



HOKKAIDO UNIVERSITY

Title	Significance of life-history strategies of arbuscular mycorrhizal fungi in a coastal dune ecosystem
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Degree Grantor	北海道大学
Degree Name	博士(農学)
Dissertation Number	甲第16094号
Issue Date	2024-09-25
DOI	https://doi.org/10.14943/doctoral.k16094
Doc URL	https://hdl.handle.net/2115/93588
Type	doctoral thesis
File Information	Anjar_Cahyaningtyas.pdf



Significance of life-history strategies of arbuscular mycorrhizal fungi in a coastal dune ecosystem

(海岸砂丘生態系におけるアーバスキュラー菌根菌の生活史戦略
の重要性)

Hokkaido University Graduate School of Agriculture

Frontiers in Bioscience Doctoral Course

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General introduction

Soil disturbance occurs through anthropogenic or natural processes and causes serious environmental issues, such as erosion (Kurothe et al. 2014; Rey 2003; Suescún et al. 2017) and eutrophication of ground and marine water ecosystems (Smith 2003). Arbuscular mycorrhizal (AM) fungi form symbiotic associations with most land plants and deliver mineral nutrients to the host (Smith and Read 2008). In the associations the fungi construct extraradical hyphal networks in the soil, from which water and nutrients are taken up and translocated to arbuscules in the root cortex where the fungal symbionts release nutrients to the host (Ezawa and Saito 2018). In exchange for the nutrients, the fungi receive lipids and sugars as carbon source (Lanfranco et al. 2018). Soil disturbance has a serious impact on mycorrhizal associations via destruction of extraradical hyphal networks, which inhibits nutrient exchange between the symbionts and reduces new colonization mediated by the hyphal networks. It has been demonstrated that soil disturbance acts as a selection pressure for the fungi that inhabit agroecosystems (Jansa et al. 2002), semi-natural grassland (Schnoor et al. 2011), coastal dunes (Corkidi and Rincón 1997; Kawahara and Ezawa 2013), and volcanic slopes (Atunnisa and Ezawa 2019; Fujiyoshi et al. 2006), indicating that sensitivity to soil disturbance is different among AM fungal species.

Ishikari coastal dunes, Japan, show typical zonal distribution of vegetation along the soil disturbance gradient caused by tides and wind; the two grass species *Leymus mollis* and *Miscanthus sinensis* are distributed in the seaward (severely disturbed) and landward (less disturbed) slopes, respectively. In this ecosystem, Kawahara and Ezawa (2013) demonstrated the enrichment of disturbance-tolerant AM fungi in the seaward slopes and the coexistence of disturbance-tolerant and -sensitive fungi in the landward slopes through the comparative analysis of the community members before and after soil disturbance. However, the

significance of life-history strategies of AM fungi in the resilience of the fungi and vegetation were not well understood. In this study, Ishikari sand dunes were employed as a model ecosystem, and the life-history strategies of AM fungi were characterized along the disturbance gradient in Chapter 1. In Chapter 2, the role of the life-history strategies in the selection of AM fungal partners by the native C₄ grass *M. sinensis* under low-light conditions were investigated.

Chapter 1

Characterization of life-history strategies of AM fungi along a disturbance gradient

