Title

Characterization of arbuscular mycorrhizal fungal communities with respect to zonal vegetation in a coastal dune ecosystem

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Abstract

Coastal dune vegetation distributes zonally along the environmental gradients of, e.g., soil disturbance. In the preset study, arbuscular mycorrhizal fungal communities in a coastal dune ecosystem were characterized with respect to tolerance to soil disturbance. Two grass species *Elymus mollis* and *Miscanthus sinensis* distribute zonally in the seaward and landward slopes, respectively, in the primary dunes in Ishikari, Japan. The seaward slope is severely disturbed by wind, while the landward slope is stabilized by the thick root system of *M. sinensis*. The roots and rhizosphere soils of the two grasses were collected from the slopes. The soils were sieved to destruct the fungal hyphal networks, and soil trap culture was conducted to assess tolerance of the communities to disturbance with parallel analysis of the field communities using a molecular ecological tool. In the landward communities large shifts in the composition and increases in diversity were observed in the trap culture compared with the field, but in the seaward communities the impact of trap culture was minimum. The landward-field community was significantly nested within the landward-trap culture community, implying that most members in the field community did not disappear in the trap culture. No nestedness was observed in the seaward communities. These observations suggest that disturbance-tolerant fungi have been preferentially selected in the seaward slope due to severe disturbance in the habitat. Whereas a limited number of fungi, which are not necessarily disturbance-sensitive, dominate in the stable landward slope, but high-potential diversity has been maintained in the habitat.

Key words

Community structure · Glomeromycota · Nestedness · Soil disturbance · Trap culture

Introduction

Coastal dune vegetation is rich in rare species and forms a unique ecosystem. Dune soil is constantly disturbed by wind-blown sand from the sea and poor in nutrients. The distribution of plant communities in coastal dunes is zonal along the coastline due to the steep gradients in sand movement i.e. soil disturbance (Doing 1985; Moreno-Casasola 1986), salinity (Wilson and Sykes 1999), and soil pathogens (van der Putten et al. 1993) from the seaward to landward slopes of dunes. On the seaward slope where soil disturbance is severe, the plant species that are specifically adapted to the environment, e.g., *Elymus mollis* (Abe et al. 1994) and *Ammophila arenaria* (Rodríguez-Echeverría and Freitas 2006), are dominated, whereas those occur on the stable landward slope are not necessarily specific to coastal dunes.

Arbuscular mycorrhizal (AM) fungi are obligate biotrophs that form symbiotic associations with a wide range of plant species (Smith and Read 2008) and considered to play key roles in the establishment of dune vegetation (Gemma and Koske 1997). AM fungi alleviate various stresses in the plants grown in coastal dunes, e.g., nutrient deficiency (Koske and Polson 1984), salinity (Yamato et al. 2008), and soil pathogens (de la Peña et al. 2006;

Little and Maun 1996). Rodríguez-Echeverría and Freitas (2006) demonstrated that the AM fungi associating with *A. arenaria* were more diverse in the well-preserved dunes than in the degraded dunes. AM fungal diversity was higher in the early plant-successional dune than in the intermediate and late successional dunes (Sikes et al. 2012). It has been suggested that the gradients in plant community and soil chemical properties towards the sea play an important role in structuring the AM fungal communities (Abe et al. 1994; Yamato et al. 2012).

Soil disturbance is not only driving the zonal distribution of plant communities, but may also be involved in structuring the AM fungal communities in primary dunes. In arable field where soil is frequently disturbed by plowing/tillage, the fungi that readily produce abundant spores for rapid colonization are more tolerant to destruction of the hyphal networks and thus dominate (Daniell et al. 2001; Jansa et al. 2003). Applying the *r*/*K* selection theory (Pianka 1970) to AM fungi, the disturbance-tolerant fungi categorized as *r*-strategists. Whereas AM fungal *K*-strategists can be defined as those that proliferate mainly via hyphal networks, e.g., in an undisturbed grassland the dominant fungi constructed large hyphal networks, which reached up to 10 m in diameter (Rosendahl and Stukenbrock 2004) and occur at later stages of succession (Sýkorová et al. 2007). These observations suggest that the AM fungi in the seaward and landward slopes in which soil disturbance is more severe in the former are likely to have different reproductive strategies.

In previous studies, soil was directly disturbed in the field to evaluate the impact of soil disturbance on AM fungal communities (Lekberg et al. 2012; Schnoor et al. 2011). This

approach is probably most appropriate for flat and stable field, e.g., grassland and arable land, but not for topographically variable and unstable field such as coastal sand dunes, in which reproducible disturbance of root-soil structure may be technically difficult. On the other hand, the soil trap culture technique has been widely employed for AM fungal ecology (e.g., An et al. 2008; Hazard et al. 2012). The community structure revealed by this approach is usually different from that revealed by direct analysis of field samples (e.g., Sýkorová et al. 2007), probably due to difference in the growth conditions, such as temperature, soil water status, and available soil volume. It is feasible, however, to standardize the process of soil disturbance in the trap culture approach, e.g., the networks could be destructed completely by sieving, which would improve reproducibility of the experiment. In addition, tolerance/sensitivity of the fungi to disturbance i.e. the difference in reproductive strategy could be evaluated using the approach in parallel with direct analysis of the field communities that are not subjected to the artificial disturbance.

The objective of the present study is to characterize the AM fungal communities in a coastal sand dune ecosystem with respect to zonal vegetation. For the characterization, we addressed the hypothesis that the communities in the seaward and landward slopes of primary dunes would show different tolerant/sensitivity to soil disturbance. As a tool for community analysis, the PCR-clone library based approach targeting large subunit ribosomal RNA gene (LSU rDNA) of the fungi was employed.

Materials and methods

Sampling site

The sampling site was coastal sand dunes (43˚25'N, 141˚35'E) in Ishikari, Hokkaido Isl. in Japan (Online Resource Fig. S1). This area belongs to the subarctic zone, and the annual mean temperature and rainfall are 8.3˚C and 651.0 mm, respectively. The primary dunes are 2–6 m in height and located about 50–100 m inland from the coastal line. The seaward slope of the dune is 40–70 m in width and predominantly covered with *Elymus mollis* Trin. (Poaceae), which is a perennial beach grass (20–30 cm in height) that proliferates clonally by developing rhizomes. Additionally, scattered distributions of *Ixeris repens* (L.) A. Gray (Asteraceae), *Arabis stelleri* DC. var. *japonica* (A. Gray) Fr. Schm. (Brassicaceae), *Carex kobomugi* Ohwi (Cyperaceae), and *Calystegia soldanella* (L.) Roem. et Schult. (Convolvulaceae) were observed. The landward slope is 80–150 m in width and largely covered with *Miscanthus sinensis* Anderson (Poaceae), which is a C_4 perennial grass (1–2 m in height) that propagates by producing seeds and widely distributed in eastern Eurasia and Pacific Asia. *Lathyrus japonicus* Willd. (Fabaceae) and *Rosa rugosa* Thunb. (Rosaceae) were also patchily distributed on the slope. The habitats of the two dominant grass species are clearly segregated between the slopes, and this typical zonal distribution is observed more than 5 km along the coastal line. The topsoil layer of the seaward slope is constantly

disturbed by wind and thus unstable. Whereas the thick root system of *M. sinensis* stabilized the landward slope, and thus an organic layer (3–5 cm) originated from *M. sinensis* litter has been developed.

Soil and root sampling

A preliminary sampling was conducted in June 2007: 3–4 kg rhizosphere soil (top 5–10 cm, 30–40 cm in diam) and root samples were collected from six plants of both *E. mollis* on the seaward slope and *M. sinensis* on the landward slope at 600 m intervals along the coastal line. The soils were used for trap culture, and the roots were used to assess mycorrhizal status of the plants in the field. A larger scale sampling was conducted in June 2008: rhizosphere soil and root samples were collected from twelve plants of each species at 300 m intervals in the same area, and these soils and roots were subjected to trap culture and direct DNA extraction, respectively. The soil samples were sieved with a 4.5-mm stainless steel mesh and stored in plastic bags at room temperature for trap culture. For *E. mollis*-root sampling, the root system of a ramet, which was connected with the above-ground part, was taken from the soil (20 cm in diam, 30 cm in depth), and about 3 g of fine roots were detached from the tap roots by scissors, collected, and stored in a plastic bag in the field. *M. sinensis* plants grown in Ishikari dune generally develop a large clump of multiple stems (30–50 cm in diam) with a dense root system in the soil, and thus root samples were collected with a soil core sampler (stainless

steel, 100 ml in vol) as follows: after removing the litter layer, two core samples were taken from each plant, and then the roots were combined (1–3 g FW), washed with tap water, and blotted on a paper towel in the laboratory. The root samples collected in 2007 were stored at –30˚C for the assessment of mycorrhizal colonization, and those collected in 2008 were frozen in liquid nitrogen immediately, freeze-dried for two days, and stored at –30˚C for DNA extraction.

