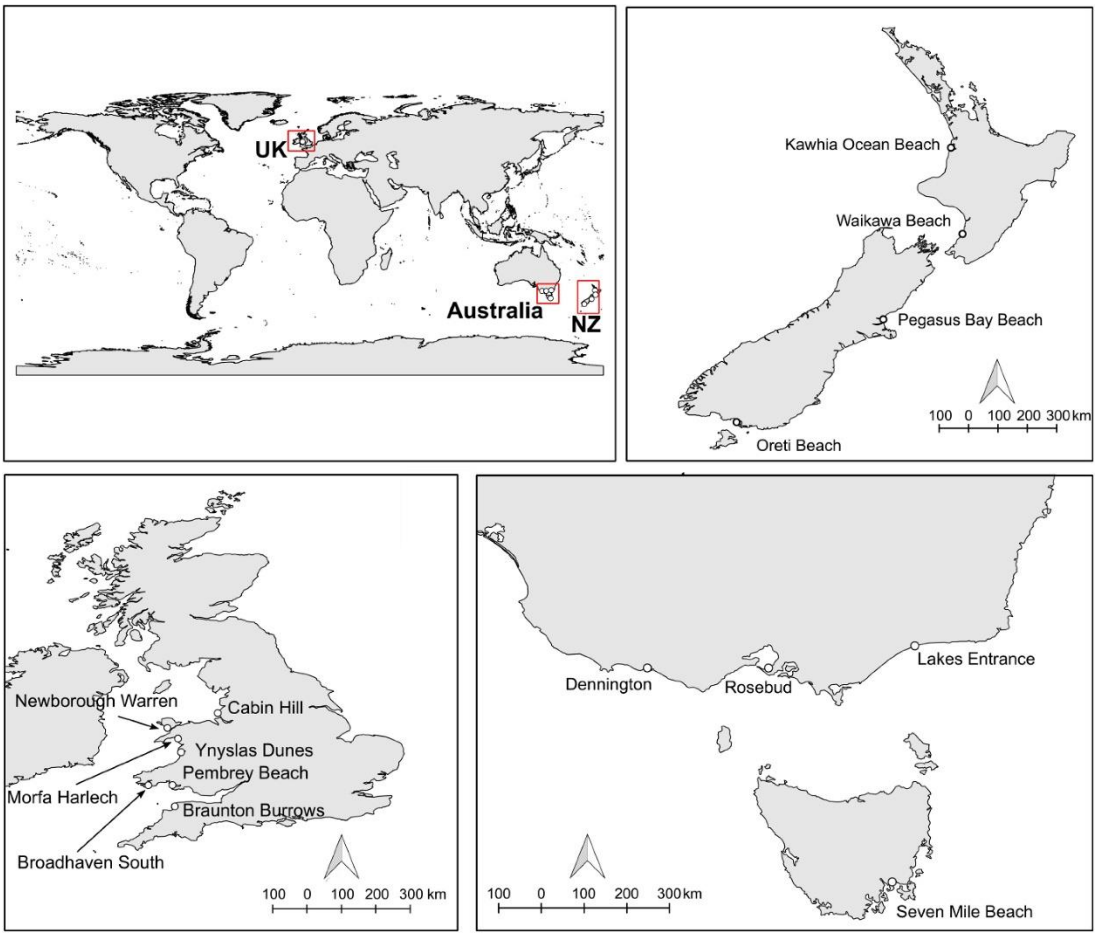
UK
Australia
NZ
Kawhia Ocean Beach
Waikawa Beach
Pegasus Bay Beach
Oreti Beach
100 0 100 200 300 km
Newborough Warren
Cabin Hill
Ynyslas Dunes
Pembrey Beach
Morfa Harlech
Braunton Burrows
Broadhaven South
100 0 100 200 300 km
Dennington
Rosebud
Lakes Entrance
Seven Mile Beach
100 0 100 200 300km