1.1 Introduction

In frequently disturbed ecosystems rapid regeneration/reconstruction of hyphal networks would be an important trait for the survival of AM fungi after disturbance. Chagnon et al. (2013) proposed a concept of AM fungal life-history strategies according to the C-S-R (competitor, stress-tolerator, and ruderal) framework, which was originally proposed by Grime (1977) to classify plant life-history strategies. Competitor (C) fungi allocate more biomass to extraradical mycelia than in the roots, constructing high-density hyphal networks in the soil, and produce spores at later growth stages (e.g., the members of the family Gigasporaceae). Stress-tolerator (S) fungi can tolerate abiotic stresses, such as soil acidity and low temperature, have long-lived mycelia, and grow slowly (e.g., the members of the Acaulosporaceae). Ruderal (R) fungi grow rapidly, show high rates of hyphal turnover, produce abundant spores at early growth stages or constitutively, and allocate more biomass in the roots than in the soil (e.g., the members of the Glomeraceae). Whereas Weber et al. (2019) classified the fungi into guilds by their patterns of biomass allocation: "edaphophilic" fungi (Gigasporaceae and Diversisporaceae) that allocate biomass more to extraradical hyphae, contributing to greater nutrient delivery, "rhizophilic" fungi [Glomeraceae, Entrophosporaceae (formerly Claroideoglomeraceae), and Paraglomeraceae] that allocate more biomass to intraradical hyphae (Hart and Reader 2005), probably for protecting the roots from pathogens, and "ancestral" fungi (Archaeosporaceae, Ambisporaceae, Pacisporaceae, and Acaulosporaceae) that lack an apparent preference for biomass allocation. In this context, responses of AM fungi to soil disturbance would be predictable at the family level. For example, Glomeraceae and

Paraglomeraceae fungi, the rhizophilic fungi that have larger biomass in the roots, could regenerate rapidly (i.e., rapid regenerators) not only from spores, but also from intraradical hyphae. Whereas the edaphophilic fungi in the Gigasporaceae and Diversisporaceae that have larger biomass in the soil would rather be susceptible to soil disturbance, that is, slow regenerators that require longer time for reestablishment, and regenerate mainly from soil-borne propagules, e.g., spores and extraradical hyphae.

In this chapter, rapid and slow regenerators were defined as those that are capable of regenerating within "two" and "six" months, respectively, after disturbance based on the following ideas. The most prevalent laboratory model *Rhizophagus* spp. are likely rhizophilic/ruderal fungi; for example, they can colonize roots within a month (Hestrin et al. 2019) and produce abundant spores within 6 – 8 weeks (Tanaka et al. 2022), suggesting that regeneration within "two months" could be one feature of rapid regenerators. Whereas in temperate/subarctic grassland slow regenerators need to regenerate at least during spring and summer, typically within "six months", before defoliation of the host in autumn because the reconstruction of hyphal networks would require substantial carbon derived from the host. A nylon mesh-separated-compartment culture system was applied for assessing the inoculum potential of extraradical and intraradical mycelia separately, addressing the following three hypotheses; i) both soil-borne propagules and intraradical hyphae play a main role in the regeneration of disturbance-tolerant fungi within two months (i.e., rapid regenerators), ii) extension of culture period to six months increases AM fungal richness due to appearance of disturbance-sensitive fungi (i.e., slow regenerators), and iii) most rapid regenerators belong to the Glomeraceae and Paraglomeraceae, whereas slow regenerators mainly belong to the Gigasporaceae and Diversisporaceae.

1.2 Materials and methods

Sampling sites

A sampling area of 100×1,000 m was defined along Ishikari coastal dune, Hokkaido, Japan (Table 1.1 and Figs. 1.1a and b). This ecosystem belongs to the subarctic zone with the annual mean temperature and rainfall being 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. Vegetation in the dune has been described in Kawahara and Ezawa (2013). Briefly, the landward slopes of the primary dune are 80 – 150 m in width and largely dominated by the C4 perennial grass *Miscanthus sinensis* Andersson (Fig. 1c). In addition, *Rosa rugosa* Thunb. and *Lathyrus japonicus* Willd. are also patchily distributed. The seaward slopes are 40 – 70 m in width and dominated by *Leymus mollis* (Trin. ex Spreng.) Pilg. (Fig. 1d). *Calystegia soldanella* (L.) Riom. et Schult., *Arabis stelleri* var. *japonica* (A. Gray) Fr. Schm., *Glehnia littoralis* F. Schmidt ex Miq., *Carex kobomugi* Ohwi., *Linaria japonica* Miq., and *Ixeris repens* (L.) A. Gray are also patchily distributed in the slopes. The habitats of *M. sinensis* and *L. mollis* 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 slopes is constantly disturbed by wind thus unstable. Whereas the thick root system of *M. sinensis* stabilized the landward slopes, and thus an organic layer (3 – 5 cm) originated from *M. sinensis* litter has been developed. The soil chemical properties in the two slopes are described in Kawahara and Ezawa (2013). Briefly, sodium concentration in the root-zone soils was not significantly different between the seaward and landward slopes (140 and 128 mg-Na kg⁻¹, respectively, on average), but organic carbon (1.4 and 5.1 g-C kg⁻¹, respectively, on average) and total nitrogen (0.18 and 0.4 g-N kg⁻¹, respectively, on average) contents were significantly different between the two slopes.