Soil trap culture

In the soil trap culture experiments *M. sinensis* was used as a host plant both for the *E. mollis*- and *M. sinensis*-rhizosphere soils, because *E. mollis* seeds were unavailable. Within two weeks after soil collection, seeds of *M. sinensis* (provided by Kaiseisha Co., Ltd., Otofuke, Hokkaido) were sown onto each of the soil samples in 9-cm plastic pots (350 mL in vol) and covered with a thin layer of autoclaved river sand to avoid soil cluster formation on the surface. The seedlings were grown only with tap water in a temperature/humidity/light-controlled greenhouse (26/20˚C, day/night temperature; 60% relative humidity; 14-h day length) with steel flooring and thinned to 10 plants per pot 2–3 weeks after sowing. The plant roots (1–3 g pot^{-1}) were harvested two months after sowing from each pot separately, washed with tap water, blotted on a paper towel, and divided into two subsamples. Then one subsample was frozen in liquid nitrogen, freeze-dried, and stored

at –30˚C for DNA extraction, and the other was stored at –30˚C for the assessment of mycorrhizal colonization.

Soil chemical properties

Subsamples of the soils were air-dried in the greenhouse, crushed, and passed through a 2-mm sieve. Soil pH (H_2O) was measured at a 1: 2.5 soil: water ratio (w/v) using an electrode after shaking for 1 h at 160 rpm. Total carbon (C) and nitrogen (N) were analyzed using the Vario MAX CNS analyzer (Elementar, Tokyo). Available phosphate (Truog-P) was measured based on the vanado-molybdate method after extraction with 1 mM sulfuric acid at a 1: 200 (w/v) ratio of soil to extraction buffer (modified from Truog 1930). Exchangeable sodium (Na), potassium (K), magnesium (Mg), and calcium (Ca) were extracted with 1 M ammonium-acetic acid (pH 7.0) at a ratio of 1: 10 (w/v) and measured by flame photometry (for Na and K) or by atomic absorption photometry (for Mg and Ca).

Assessment of AM fungal colonization

The frozen root subsamples were thawed in tap water, cleared in 10% (w/v) KOH at 80˚C for 90 min, stained with 0.05% trypan blue in the lactoglycerol (lactic acid/glycerol/water = 1/1/1) at 80˚C for 30 min, and destained in the lactoglycerol at 80˚C for 30 min. The percentage

colonization of the roots was estimated under microscope with 10–40 magnification by the gridline intersect method (Giovannetti and Mosse 1980).

LSU rDNA sequencing

The freeze-dried whole root sample (about 0.3 g DW) was first cut into fine segments (less than 3 mm) by scissors and mixed thoroughly in a plastic tube. Then 10–20 mg subsample was transferred to a 2-mL tube with an O-ring sealed cap (Yasui Kikai, Osaka) and ground using Multi-Beads Shocker (Yasui Kikai) with a metal cone at 2500 rpm for 2 × 60 s at room temperature. DNA was extracted from the ground samples using DNeasy Plant Mini Kit (Qiagen, Tokyo) according to the manufacturer's instructions, and stored at −30°C. A part of large subunit ribosomal RNA gene (LSU rDNA) was amplified in a 25 µL reaction mixture of Expand High-Fidelity PLUS PCR System (Roche Diagnostics, Tokyo), 0.5 nmol μL^{-1} each of the forward LR1 (van Tuinen et al. 1998) and reverse FLR2 (Trouvelot et al. 1999) primers, and 0.1–1 µL template DNA. PTC-225 DNA Engine Tetrad thermal cycler (MJ Research, Tokyo) was used for amplification with the following program: initial denaturation at 94˚C for 2 min, followed by 30 cycles of denaturation at 94°C for 15 s, annealing at 50˚C for 40 s, and polymerization at 72°C for 80 s, and final extension at 72°C for 10 min. The PCR products were analyzed by electrophoresis on 1.5% agarose gels and visualized on Safe Image Blue-Light Transilluminator (Invitrogen, Tokyo) after staining with SYBR Safe DNA

gel stain (Invirtogen). Since the sizes of PCR products from AM fungal LSU rDNA were expected to be 680–780 bp, those shorter than 680 bp were excluded by cutting the gel if considerable amounts of short fragments were observed in electrophoresis. Each PCR products was purified with MonoFas DNA purification Kit (GL Sciences, Tokyo) and cloned into pT7Blue T-vector (Novagen/Merch, Tokyo) according to the manufacturer's instructions. Nucleotide sequences of an average of 25 clones randomly chosen from each library were determined using BigDye Terminator v3.1 Cycle Sequencing Kit with ABI PRISM 3130*xl* Genetic Analyzer (Applied Biosystems, Tokyo). LSU rDNA sequences were trimmed and manually edited using Vector NTI (Invitrogen).

Definition of phylotypes and phylogeny

In a preliminary analysis the DNA sequences were aligned together with several published AM fungal sequences across all families (reference sequences) using Clustal X ver. 1.81 (Thompson et al. 1997) to construct a preliminary neighbor-joining tree, and the sequences that were unlikely to belong to Glomeromycota were excluded at this step. For phylotype definition, we employed $\geq$ 95%-sequence similarities in the region of LSU rDNA as a species boundary in the fungi. The AM fungal sequences were grouped based on $\geq$ 95% similarities using Sequencher v. 5.0 software (Gene Codes Corporation, Ann Arbor, MI). Representative sequences that were randomly chosen from each of the 95%-similarity groups and those did

not form a group i.e. singletons were subjected to BLAST searches, and validity of the sequences (groups), e.g., whether they were chimeric or not, was carefully assessed by comparing published sequences. To construct the neighbor-joining tree for phylogenetic analysis the representative sequences were aligned together with the reference sequences using Clustal X, and the confidence limits of each branch in the phylogeny were assessed by 1000 bootstrap replications and expressed as percentage values. The tree was displayed using NJplot software (Perriere and Gouy 1996). The tree topologies were generally in good agreement with the 95%-similarity groups with respect to the bootstrap values (> 70%), and thus each group was defined as a single phylotype. The representative sequences were deposited in DNA Data Bank of Japan (Online Resource, Table S1).

Statistical analysis

In statistical analysis of soil chemical properties, all pH data, which are logarithmic values, were transformed to real numbers before statistical treatments. Correlation analysis and Student′s *t*-test were performed with StatView (SAS institute Japan, Tokyo). Rarefaction curves were computed based both on root sample numbers and sequenced clone numbers using EstimateS 8.2 (http://viceroy.eeb.uconn.edu/estimates) (Colwell 2009) and Analytic Rarefaction 1.3 (http://www.uga.edu/strata/software/index.html) (Holland 2003), respectively. Detrended correspondence analysis (DCA) and canonical correspondence analysis (CCA)

were performed with CANOCO 4.5 (Microcomputer Power, Ithaca, NY) using the presence/absence data of phylotypes in individual samples. Rare phylotypes that were defined as those detected only from one sample were excluded in the ordination analyses. In CCA, soil chemical properties, sample collection year, AM fungal habitat/origin (seaward *E. mollis* or landward *M. sinensis* rhizosphere), host plant species (*E. mollis* or *M. sinensis*), and growth conditions (field or trap culture) were employed as environmental factors. Among these factors, AM fungal habitat/origin, host plant species, and growth conditions were represented by dummy values (1 or 0). The experimental setup and definition of these terms are summarized in Table 1. The significance of environmental factors was assessed by forward selection procedure by means of Monte Carlo permutation test (999 permutations).

Morisita-Horn similarity index was calculated with EstimateS 8.2, in which the presence/absence data of phylotypes in each sample were pooled within the same sample types and treated as frequency (abundance) data. AM fungal richness was expressed as a mean value of phylotype number per sample, and β-diversity was represented by Jaccard distance calculated based on pairwise comparison between two samples. Differences in these diversity indices were assessed by Student′s *t*-test.