Fig. 1

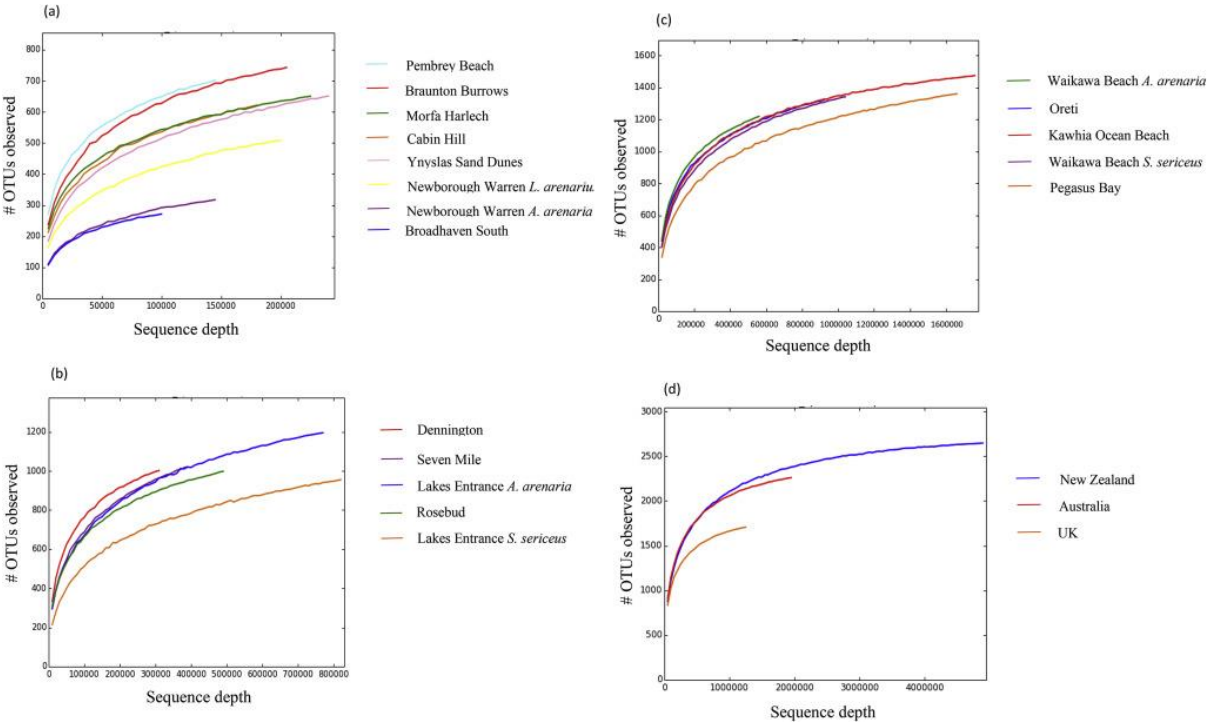
(a)
OTUs observed
Sequence depth
Pembrey Beach
Braunton Burrows
Morfa Harlech
Cabin Hill
Ynyslas Sand Dunes
Newborough Warren L. arenariu
Newborough Warren A. arenaria
Broadhaven South
(b)
OTUs observed
Sequence depth
Dennington
Seven Mile
Lakes Entrance A. arenaria
Rosebud
Lakes Entrance S. sericeus
(c)
OTUs observed
Sequence depth
Waikawa Beach A. arenaria
Oreti
Kawhia Ocean Beach
Waikawa Beach S. sericeus
Pegasus Bay
(d)
OTUs observed
Sequence depth
New Zealand
Australia
UK