Soil sampling

Five sampling plots were designated at 200 m intervals along the coastal line in each of the seaward and landward slopes (Fig. 1b). Four individuals of *M. sinensis* and *L. mollis* in landward and seaward, respectively, were selected in each sampling plot (5×5 m), and root-zone soil samples, including root fragments, were collected from the four on June 17, 2020, as follows. For *M. sinensis*, a stainless-steel core sampler [5×5 cm (diam/height), 100 mL in vol] was used to collect after removing the litter layer. The core sampler could not be applied to *L. mollis* sampling because the root-zone soil (sand) was too fragile to collect with the sampler due to the low density of the roots, but instead, root-soil samples were collected by using a small shovel from an area of 10×10 cm at a depth of 10 cm (ca. 100 – 200 g plant⁻¹). Twenty kilograms of sand was also collected from the seaward slope where no vegetation was present and autoclaved for 2 h as a "base medium" for soil trap cultures.

Trap cultures

The root-soil samples collected by the core samplers (landward slopes) and those collected by the shovel (seaward slopes) were transferred to cylinder-shaped 37- μ m nylon mesh bags [5×5 cm (diam/height)] (non-destructively for the landward samples) in 400-mL plastic pots in which the base medium was filled around the mesh bag (four-replicated samples per plot and five plots in each of the two slopes in a total of 40 pots). *M. sinensis* was used as a host plant for the samples from both slopes based on the following two reasons; i) *L. mollis* seeds were unavailable, and ii) no significant differences in the composition and diversity were observed between the communities of *L. mollis* field roots and trap cultures with *M. sinensis* in the previous study (Kawahara and Ezawa 2013). *M. sinensis* seeds (Snow Brand Seed Co., Ltd., Sapporo) were sown onto all soil samples in

the nylon mesh bag, covered with a thin layer of sterilized sand, and grown in a temperature/light/humidity-controlled greenhouse (day/night temperature 26/20 °C; 14-h day length; 60% relative humidity). The seedlings were grown with tap water for the first month and with liquid fertilizer made of Peters Professional 25-5-20 (ICL, St. Louis, MO) at 50 µM phosphate once a week thereafter and thinned to three plants per pot one month after sowing. After two months, the seedlings were removed together with the mesh bag from two out of the four-replicated pots. These seedlings were used as donor plants of "root-direct regenerator (RD)" fungi, whereas spore/hyphae formed in the medium outside the mesh bag (remained in the pots) were used as inocula of "soil propagule-mediated regenerator" (SP) fungi as follows. The hole formed after the removal of the mesh bag was filled with the base medium to which five three-week-old new seedlings of *M. sinensis* grown in the base medium were transplanted and grown for two months as assessment plants for soil propagule-mediated regenerators (two pots per plot) (Fig. 1.2). Roots of the donor plants were washed on a stainless mesh with gently pressured tap water to remove adhering spores and extraradical mycelia, and the whole plants were transplanted outside the mesh bag of a new pot filled with the base medium. Then five three-week-old *M. sinensis* seedlings were transplanted inside the mesh bag and grown for two months as assessment plants for root-direct regenerators (two pots per plot). To raise "slow regenerator" (SL) fungi, five three-week-old *M. sinensis* seedlings were transplanted outside the mesh bag in the remaining two pots (per plot) and grown for further four months without removing the donor plants in the mesh bag. In all treatments the two pots from each sampling plot were treated as technical replication, and thus the sequence read data from the two were summed prior to analysis as described in the later section.