Nestedness among AM fungal communities was analyzed based on nested overlap and decreasing fill (NODF) (Almeida-Neto et al. 2008) by using the NODF-program (Almeida-Neto and Ulrich 2011). In this analysis, columns (sample types) and rows (phylotypes) of a matrix were sorted according to their total incidences and abundances to

generate a maximally stacked matrix, and then NODF and weighted NODF (WNODF) of the matrix were calculated independently among the columns and among the rows based on the presence/absence and abundance data, respectively. The value of (W)NODF ranges from 0 for non-nested to 100 for fully nested matrices. The significance of nestedness was assessed by comparing the observed value of (W)NODF with the expected value obtained from 1000 matrices randomly generated under null models. We chose the fixed row and equiprobable column totals constraint null model for the randomization test based on the presence/absence data, because it is an appropriate null model for detecting patterns caused by phylotype interactions (Gotelli 2000). The fixed row and column total abundances constraint null model was chosen for the randomization test based on the abundance data because of its low Type I error rates and good power for detecting nestedness (Ulrich and Gotelli 2010). Modularity was assessed with the index *M*, which ranges from 0 for no module to 1 totally separated modules, using a simulated annealing algorithm with Netcarto (Guimerà and Amaral 2005). Significance was estimated with a Monte Carlo procedure with 100 randomizations. The presence/absence data of the bipartite (phylotype-sample type) matrix that was used in the nestedness analysis was also used in this analysis according to Mello et al. (2011).

Results

Chemical properties of rhizosphere soils and mycorrhizal status

The chemical properties of the rhizosphere soils are summarized in Table 2. Among these properties, the levels of pH were negatively correlated with those of N, C, and Mg, and the levels of N, C, Mg, and Ca were positively correlated each other (Online Resource, Table S2). The mean value of pH was significantly lower in the *M. sinensis*-rhizosphere soils than in the *E. mollis*-rhizosphere soils, whereas those of N, C, Mg, and Ca were significantly higher in the *M. sinensis*-rhizosphere soils. These properties were employed for subsequent CCA as environmental variables.

Percentages of AM fungal colonization (± SE) in the field roots were 32.7 ± 4.8 and 18.6 ± 2.5% in seaward and landward samples, respectively (n = 6). In the trap culture experiment the levels of colonization in the seaward and landward samples were 34.8 ± 6.8 (n = 6) and 55.7 ± 4.5% (n = 4), respectively, in 2007 and 35.1 ± 10.7 and 26.5 ± 4.5%, respectively, in 2008 (n = 12). Note that the plants grown in 2 pots out of the 6 of the landward-trap culture in 2007 died before harvest.

Arbuscular mycorrhizal fungal phylotypes

DNA was extracted from 12 samples for each of the seaward- and landward-field roots and from 16 (4 and 12 samples grown in 2007 and 2008, respectively) and 18 (6 and 12 samples grown in 2007 and 2008, respectively) samples for the seaward- and landward-trap cultures.

Fungal LSU rDNA was successfully amplified by one-step PCR from 10 samples of each of the field and trap culture samples, and clone libraries were raised from each PCR product (10 libraries per sample type, 40 libraries in total). Numbers of the clones for sequencing were increased until the 95% confidential interval in the rarefaction analysis reached within ± 0.4 phylotype in each sample type. In total, 135, 256, 159, and 390 AM fungal sequences were obtained for the seaward-field, seaward-trap culture, landward-field roots, and landward-trap culture, respectively. In this sequencing, non-AM fungal sequences were less than 3% of all sequences. Overall, 34 phylotypes were defined based on ≥ 95%-sequence similarities, and 32 out of the 34 types were assigned to six families: Glo1–17 in Glomeraceae, Aca1–4 in Acaulosporaceae, Div1–4 in Diversisporaceae, Gig1–2 in Gigasporaceae, Cla1–2 in Claroideoglomeraceae, and Par1–3 in Paraglomeraceae (Fig. 1). The two phylotypes Unc1 and Unc2 were likely to belong to Glomeromycota, but could not be assigned to any of the known families. Total phylotype richness was highest in the landward-trap culture (24), followed by the seaward-field (17) and seaward-trap culture (14), and lowest in the landward-field (11). The presence/absence data of the phylotypes in each sample, which were used in subsequent community analysis, were presented in Tables S3–6 in Online Resource. In this sequencing all rarefaction curves showed signs of leveling off (Online Resource, Fig. S2).

Community analysis

DCA was first applied to predict environmental factors that drive the communities (Fig. 2). The axes 1 and 2 of the sample plot explained 13.9 and 11.3% of total variance. Overall, the plots were separated into two groups along the axis 1 on the basis of AM fungal habitat/origin: one group originated from the seaward slope (*E. mollis*-rhizosphere soils) showed higher axis 1 scores, and the other originated from the landward slope (*M. sinensis*-rhizosphere soils) showed lower scores. Along the axis 2, the communities of landward-field and -trap culture were clearly separated, whereas those of the seaward-field and -trap culture were not. No clear separation between the trap culture communities using the soils collected in the different years 2007 and 2008 was observed. For further confirmation, Monte-Carlo permutation test on the collection years was conducted for the seaward- and landward-trap cultures separately, but no significant effect was observed both in the seaward ($P = 0.45$) and landward ($P = 0.49$) communities.

The unimodal method CCA was employed as a direct ordination method to assess correlations between community compositions and environmental factors, because the DCA suggested that the distributions of the AM fungal phylotypes along the environmental gradients were likely to be unimodal (the gradient lengths of axes 1 and 2 were 4.14 and 3.46, respectively). In this analysis the edaphic factors C, Mg, and Ca were represented by N, because these factors were highly correlated each other. The two main axes of CCA explained 18.3% of total variance (Fig. 3). Monte Carlo permutation test confirmed that AM fungal

habitat/origin (seaward or landward slope, $F = 3.63$, $P \leq 0.001$), host plant species (*E. mollis* or *M. sinensis*, $F = 2.93$, $P \leq 0.001$) and growth conditions (field or trap culture, $F = 2.52$, $P \leq 0.001$) were the significant environmental factors, but all the edaphic factors ($P > 0.3$) and collection year ($P = 0.468$) were not. As predicted by the DCA, the seaward slope- and landward slope-originated communities were clearly differentiated along the habitat/origin arrows as well as along the host plant arrows. The seaward-trap culture communities in which *M. sinensis* was used as a host plant were slightly shifted towards the *M. sinensis*-direction along the host plant arrows. Separation along the growth condition arrows was quite evident between the landward-field and -trap culture communities, but not between the seaward-field and -trap culture communities.

Morisita-Horn similarity indices were calculated to compare relative impact of trap culture between the seaward and landward communities. In this analysis the factor collection year for soil sampling was excluded. The indices between the field and trap culture communities were higher in the seaward communities (0.72) than in the landward communities (0.43).

AM fungal diversity (richness and β-diversity) was also analyzed to assess the relative impact of trap culture on the communities (Fig. 4). No significant impact of trap culture on the diversity was observed in the seaward communities. In contrast, both richness and β-diversity were significantly increased in the trap culture compared with the field in the landward communities.

For further characterization, nestedness analysis was conducted (Fig. 5). In the maximally stacked matrix a significant nestedness pattern was observed among the sample types (columns) based on the presence/absence data ($NODF_{column}$ = 64.2, P = 0.008). The column rank illustrates that the communities of the lower-ranking sample types (localized to the right) were nested within those of the higher-ranking sample types (localized to the left). The landward-trap culture community was located at the left end column, while the landward-field community was located at the right end column. The two seaward communities were located in between the two landward communities, although no significant nestedness was observed between the two seaward communities ($NODF_{column}$ = 64.3, P = 0.50). On the other hand, a significant nestedness pattern was also observed among the phylotypes (rows) based on the presence/absence data ($NODF_{row}$ = 54.9, P = 0.025), but not based on the abundance data ($WNODF_{row}$ = 30.2, P = 0.065). The row rank indicates that the higher-ranking phylotypes (located in the upper rows) occurred more commonly across the samples than the lower-ranking phylotypes (located in the lower rows). These results imply that many of the phylotypes occurred in the landward-field community were shared across the sample types, but their abundances were not necessarily consistent among the sample types. The matrix could also show an overview of the changes in the composition involved in the increases in the richness by the trap culture in the landward communities: among a total of 28 phylotypes occurred in both communities, 7 types were shared between the two communities, and 4 and 17 were non-shared in the field and trap culture, respectively, resulted in a net

increase of 13 types in the trap culture. In the field community 3 out of the 4 non-shared types were shared with the seaward communities, whereas 7 out of the 17 non-shared types in the trap culture community were shared with the seaward communities.