Fig. 2

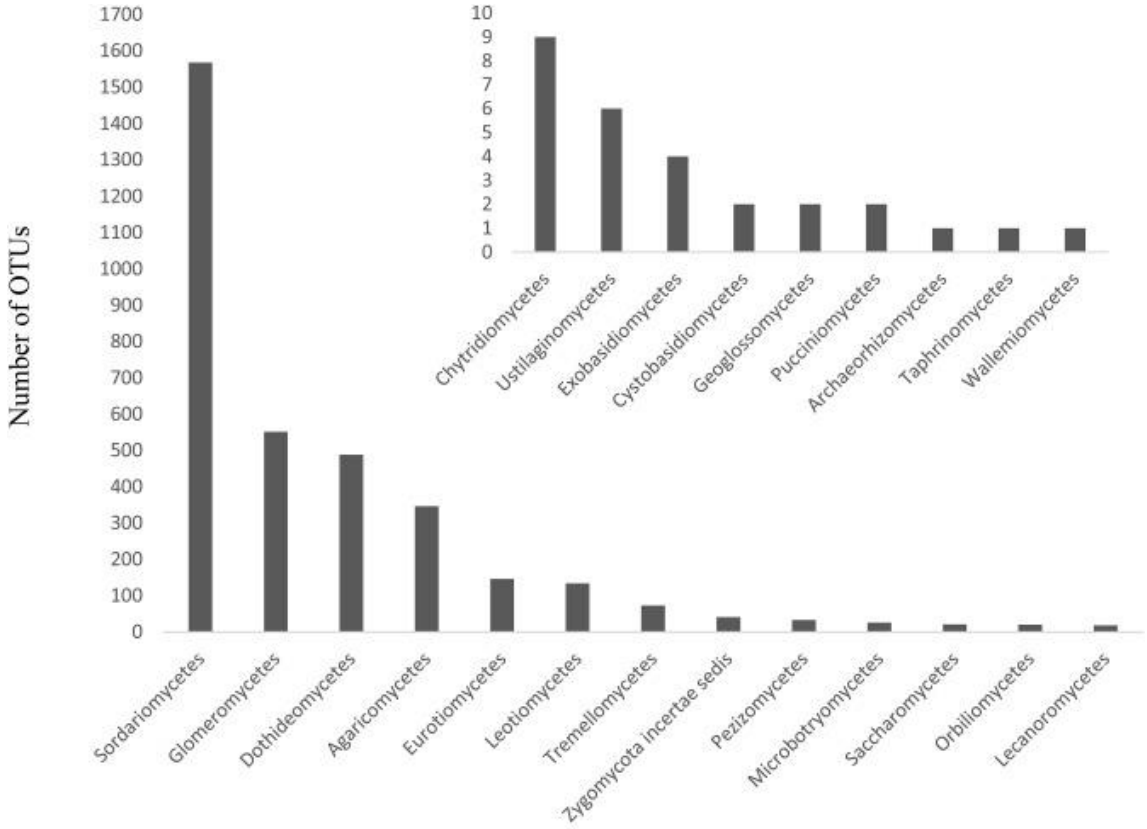
Number of OTUs
1700
1600
1500
1400
1300
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
Sordariomycetes
Glomeromycetes
Dothideomycetes
Agaricomycetes
Eurotiomycetes
Leotiomycetes
Tremellomycetes
Zygomycota incertae sedis
Pezizomycetes
Microbotryomycetes
Saccharomycetes
Orbiliomycetes
Lecanoromycetes
10
9
8
7
6
5
4
3
2
1
0
Chytridiomycetes
Ustilaginomycetes
Exobasidiomycetes
Cystobasidiomycetes
Geoglossomycetes
Pucciniomycetes
Archaeorhizomycetes
Taphrinomycetes
Wallemiomycetes
Class

Fig. 3

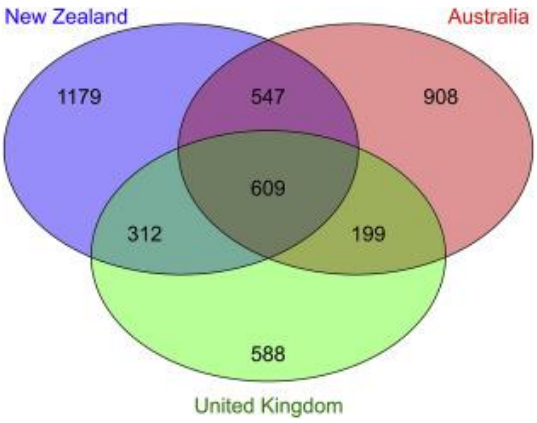
New Zealand
Australia
1179
547
908
609
312
199
588
United Kingdom

Fig. 4

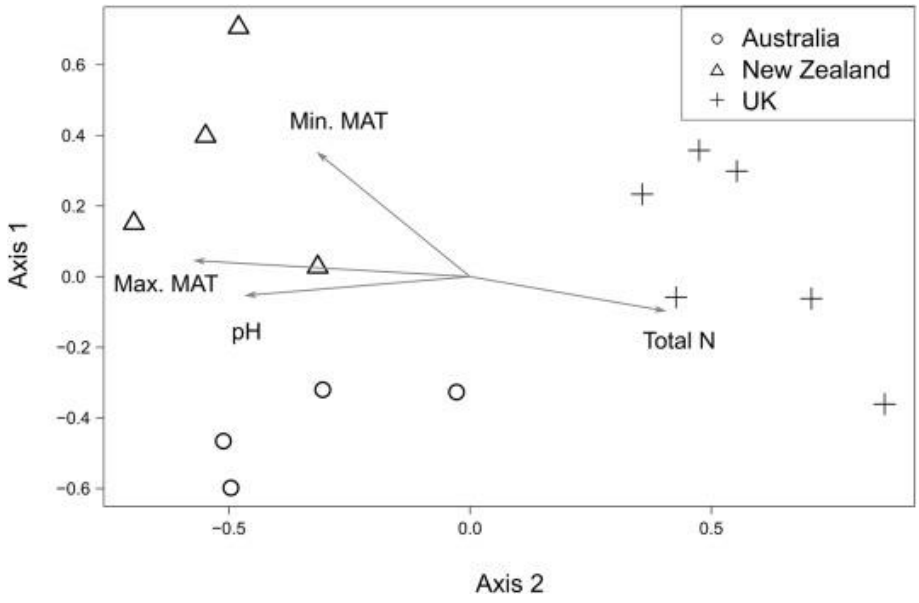
Australia
New Zealand
UK
Min. MAT
Max. MAT
pH
Total N
Axis 1
Axis 2
0.6
0.4
0.2
0.0
−0.2
−0.4
−0.6
−0.5
0.0
0.5