After harvesting the assessment plants, the roots were washed with pressured tap water, cut into 1-cm segments, randomized in water, blotted on a paper towel, immersed

in RNAlater (Thermo Fisher Scientific, Tokyo) for more than 48 h at room temperature, by which not only RNA but also DNA are preserved, blotted on a paper towel to remove excess RNAlater, transferred to a 3-mL tube with an O-ring sealed cap (Yasui Kikai, Osaka), and stored at -80 °C for molecular analysis.

Molecular identification and life-history strategy assignment

The frozen roots in the 3-mL tube were ground with a metal cone in the presence of liquid nitrogen at 2,500 rpm for 2×5 s using Multi-Beads Shocker (Yasui Kikai, Osaka), and DNA was extracted and purified from approx. 100 mg of ground sample by Maxwell RSC Instrument (Promega, Madison, WI) using Maxwell RSC PureFood GMO and Authentication Kit (Promega) according to the manufacturer's instructions, stored at -30°C, and used as template for PCR amplification. The divergent domain 2 of the large-subunit ribosomal RNA gene (LSU rDNA) was amplified in a 25- μ L reaction mixture of Expand High-Fidelity PCR System (Roche Diagnostics, Tokyo), 0.5 nmol μ L⁻¹ each of FLd3 (forward) and FLR2 (reverse) primers that were linked to TruSeq-type forward- and reverse-adaptor sequences (Illumina, Tokyo), respectively, at the 5'-end (Niwa et al. 2018), and 0.2–2 μ L template DNA using C1000 Touch™ Thermal Cycler (BIO-RAD, Tokyo) 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 48 °C for 40 s, polymerization at 72 °C for 1 min, and final elongation at 72 °C for 10 min. PCR products were obtained from all samples from the landward slopes, except for one of the RD samples, and from five SP, five RD, and 10 SL samples from the seaward slopes and sequenced on the Illumina MiSeq platform (2×300 bp), and high quality paired end reads (read 1 and read 2) of which Phred quality score ≥ 20 were merged with a minimum overlap length of 10 nt using FLASH (<http://ccb.jhu.edu/software/FLASH/>) at Bioengineering Lab (Sagamihara, Kanagawa,

Japan). The merged reads were subjected to blastn searches against the fungal LSU rDNA database consisted of 82,606 operational taxonomic units (OTUs) of fungi, including 524 and 82,082 OTUs of glomeromycotinian (AM) and non-glomeromycotinian fungi, respectively, constructed by Niwa et al. (2018) (<http://amfungi.kazusa.or.jp/>) and assigned to the OTUs at $\geq 95\%$ similarities over 330-bp alignment with an E-value cut-off of $1e-30$. The maximum likelihood tree of the 524 AM fungal OTUs with the 174 reference species of AM fungi (Delavaux et al. 2022) is available at <http://amfungi.kazusa.or.jp/>. The two sets of the read count data from the same sampling plots (i.e., two technical replication) were summed and treated as "one sample", whereas in the samples from which no PCR product was obtained zero-data were assigned to all OTUs. Then the read count data were normalized to 10^6 reads sample⁻¹ and transformed to logarithmic values (\log_2) or binary data (presence/absence) after removing the read counts of non-glomeromycotinian fungi. At this stage, samples in which all count data were zero were removed in subsequent analyses.

The OTUs that occurred uniquely in the SP and RD trap cultures, irrespective of the occurrence in the SL trap cultures, were assigned to SP and RD fungi, respectively, and those that occurred in both SP and RD trap cultures were defined as SP/RD fungi. Only the OTUs that occurred uniquely in the SL trap cultures were assigned to SL fungi. Approximate phylogenetic positions of the OTUs detected in this study were indicated by the maximum likelihood tree as follows. The OTU sequences were aligned with the LSU rDNA sequences of the ascomycotan fungus *Exophiala spinifera* (MH876260) and mucoromycotan fungus *Mortierella elongata* (MH047197) as outgroup with MAFFT ver. 7 (Katoh et al 2019), and the tree was inferred with raxmlGUI ver. 2.0.10 (Silvestro and Michalak 2012) in which the GTR + I + G4 model was selected. Closest know species or

genera of the OTUs were assumed in reference to the phylogenetic tree of the 524 AM fungal OTUs with the 174 reference species at <http://amfungi.kazusa.or.jp/>.