In modularity analysis, no significant pattern was observed among the sample types and also among the phylotypes ($P > 0.05$): M value of the original matrix (0.284) was not higher than that of the randomized matrices (0.309).

Discussion

In the present study, the ordination analyses revealed clear differentiation of AM fungal community compositions between the two habitats seaward and landward slopes, which is highly likely to be a reflection of the zonal distribution in which physical, chemical, and biological (including host plant species) environments were largely different. Edaphic factors, in particular pH (An et al. 2008; Dumbrell et al. 2010b) and available phosphate (Cheng et al. 2012), are generally strong drivers for AM fungal communities, although the CCA showed that none of the edaphic factors were significant in the present study. The soil chemical properties, however, were distinctively different between the two slopes, suggesting that the edaphic factors were represented by the factor AM fungal habitat/origin and thus were not significant. The CCA also indicated that host plant is a significant factor for driving the

communities. Host preference in AM fungal associations has been reported in previous studies (e.g., Vandenkoornhuyse et al. 2003), whereas lack of the preference has also been reported (e.g., Santos et al. 2006). In the CCA the host plant arrows were highly correlated with the habitat arrows i.e. these arrows pointed in almost the same directions. These results suggest that the large separation between the seaward and landward communities along the host plant arrows may reflect the long-term effect of the selection not only by the host plants but also by the habitats on the communities. On the other hand, slight shifts in the composition along the host plant arrows were also observed between the seaward-field and -trap culture communities. It seems likely that these shifts may represent a short-term selective effect of *M. sinensis* on the seaward communities that originally associated with *E. mollis* in the field.

The trap culture approach in parallel with the direct analysis of field samples enabled us to characterize the seaward and landward communities in terms of tolerance/sensitivity to soil disturbance. The seaward communities were unresponsive i.e. tolerant to trap culture, which is based on the observations that i) shifts in the community compositions were minimum as shown by the CCA, ii) the higher similarity index between the field and trap culture communities, and iii) no significant change in the diversity. It has been known that the intensification of land use and disturbance preferentially select disturbance-tolerant fungi (König et al. 2010; Oehl et al. 2003) i.e. the *r*-strategists. These observations suggest that the *r*-strategists are preferentially selected in the seaward slope due to severe soil disturbance in

the habitat.

In contrast to the seaward communities, the ordination and similarity analyses indicated that the trap culture had a significant impact on the landward communities i.e. the shifts in the community composition were much larger in the landward communities than in the seaward communities. However, the subsequent nestedness/modularity analyses suggest that the fungi in the landward communities were not necessarily disturbance-sensitive: the landward-field community was not a specific group (module) but a subset of the landward-trap culture community, although the abundance of the shared phylotypes was different between the two communities. These results imply that the large shifts of the landward communities by the trap culture were unlikely due to replacement of disturbance-sensitive fungi by disturbance-tolerant fungi, but likely due to i) appearance of the members hidden in the field and ii) the changes in the abundance of the shared phylotypes. Lekberg et al. (2012) observed high resilience of the AM fungal communities after severe disturbance in a semi-natural grassland and suggested that they were unexpectedly disturbance-tolerant. These observations suggest that most of the field members in the landward habitat were to some extent disturbance-tolerant: they are able to colonize the roots rapidly and dominate in later stages, probably not only via their hyphal networks but also by a spore, which are not fully consistent with the features of the *K*-strategist that we expected.

The trap culture increased phylotype richness in the landward communities. This implies that the landward-field communities were potentially highly diverse, although the

rarefaction analysis suggested that our sampling provided a reasonable coverage of the richness. In the field, only a limited number of the phylotypes that are highly adapted to the environment dominated in the landward slope as indicated by the lower richness and β-diversity in the field communities. Applying the intermediate disturbance hypothesis by Connell (1978), the destruction of hyphal networks by the trap culture might reset competition among the fungi, which could increase AM fungal richness via providing an equal chance to colonize the roots not only for the dominants but also for the rare species. In this context, the trap culture in which destruction of hyphal networks was conducted only once at the beginning may represent the intermediate level of disturbance. On the other hand, Hazard et al. (2012) observed higher AM fungal richness in the field roots than in the trap culture in *Trifolium repens* and suggested that longer exposure of the roots to larger quantity of AM fungal propagule in the field might be a reason for the higher richness. Schnoor et al. (2011) suggested that the decreases in fungal richness by soil disturbance (plowing) in a semi-natural grassland were due to disappearance of the disturbance-sensitive fungi that require a longer culture period to occur. In the present study, the nestedness analysis revealed that most of the fungi that occurred in the field did not disappear in the trap culture, probably because they are disturbance-tolerant. In addition, it seems likely that AM fungal propagule has been supplied from the neighboring habitats that differ in successional stage (Sikes et al. 2012), such as the seaward slope and inner land dunes, to the landward slope. This idea is supported by the observations that some of the phylotypes that were responsible for the net increase in the

richness were shared with the seaward communities, and the rest of the phylotypes were unique in the trap culture.

The trap culture also increased β-diversity in the landward communities. Increases in β-diversity by a disturbance treatment were also observed in semi-natural grasslands (Lekberg et al. 2012; Schnoor et al. 2011). The mechanism underlying could be interpreted by the stochastic processes of fungal colonization as proposed by Dumbrell et al. (2010a). In the landward communities the reset of competition (destruction of hyphal networks by the trap culture) might reduce competitiveness of the field dominants (Jasper et al. 1991). Thereafter, fungal colonizers were selected through stochastic processes, leading to the development of patchy distribution of the species i.e. increases in β-diversity (Chagnon et al. 2012).

The present study characterized the AM fungal communities with respect to zonal distribution in a coastal sand dune ecosystem. The communities in the seaward slope were rich in the *r*-strategists that are disturbance-tolerant. Whereas the communities in the landward slope were dominated by a limited number of fungi that were not necessarily disturbance-sensitive, but maintained high-potential diversity. Interestingly, the AM fungal assemblage observed in the present study was similar to that associated with the dune grass *A. arenaria* in Denmark (Błaszkowski and Czerniawska 2011), although they employed the spore morphology-based approach (data not shown). The similarity between the two assemblages supports, at least partially, the idea that AM fungal community compositions among the same habitat types are similar to each other on a global scale (Öpik et al. 2006). To

examine the idea, geographically distant coastal dunes could be a model ecosystem to test the linkage between AM fungal assemblages and habitat types, because dune ecosystems share similarities not only in the soil chemical and physical properties but also in the plant community, which is one major driver for AM fungal communities (Johnson et al. 2004).

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1

Table 1 Experimental setup and definition of terms.

Sample type	AM fungal habitat/origin	Host plant	Collected sample	Growth condition	Purpose (no. of sample)	
					2007 collection	2008 collection
Seaward field	Seaward slope	*E. mollis*	Roots	Field	Assessment of colonization (6)	Direct DNA extraction (12)
Seaward-trap culture	Seaward slope	*M. sinensis*	Rhizosphere soil	Trap culture	Assessment of colonization & DNA extraction (6)	Assessment of colonization & DNA extraction (12)
Landward field	Landward slope	*M. sinensis*	Roots	Field	Assessment of colonization (6)	Direct DNA extraction (12)
Landward-trap culture	Landward slope	*M. sinensis*	Rhizosphere soil	Trap culture	Assessment of colonization & DNA extraction (6)	Assessment of colonization & DNA extraction (12)

Table 2 Chemical properties of the rhizosphere soils of *E. mollis* and *M. sinensis* collected from seaward and landward slopes of Ishikari primary dunes.

Plant species/habitat	pH (H_2O)[a]	Truog-P (mg kg^{-1})	Total N (g kg^{-1})	Total C (g kg^{-1})	Na (mg kg^{-1})	K (mg kg^{-1})	Mg (mg kg^{-1})	Ca (mg kg^{-1})
E. mollis (seaward slope)								
Range (min–max)	7.0–9.0	7.9–28	0.05–0.26	0.8–2.6	140–209	120–283	246–279	155–216
Mean	7.5	15	0.18	1.4	140	193	246	155
M. sinensis (landward slope)								
Range (min–max)	5.9–7.3	10–29	0.19–0.63	2.3–8.5	94.3–172	150–202	268–400	203–574
Mean	6.5	18	0.40	5.1	128	174	316	348
t-Test[b]	***	ns	***	***	ns	ns	*	**

[a]The pH values were transformed to real numbers before calculating mean values.