Fig. 5

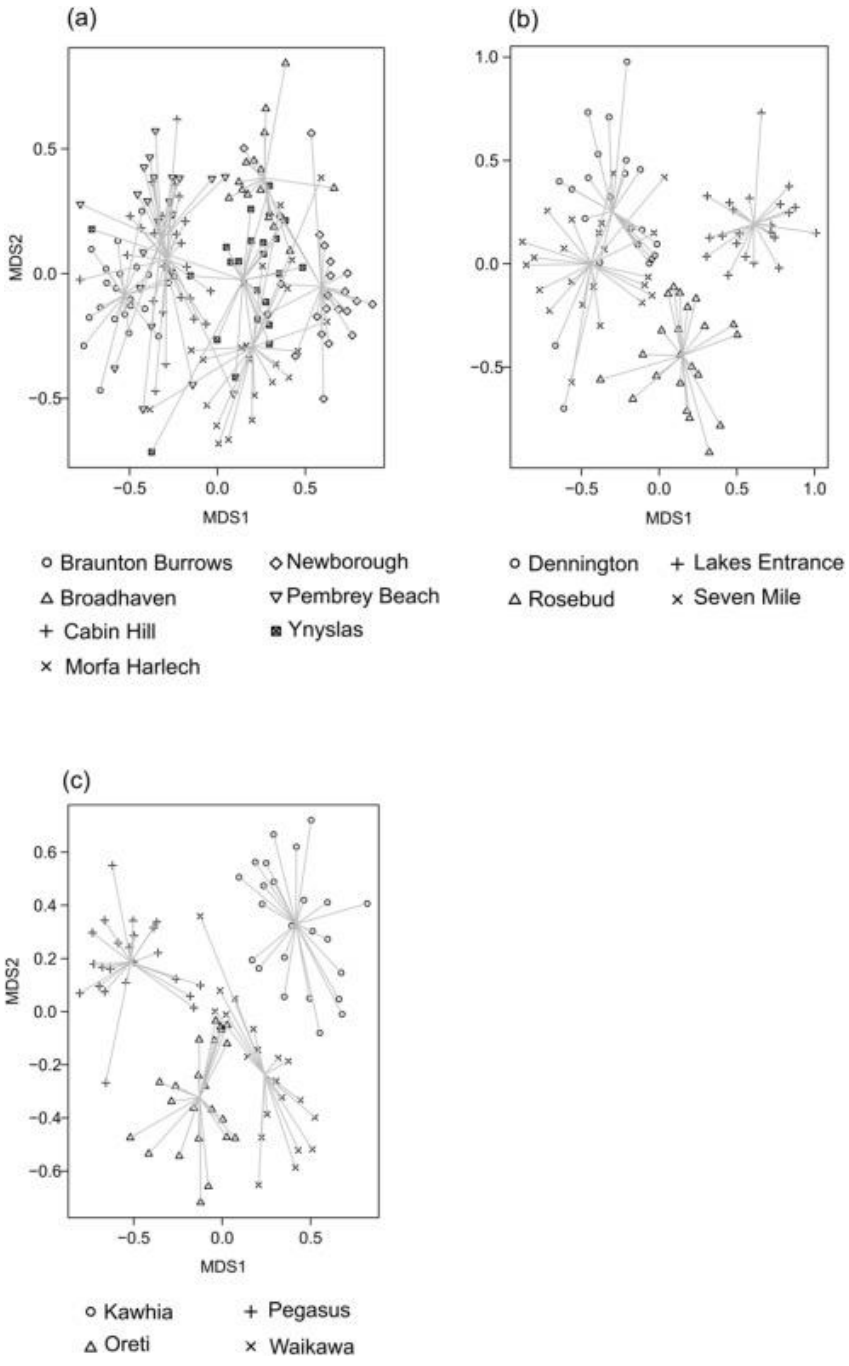
(a)
(b)
MDS1
MDS2
Braunton Burrows
Broadhaven
Cabin Hill
Morfa Harlech
Newborough
Pembrey Beach
Ynyslas
Dennington
Rosebud
Lakes Entrance
Seven Mile
(c)
Kawhia
Oreti
Pegasus
Waikawa

Fig. 6