To assess the possible biases in the community compositions revealed by the trap cultures, the sequence data obtained by Kawahara and Ezawa (2013) were subjected to blastn searches and reassigned to the recent AM fungal OTUs with the same criteria applied to the present sequence reads.

Statistical analysis

F-test for equality of variance and Welch's *t*-test were conducted on the R 4.2.1 platform (R Core Team 2020), in which Bonferroni correction was applied for multiple comparisons. Rarefaction analysis, nonmetric multidimensional scaling (NMDS) using Bray–Curtis dissimilarity index as a distance metric, β -diversity analysis (Jaccard index), and permutational analysis of variance (PERMANOVA) were performed with the vegan package (Oksanen et al. 2022) on the R platform. β -diversity analysis was applied only to the OTUs that were detected from three or more samples. Scatter plots were drawn with the dabestr package on the R platform (Ho et al. 2019). Venn diagrams were drawn with Google Charts (<https://developers.google.com/chart>). To assess preferences for the SP, RD, and SL strategies at the family and genus levels, as well as preferences for the habitats, the numbers of samples in which the individual OTUs were detected in each trap culture were summed within the same families/genera, normalized, and subjected to principal component analysis using the vegan package.

Table 1.1 GPS data of the sampling plots

Plot ID	Soil sample ID	Latitude	Longitude
Landward slope			
1L	1L1 - 1L4	43°14'33"N	141°20'40"E
2L	2L1 - 2L4	43°14'27"N	141°20'34"E
3L	3L1 - 3L4	43°14'22"N	141°20'28"E
4L	4L1 - 4L4	43°14'18"N	141°20'23"E
5L	5L1 - 5L4	43°14'13"N	141°20'17"E
Seaward slope			
1S	1S1 - 1S4	43°14'34"N	141°20'37"E
2S	2S1 - 2S4	43°14'29"N	141°20'31"E
3S	3S1 - 3S4	43°14'24"N	141°20'26"E
4S	4S1 - 4S4	43°14'20"N	141°20'21"E
5S	5S1 - 5S4	43°14'14"N	141°20'15"E