[b]Student's *t*-test was performed between the values of *E. mollis*- and *M. sinensis*-rhizosphere soils: ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$; ns, not significant.

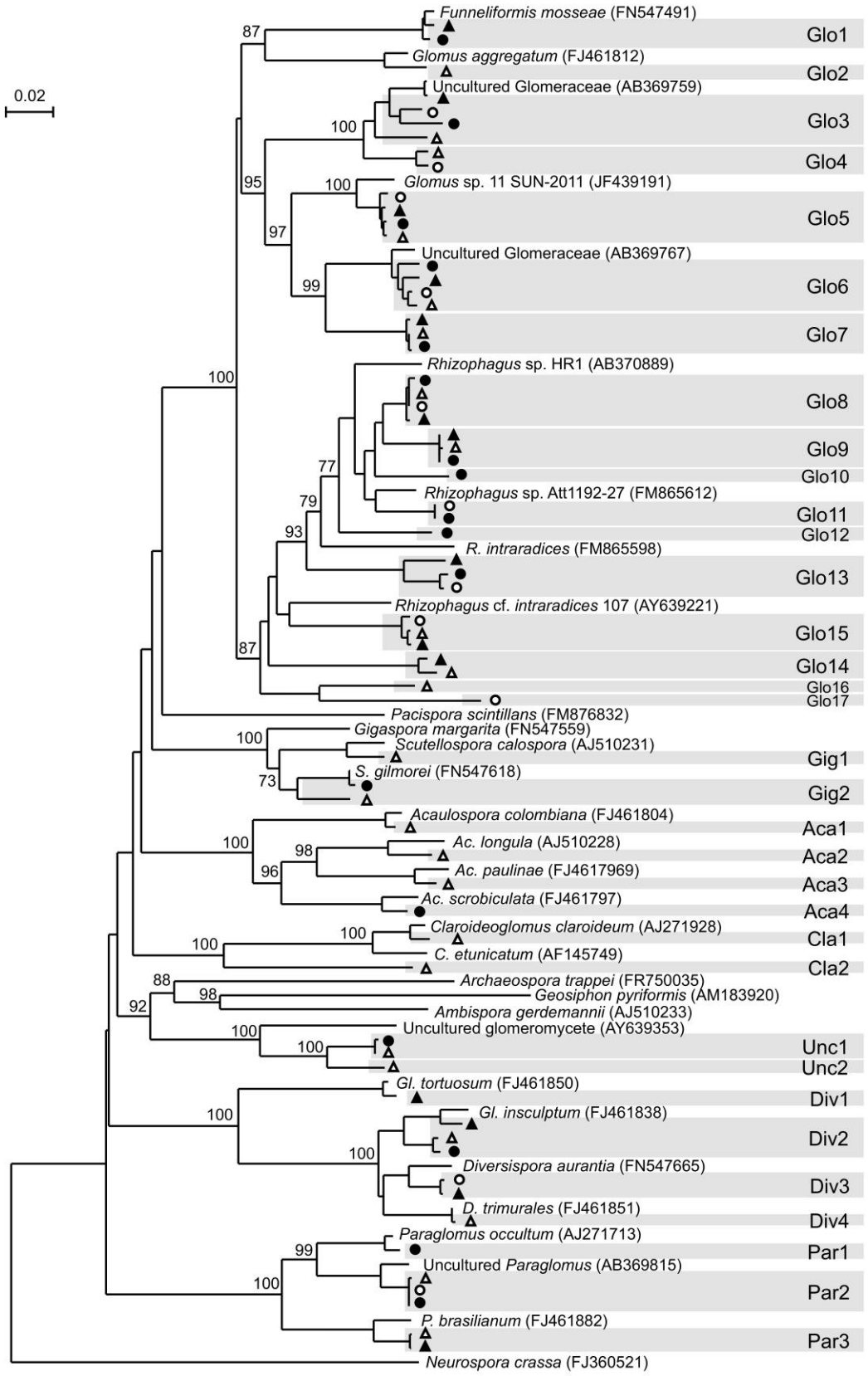

0.02
Funneliformis mosseae (FN547491)
Glo1
Glomus aggregatum (FJ461812)
Glo2
Uncultured Glomeraceae (AB369759)
Glo3
Glo4
Glomus sp. 11 SUN-2011 (JF439191)
Glo5
Uncultured Glomeraceae (AB369767)
Glo6
Glo7
Rhizophagus sp. HR1 (AB370889)
Glo8
Glo9
Glo10
Rhizophagus sp. Att1192-27 (FM865612)
Glo11
Glo12
R. intraradices (FM865598)
Glo13
Rhizophagus cf. intraradices 107 (AY639221)
Glo15
Glo14
Glo16
Glo17
Pacispora scintillans (FM876832)
Gigaspora margarita (FN547559)
Scutellospora calospora (AJ510231)
Gig1
S. gilmorei (FN547618)
Gig2
Acaulospora colombiana (FJ461804)
Aca1
Ac. longula (AJ510228)
Aca2
Ac. paulinae (FJ4617969)
Aca3
Ac. scrobiculata (FJ461797)
Aca4
Claroideoglomus claroideum (AJ271928)
Cla1
C. etunicatum (AF145749)
Cla2
Archaeospora trappei (FR750035)
Geosiphon pyriformis (AM183920)
Ambispora gerdemannii (AJ510233)
Uncultured glomeromycete (AY639353)
Unc1
Unc2
Gl. tortuosum (FJ461850)
Div1
Gl. insculptum (FJ461838)
Div2
Diversispora aurantia (FN547665)
Div3
D. trimurales (FJ461851)
Div4
Paraglomus occultum (AJ271713)
Par1
Uncultured Paraglomus (AB369815)
Par2
P. brasilianum (FJ461882)
Par3
Neurospora crassa (FJ360521)

Fig. 1 Neighbor-joining tree based on the partial LSU rDNA sequences of AM fungi detected in the seaward-field (*filled circles*), seaward-trap culture (*filled triangles*), landward-field (*open circles*), and landward-trap culture (*open triangles*). *Grey boxes* indicate AM fungal phylotypes that were defined based on sequence similarity of 95–100%. Representative sequences of each phylotype were aligned together with published AM fungal (reference) sequences using Clustal X, and the tree was drawn with NJplot. Bootstrap values more than 70% are indicated. Genbank accession numbers of the reference and representative sequences are indicated in *parentheses* and Table S1 in Online Resource, respectively.

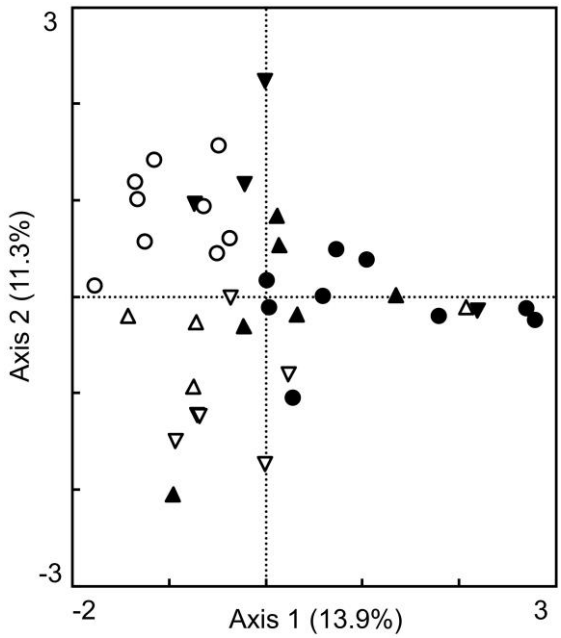
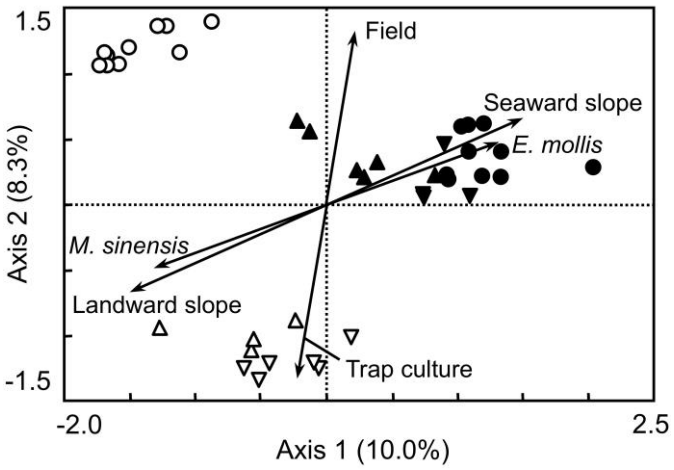


Fig. 2 Sample plot of detrended correspondence analysis on AM fungal communities in the seaward-field (*filled circles*), seaward-trap culture (*filled triangles*), landward-field (*open circles*), and landward-trap culture (*open triangles*). The trap cultures were conducted using the soils collected in 2007 (*regular triangles*) and 2008 (*upside-down triangles*).