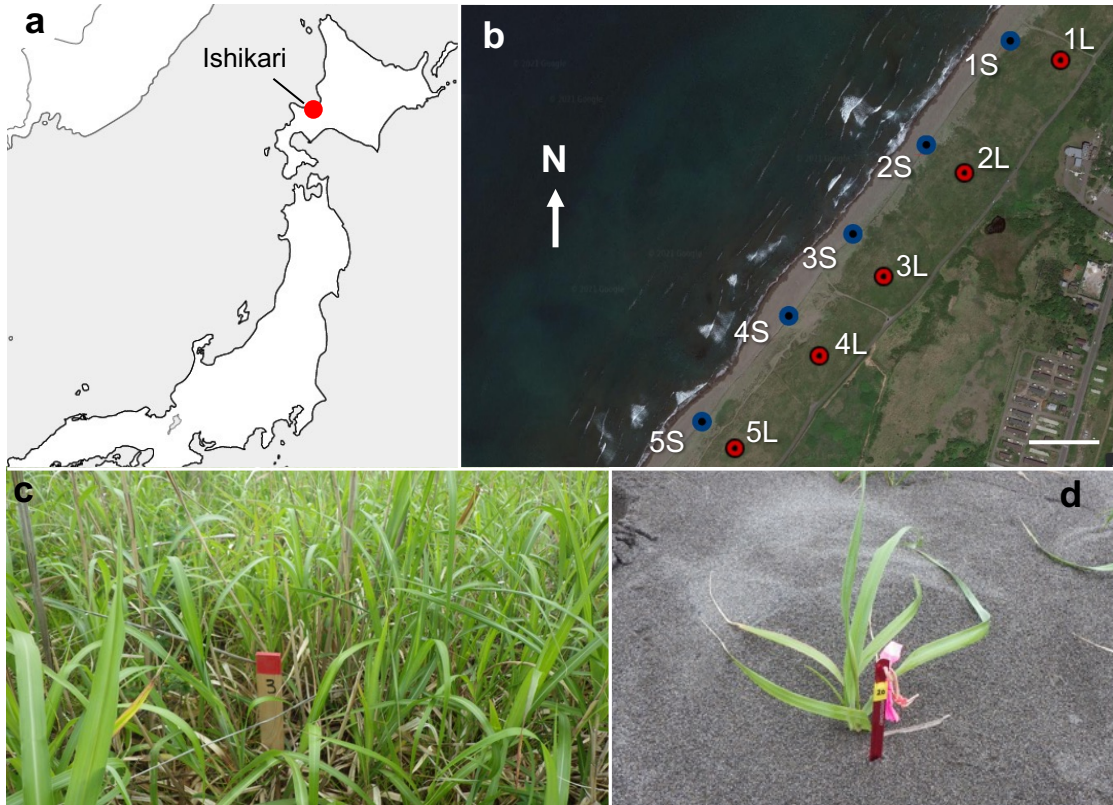


Fig. 1.1 a) Location of Ishikari coastal dune in Hokkaido, Japan. Scale, 250 km. b) Sampling plots and ID in the seaward (1S – 5S) and landward (1L – 5L) slopes in the dunes. The image was generated by Google Earth (<https://www.google.co.jp/intl/ja/earth/>) using the GPS data of the plots (Table 1.1). Scale, 100 m. c) *Miscanthus sinensis* grown in a landward slope. d) *Leymus mollis* grown in a seaward slope.

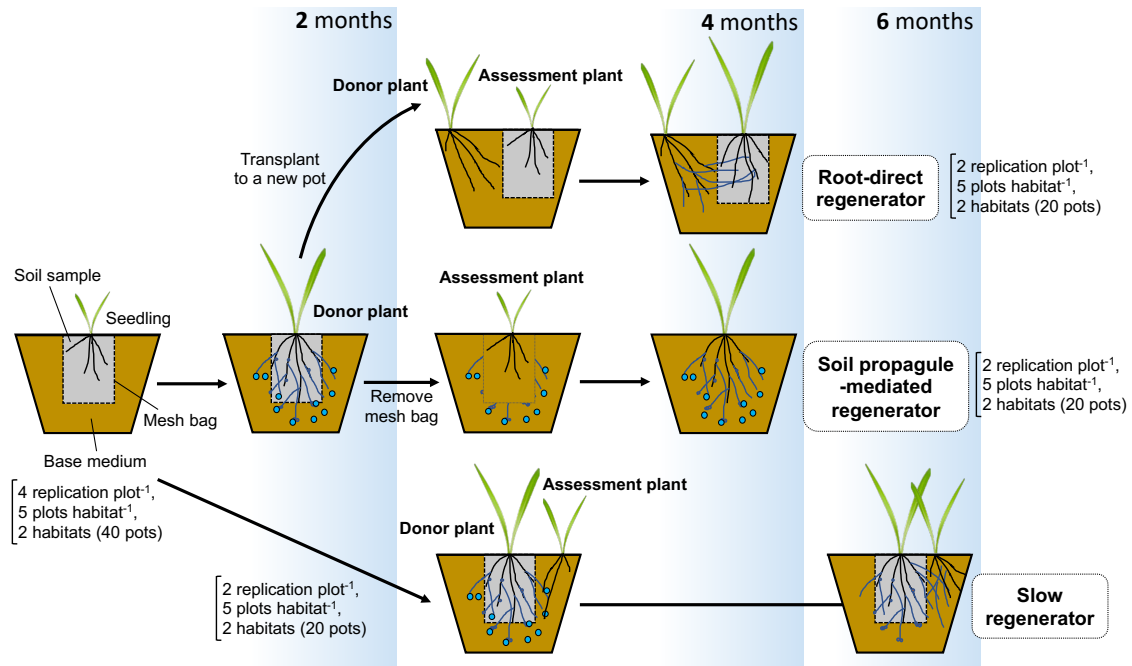


Fig. 1.2 Experimental procedure to separate disturbance-tolerant (rapid regenerator) and -sensitive (slow regenerator) arbuscular mycorrhizal fungi in *M. sinensis* trap culture. *M. sinensis* was sown onto the soil samples in cylindrical-shaped-nylon mesh bags surrounded by sterilized dune soil (base medium) and grown in a greenhouse for two months. To raise rapid regenerators, the (donor) plants were removed with the mesh bag, and the remaining hole was filled with the base medium, to which new seedlings of *M. sinensis* (assessment plants) were grown for further two months (spore propagule-mediated regenerators). The roots of the donor plants were excised from the mesh bag, washed to remove adhering spores and hyphae, and then transplanted to a new pot in which assessment plants were grown in a new mesh bag and grown for further two months (root-direct regenerators). To raise slow regenerators, assessment plants were grown outside of the mesh bag without removing the donor plants for further four months.

1.3 Results

Trap culture overview

The amplicon sequences generated over 50,000 paired reads for each sample, which were merged and subjected to blastn searches to assign to the fungal OTUs. Rarefaction analysis indicated that AM fungal OTU-accumulation curves were leveling off, suggesting that the present sampling strategy (5 plots \times 2 technical replication = 10 samples per habitat) provided reasonable coverages of AM fungal diversity in the two slope habitats (Fig. 1.3). Combining of the two datasets of the technical replication generated 15 community data from the landward samples (5 each from the three trap cultures) and 12 community data from the seaward samples (3, 4, and 5 data from the SP, RD, and SL trap cultures, respectively). These data consisted of 33,000 – 109,000 of fungal OTU-assigned reads (ca. 76,000 reads per sample on average) in which 23 – 100% (85% on average) were of AM fungi (Supplementary material Table S1.1).

Overall, 83 AM fungal OTUs were detected in total; 82 and 18 AM fungal OTUs occurred in the landward and seaward samples, respectively, in which 17 OTUs were common between the two slope habitats. The NMDS sample plot suggested that difference in the community compositions between the two habitats were obvious, but that among the trap cultures were ambiguous (Fig. 1.4a). Two-way PERMANOVA, however, indicated that both the habitat factor ($R^2 = 0.387/ P < 0.001$) and the trap culture factor ($R^2 = 0.100/ P < 0.027$) were significant. Subsequent one-way PERMANOVA within each habitat, as expected, showed that the trap culture factor was significant only in the landward samples ($R^2 = 0.242/ P = 0.044$) and not in the seaward samples ($R^2 = 0.279/ P = 0.094$). OTU richness in the SL trap cultures was significantly higher than those in the SP and RD trap cultures in the landward samples, but no significant differences were observed among the seaward trap cultures (Fig. 1.4b). Total OTU richness was also highest

in the SL trap cultures (72 OTUs), followed by the SP trap cultures (51 OTUs), and lowest in the RD trap cultures (28 OTUs), in which 84 and 89% of the OTUs in the SP and RD trap cultures, respectively, were also detected in the SL trap cultures; that is, they remained colonized six months after disturbance (Fig. 1.4c). These results imply that the extension of culture period to six months assisted the appearance of new OTUs, even in the presence of the rapid regenerators.

Characterization of life-history strategies for disturbance tolerance

Based on the occurrence patterns of the OTUs in the trap cultures, 23, 28, 5, and 27 OTUs were assigned to SP/RD, SP, RD, and SL strategists (Supplementary material Table S1.2). Subsequently, their phylogenetic positions at the genus level were inferred in reference to the maximum likelihood tree (<http://amfungi.kazusa.or.jp/>) as follows: 20 SP/RD, 12 SP, 4 RD, and 14 SL fungi in the 8 genera of the Glomeraceae; 2 SP/ RD, 11 SP, and 1 SL fungi in the 5 genera of the Gigasporaceae; 2 SP and 1 SL fungi in the genus *Acaulospora* (Acaulosporaceae); 2 SL fungi in the genus *Archaeospora* (Archaeosporaceae); 2 SP, 1 RD, and 4 SL fungi in the genus *Entrophospora* (Entrophosporaceae); 3 SL fungi in the genus *Diversispora* (Diversisporaceae); 1 SP/RD, 1 SP, and 2 SL fungi in the genus *Paraglomus* (Paraglomeraceae) (Fig. 1.5 and Supplementary material Table S1.2).