1.5
-1.5
-2.0
2.5
Axis 2 (8.3%)
Axis 1 (10.0%)
Field
Seaward slope
E. mollis
M. sinensis
Landward slope
Trap culture

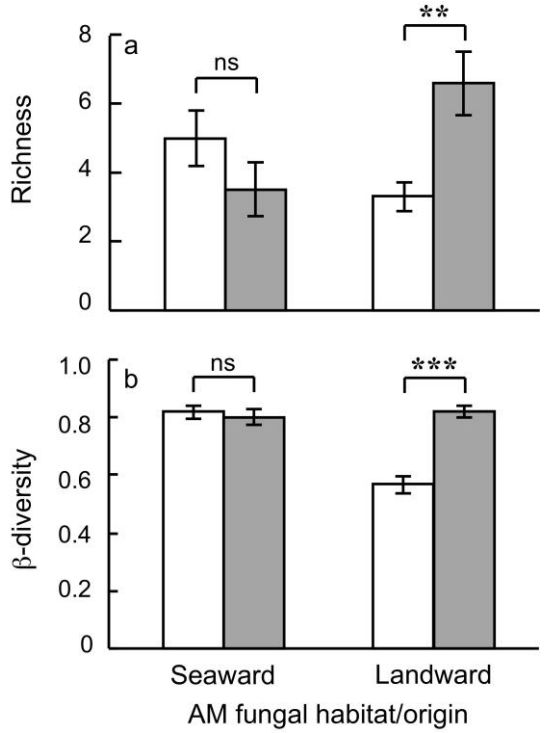


Fig. 3 Biplot of canonical correspondence analysis on AM fungal communities in the seaward-field (*filled circles*), seaward-trap culture (*filled triangles*), landward-field (*open circles*), and landward-trap culture (*open triangles*). The trap cultures were conducted using the soils collected in 2007 (*regular triangles*) and 2008 (*upside-down triangles*). Only the significant environmental factors (Monte-Carlo permutation test, $P \leq 0.001$) are indicated as *arrows*: AM fungal habitat/origin (seaward or landward), host plant species (*E. mollis* or *M. sinensis*), and growth conditions (field or trap culture). Soil chemical properties and collection year were not significant ($P > 0.3$).

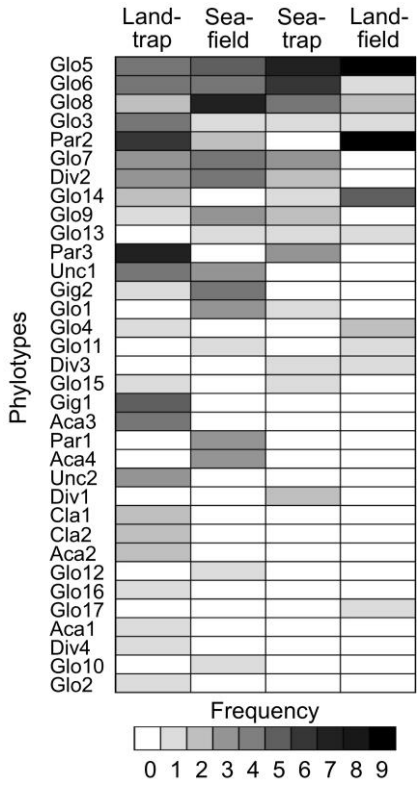


Fig. 4 AM fungal **a** phylotype richness and **b** β-diversity in the seaward and landward communities of the field (*open bars*) and trap culture (*gray bars*) samples. β-diversity is represented by Jaccard distance. The values represent the mean ± SE. Student′s *t*-tests were performed: ***, $P < 0.001$; **, $P < 0.01$; ns, not significant.

Fig. 5 Maximally stacked matrix of the AM fungal communities of the seaward and landward slopes in the field and trap culture. The rows represent AM fungal phylotypes, and the columns represent the sample types: the seaward field, Sea-field; seaward-trap culture, Sea-trap; landward field, Land-field; landward-trap culture, Land-trap. The rows and columns are sorted in the order of decreasing total incidences and then total abundances. Darkness of the cells indicates the frequency (number) of samples from which the individual phylotypes were detected (raw data are presented in Tables S3–6). Significant nestedness was observed both among the columns ($NODF_{column}$ = 64.2, P = 0.008) and the rows ($NODF_{row}$ = 54.9, P = 0.025) based on the presence/absence data. No nestedness was observed among the rows based on the abundance data ($WNODF_{row}$ = 30.2, P = 0.065).

Online Resource

Characterization of arbuscular mycorrhizal fungal communities with respect to zonal vegetation in a coastal dune ecosystem.

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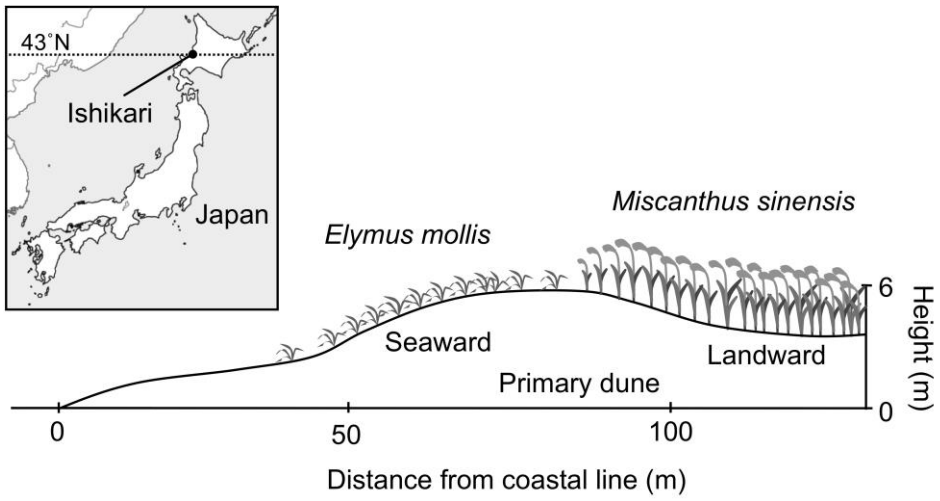

Fig. S1 Location of Ishikari sand dune in Japan (in square) and cross-section diagram of the dune. The primary dune was 2–6 m in height and located about 50–100 m inland from the coastal line. The seaward slope was 40–70 m in width and dominated by *Elymus mollis*, whereas the landward slope was 80–150 m in width and dominated by *Miscanthus sinensis*.

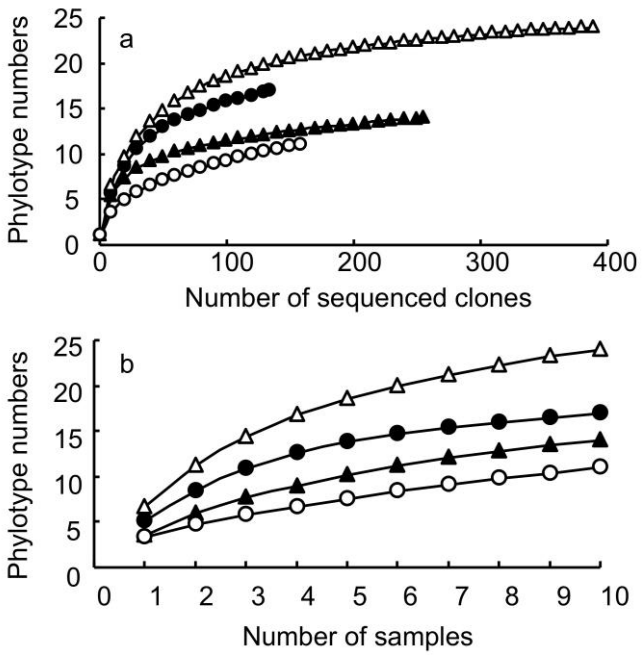

Fig. S2 Rarefaction analysis on AM fungal phylotype richness in the seaward-field (*filled circles*), seaward-trap culture (*filled triangles*), landward-field (*open circles*), and landward-trap culture (*open triangles*) based on the numbers of **a** sequenced clone and **b** root sample with Analytic Rarefaction 1.3 (**a**) and Estimate S 8.2 (**b**), respectively.

Table S1 GenBank accession numbers of nucleotide sequences of the clones in Fig. 2.

Phylotype	AM fungal habitat/origin	Host plant	Growth condition	Clone name	Accession no.
Glo1	Seaward slope	*M. sinensis*	Trap culture	KA1-64	AB561113
Glo1	Seaward slope	*E. mollis*	Field	KAE9-3	AB561114
Glo2	Landward slope	*M. sinensis*	Trap culture	08KB9-3	AB640731
Glo3	Seaward slope	*M. sinensis*	Trap culture	KA1-55	AB561099
Glo3	Landward slope	*M. sinensis*	Field	KBM6-8	AB643635
Glo3	Seaward slope	*E. mollis*	Field	KAE6-11	AB561100
Glo3	Landward slope	*M. sinensis*	Trap culture	KB6-2-8	AB561101
Glo4	Landward slope	*M. sinensis*	Trap culture	08KB9-23	AB643636
Glo4	Landward slope	*M. sinensis*	Field	KBM9-8	AB561102
Glo5	Landward slope	*M. sinensis*	Field	KBM5-1	AB561104
Glo5	Seaward slope	*M. sinensis*	Trap culture	KA5-5	AB561105
Glo5	Seaward slope	*E. mollis*	Field	KAE4-19	AB561103
Glo5	Landward slope	*M. sinensis*	Trap culture	KB4-22	AB561106
Glo6	Seaward slope	*E. mollis*	Field	KAE12-14	AB561107
Glo6	Seaward slope	*M. sinensis*	Trap culture	KA6-24	AB561108
Glo6	Landward slope	*M. sinensis*	Field	KBM12-5	AB561109
Glo6	Landward slope	*M. sinensis*	Trap culture	KB5-33	AB561110
Glo7	Seaward slope	*M. sinensis*	Trap culture	KA5-2-11	AB640742
Glo7	Landward slope	*M. sinensis*	Trap culture	08KB5-33	AB561111
Glo7	Seaward slope	*E. mollis*	Field	KAE8-17	AB561112
Glo8	Seaward slope	*E. mollis*	Field	KAE3-10	AB640744
Glo8	Landward slope	*M. sinensis*	Trap culture	KB6-21	AB561097
Glo8	Landward slope	*M. sinensis*	Field	KBM3-26	AB561095
Glo8	Seaward slope	*M. sinensis*	Trap culture	KA3-43	AB640738
Glo9	Seaward slope	*M. sinensis*	Trap culture	08KA6-14	AB640739
Glo9	Landward slope	*M. sinensis*	Trap culture	KB6-2-4	AB640732
Glo9	Seaward slope	*E. mollis*	Field	KAE2-27	AB640745
Glo10	Seaward slope	*E. mollis*	Field	KAE3-3	AB643805
Glo11	Landward slope	*M. sinensis*	Field	KBM6-5	AB640749
Glo11	Seaward slope	*E. mollis*	Field	KAE3-1	AB640746
Glo12	Seaward slope	*E. mollis*	Field	KAE10-12	AB640747
Glo13	Seaward slope	*M. sinensis*	Trap culture	08KA7-10	AB640740
Glo13	Seaward slope	*E. mollis*	Field	KAE9-13	AB640748
Glo13	Landward slope	*M. sinensis*	Field	KBM2-28	AB640750
Glo14	Landward slope	*M. sinensis*	Field	KBM2-5	AB561092

Table S1 (continued)

Phylotype	AM fungal habitat/origin	Host plant	Growth condition	Clone name	Accession no.
Glo14	Landward slope	*M. sinensis*	Trap culture	08KB9-22	AB561094
Glo14	Seaward slope	*M. sinensis*	Trap culture	KA3-12	AB561093
Glo15	Seaward slope	*M. sinensis*	Trap culture	KA1-22	AB640741
Glo15	Landward slope	*M. sinensis*	Trap culture	08KB4-33	AB640733
Glo16	Landward slope	*M. sinensis*	Trap culture	KB5-3-9	AB640734
Glo17	Landward slope	*M. sinensis*	Field	KBM11-75	AB640751
Aca1	Landward slope	*M. sinensis*	Trap culture	KB5-3	AB561120
Aca2	Landward slope	*M. sinensis*	Trap culture	KB3-48	AB561121
Aca3	Landward slope	*M. sinensis*	Trap culture	KB4-10	AB561122
Aca4	Seaward slope	*E. mollis*	Field	KAE9-1	AB561123
Div1	Seaward slope	*M. sinensis*	Trap culture	KA1-86	AB561115
Div2	Seaward slope	*M. sinensis*	Trap culture	KA5-17	AB561117
Div2	Landward slope	*M. sinensis*	Trap culture	08KB4-21	AB640737
Div2	Seaward slope	*E. mollis*	Field	KAE4-1	AB561116
Div3	Landward slope	*M. sinensis*	Field	KBM6-2	AB561118
Div3	Seaward slope	*M. sinensis*	Trap culture	08KA7-1	AB640743
Div4	Landward slope	*M. sinensis*	Trap culture	KB5-3-33	AB561119
Gig1	Landward slope	*M. sinensis*	Trap culture	KB3-2-43	AB561124
Gig2	Landward slope	*M. sinensis*	Trap culture	08KB6-47	AB561126
Gig2	Seaward slope	*E. mollis*	Field	KAE6-16	AB561125
Cla1	Landward slope	*M. sinensis*	Trap culture	08KB5-3	AB561128
Cla2	Landward slope	*M. sinensis*	Trap culture	08KB4-14	AB561129
Unc1	Seaward slope	*E. mollis*	Field	KAE5-15	AB561130
Unc1	Landward slope	*M. sinensis*	Trap culture	KB3-2-29	AB561131
Unc2	Landward slope	*M. sinensis*	Trap culture	KB3-2-18	AB640736
Par1	Seaward slope	*E. mollis*	Field	KAE9-4	AB561132
Par2	Landward slope	*M. sinensis*	Trap culture	KB3-27	AB561133
Par2	Landward slope	*M. sinensis*	Field	KBM4-13	AB561134
Par2	Seaward slope	*E. mollis*	Field	KAE1-17	AB561135
Par3	Landward slope	*M. sinensis*	Trap culture	KB3-2-55	AB561136
Par3	Seaward slope	*M. sinensis*	Trap culture	KA2-22	AB561137

Table S2 Correlation coefficients among the soil chemical properties of *E. mollis*- and *M. sinensis*-rhizosphere soils collected from the seaward and landward slopes of Ishikari sand dune.

	Truog-P	Total N	Total C	Na	K	Mg	Ca
pH	-0.285	-0.841***	-0.817***	0.517	0.376	-0.842***	-0.554
Truog-P	-	0.328	0.389*	-0.264	0.007	0.568	0.711**
Total N		-	0.973***	-0.257	-0.338	0.675*	0.725**
Total C			-	-0.266	-0.259	0.665*	0.758**
Na				-	0.105	-0.309	-0.063
K					-	-0.256	-0.123
Mg						-	0.482

*** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$.

Table S3 Frequencies of AM fungal phylotypes detected in the seaward-field samples (*E. mollis*-field roots) collected in 2008.

	Sample ID									
Phylotype	1	2	3	4	5	6	7	8	9	10
Glo1	-	-	-	-	-	-	-	+	+	+
Glo2	-	-	-	-	-	-	-	-	-	-
Glo3	-	-	-	-	-	+	-	-	-	-
Glo4	-	-	-	-	-	-	-	-	-	-
Glo5	+	-	-	+	-	+	-	+	-	+
Glo6	-	-	+	-	-	+	-	-	+	+
Glo7	-	-	-	-	-	+	+	+	-	+
Glo8	+	+	+	-	-	+	+	+	-	+
Glo9	-	+	+	-	-	-	-	-	+	-
Glo10	-	-	+	-	-	-	-	-	-	-
Glo11	-	-	+	-	-	-	-	-	-	-
Glo12	-	-	-	-	-	-	-	-	+	-
Glo13	-	-	-	-	-	-	-	+	-	-
Glo14	-	-	-	-	-	-	-	-	-	-
Glo15	-	-	-	-	-	-	-	-	-	-
Glo16	-	-	-	-	-	-	-	-	-	-
Glo17	-	-	-	-	-	-	-	-	-	-
Aca1	-	-	-	-	-	-	-	-	-	-
Aca2	-	-	-	-	-	-	-	-	-	-
Aca3	-	-	-	-	-	-	-	-	-	-
Aca4	-	-	-	-	-	-	-	+	+	+
Div1	-	-	-	-	-	-	-	-	-	-
Div2	-	-	+	+	+	+	-	-	-	-
Div3	-	-	-	-	-	-	-	-	-	-
Div4	-	-	-	-	-	-	-	-	-	-
Gig1	-	-	-	-	-	-	-	-	-	-
Gig2	-	-	-	-	+	+	-	+	-	+
Cla1	-	-	-	-	-	-	-	-	-	-
Cla2	-	-	-	-	-	-	-	-	-	-
Unc1	-	-	-	-	+	-	+	+	-	-
Unc2	-	-	-	-	-	-	-	-	-	-
Par1	-	-	-	-	-	-	+	+	-	+
Par2	+	-	-	-	-	-	+	-	-	-
Par3	-	-	-	-	-	-	-	-	-	-
No. of phylotypes	3	2	6	2	3	7	5	9	5	8

Table S4 Frequencies of AM fungal phylotypes detected in the seaward-trap culture (*E. mollis*-rhizosphere soils).

	Sample ID									
	Collected in 2007						Collected in 2008			
Phylotype	1	2	3	4	5	6	7	8	9	10
Glo1	+	-	-	-	-	-	-	-	-	-
Glo2	-	-	-	-	-	-	-	-	-	-
Glo3	+	-	-	-	-	-	-	-	-	-
Glo4	-	-	-	-	-	-	-	-	-	-
Glo5	+	-	+	+	+	+	+	+	-	-
Glo6	+	-	+	+	+	+	-	-	+	-
Glo7	-	-	+	-	+	-	-	-	-	+
Glo8	+	-	+	-	+	-	-	-	+	-
Glo9	+	-	-	-	-	-	-	-	+	-
Glo10	-	-	-	-	-	-	-	-	-	-
Glo11	-	-	-	-	-	-	-	-	-	-
Glo12	-	-	-	-	-	-	-	-	-	-
Glo13	-	-	-	-	-	-	-	-	-	+
Glo14	-	-	+	-	-	-	-	-	-	-
Glo15	+	-	-	-	-	-	-	-	-	-
Glo16	-	-	-	-	-	-	-	-	-	-
Glo17	-	-	-	-	-	-	-	-	-	-
Aca1	-	-	-	-	-	-	-	-	-	-
Aca2	-	-	-	-	-	-	-	-	-	-
Aca3	-	-	-	-	-	-	-	-	-	-
Aca4	-	-	-	-	-	-	-	-	-	-
Div1	+	-	-	-	-	-	-	+	-	-
Div2	+	-	-	-	+	-	-	-	-	-
Div3	-	-	-	-	-	-	-	-	-	+
Div4	-	-	-	-	-	-	-	-	-	-
Gig1	-	-	-	-	-	-	-	-	-	-
Gig2	-	-	-	-	-	-	-	-	-	-
Cla1	-	-	-	-	-	-	-	-	-	-
Cla2	-	-	-	-	-	-	-	-	-	-
Unc1	-	-	-	-	-	-	-	-	-	-
Unc2	-	-	-	-	-	-	-	-	-	-
Par1	-	-	-	-	-	-	-	-	-	-
Par2	-	-	-	-	-	-	-	-	-	-
Par3	-	+	-	-	+	+	-	-	-	-
No. of phylotypes	9	1	5	2	6	3	1	2	3	3

Table S5 Frequencies of AM fungal phylotypes detected in the landward-field samples (*M. sinensis*-field roots) collected in 2008.

	Sample ID									
Phylotype	1	2	3	4	5	6	7	8	9	10
Glo1	-	-	-	-	-	-	-	-	-	-
Glo2	-	-	-	-	-	-	-	-	-	-
Glo3	-	-	-	-	-	+	-	-	-	-
Glo4	-	-	-	-	-	+	+	-	-	-
Glo5	+	+	+	+	+	+	+	-	+	+
Glo6	-	-	-	-	-	-	-	-	-	+
Glo7	-	-	-	-	-	-	-	-	-	-
Glo8	+	-	+	-	-	-	-	-	-	-
Glo9	-	-	-	-	-	-	-	-	-	-
Glo10	-	-	-	-	-	-	-	-	-	-
Glo11	-	-	-	-	-	+	-	-	-	-
Glo12	-	-	-	-	-	-	-	-	-	-
Glo13	-	+	-	-	-	-	-	-	-	-
Glo14	+	+	-	-	+	+	+	-	-	-
Glo15	-	-	-	-	-	-	-	-	-	-
Glo16	-	-	-	-	-	-	-	-	-	-
Glo17	-	-	-	-	-	-	-	-	+	-
Aca1	-	-	-	-	-	-	-	-	-	-
Aca2	-	-	-	-	-	-	-	-	-	-
Aca3	-	-	-	-	-	-	-	-	-	-
Aca4	-	-	-	-	-	-	-	-	-	-
Div1	-	-	-	-	-	-	-	-	-	-
Div2	-	-	-	-	-	-	-	-	-	-
Div3	-	-	-	-	-	+	-	-	-	-
Div4	-	-	-	-	-	-	-	-	-	-
Gig1	-	-	-	-	-	-	-	-	-	-
Gig2	-	-	-	-	-	-	-	-	-	-
Cla1	-	-	-	-	-	-	-	-	-	-
Cla2	-	-	-	-	-	-	-	-	-	-
Unc1	-	-	-	-	-	-	-	-	-	-
Unc2	-	-	-	-	-	-	-	-	-	-
Par1	-	-	-	-	-	-	-	-	-	-
Par2	+	+	+	+	+	-	+	+	+	+
Par3	-	-	-	-	-	-	-	-	-	-
No. of phylotypes	4	4	3	2	3	6	4	1	3	3

Table S6 Frequencies of AM fungal phylotypes detected in the landward-trap culture (*M. sinensis*-rhizosphere soils).

	Sample ID									
	Collected in 2007				Collected in 2008					
Phylotype	1	2	3	4	5	6	7	8	9	10
Glo1	-	-	-	-	-	-	-	-	-	-
Glo2	-	-	-	-	-	-	-	-	-	+
Glo3	-	-	+	+	+	-	-	-	-	+
Glo4	-	-	-	-	-	-	-	-	-	+
Glo5	+	+	+	-	-	+	-	-	-	-
Glo6	+	-	+	-	-	-	-	-	+	+
Glo7	-	-	-	-	-	-	+	-	+	+
Glo8	-	-	-	+	-	-	+	-	-	-
Glo9	-	-	-	+	-	-	-	-	-	-
Glo10	-	-	-	-	-	-	-	-	-	-
Glo11	-	-	-	-	-	-	-	-	-	-
Glo12	-	-	-	-	-	-	-	-	-	-
Glo13	-	-	-	-	-	-	-	-	-	-
Glo14	-	-	+	-	-	-	-	-	-	+
Glo15	-	-	-	-	-	+	-	-	-	-
Glo16	-	-	+	-	-	-	-	-	-	-
Glo17	-	-	-	-	-	-	-	-	-	-
Aca1	-	-	+	-	-	-	-	-	-	-
Aca2	+	-	-	-	-	-	-	-	+	-
Aca3	-	+	+	-	-	-	+	+	-	-
Aca4	-	-	-	-	-	-	-	-	-	-
Div1	-	-	-	-	-	-	-	-	-	-
Div2	-	-	-	-	+	+	-	-	+	-
Div3	-	-	-	-	-	-	-	-	-	-
Div4	-	-	+	-	-	-	-	-	-	-
Gig1	+	-	-	-	+	+	+	+	-	-
Gig2	-	-	-	-	-	-	-	+	-	-
Cla1	-	-	-	-	+	-	+	-	-	-
Cla2	-	-	-	-	-	+	+	-	-	-
Unc1	+	-	+	-	-	+	-	+	-	+
Unc2	+	-	+	-	-	-	-	-	-	+
Par1	-	-	-	-	-	-	-	-	-	-
Par2	+	+	+	-	-	+	+	+	-	-
Par3	+	-	+	-	-	+	+	+	+	+
No. of phylotypes	8	3	12	3	4	8	8	6	5	9