In this ecosystem SP/RD strategists were most prevalent (Fig. 1.6a), and among them, the five OTUs 216_Par, 007_Rhz, 049_Rhz, 122_UnG, and 064_UnG that are close relatives of *Paraglomus brasilianum*, *R. irregularis*, *Halonatospora panshihalos*, *Dominikia achra*, and *Nanoglomus sp.*, respectively, occurred most frequently across the two slope habitats (Fig. 1.5). The occurrences of SP, RD, and SL strategists were less than that of the SP/RD strategists and not significantly different among them (Fig. 1.6a). The family level analysis on the preferences for the strategies, which was calculated based on

the prevalence of each family among the samples, indicated that the Glomeraceae and Paraglomeraceae OTUs showed higher preferences for the RD strategy to the SP strategy, whereas the Gigasporaceae OTUs showed a distinct preference for the SP strategy (Fig. 1.6b), illustrating clear differentiation of the life-history strategies at the family level. The Entrophosporaceae (*Entrophospora*), Acaulosporaceae (*Acaulospora*), Archaeosporaceae (*Archaeospora*), and Diversisporaceae (*Diversispora*) OTUs preferred the SL strategy, although some OTUs in the former two family (genera) showed the SP strategy. The genus level analysis was conducted for more detailed characterization of the preferences not only for strategy but also for the habitats, mainly focusing on the Glomeraceae OTUs that employ diverse strategies and showed distinct distribution patterns (i.e., habitat preferences). The *Glomus* and *Microdominikia* OTUs preferred the SP strategy and mainly occurred in the landward habitat (Fig. 1.6c). Whereas the *Halonatospora*, *Dominikia*, *Nanoglomus*, and *Rhizophagus* OTUs in addition to the *Paraglomus* OTU, which include the dominant five OTUs, preferred the RD strategy and showed broad distribution across the habitats. It is noteworthy that, in this PCA plot, the two factors RD strategy and occurrences in the seaward habitat are correlated (i.e., "RD" and "Sea" arrows point the same direction in the plot), implying that those that occurred in the seaward habitat preferred the RD strategy. The *Gigaspora*, *Scutellospora*, and *Racocetra* OTUs in the Gigasporaceae showed strong preferences for the SP strategy and mainly occurred in the landward habitat.

OTU richness of the SP/RD strategists in the landward samples was not significantly different from that of the SP strategists in the same habitat but was higher than that of the other strategists in both habitats (Fig. 1.7a). Richness of the SP and SL strategists in the landward samples and that of the SP/RD strategists in the seaward samples were not different from each other, but higher than that of the RD strategists in

the landward and the SP strategists in the seaward samples. RD and SL strategists were absent in the seaward samples. β -diversity was highest among the SL fungal communities, followed by the SP and RD communities in the landward and the SP communities in the seaward samples, and lowest in the SP/RD strategists in the samples from both habitats (Fig. 1.7b).

Potential biases of the community compositions caused by the destructive collection of the seaward samples, as well as by the trap culture approach itself, were assessed by re-analyzing the previous data (Kawahara and Ezawa 2013) together with the present data, addressing the following possibilities that i) the destructive collection of the root-soil samples damaged SL fungi thus inhibited their occurrence, that ii) the employment of *M. sinensis* that is not a native host in the habitat as a trap culture host inhibited the colonization of those that have strong preferences to *L. mollis*, and that iii) the fungi that can occur in trap culture are inherently disturbance tolerant. To approach these possibilities, the OTUs that occurred uniquely in the field roots but were absent in the present trap cultures were searched, which are likely disturbance-sensitive (SL) fungi. The re-assignment of the previous sequence data to the present OTUs identified 278_Rhz, 019_Rhz, 040_Rhz, 188_Aca, and 378_Unc, close relatives of *Epigeocarpum japonicum*, *R. fasciculatus*, and *Sclerocystis sinuosa* in the Glomeraceae, *Ac. scrobiculata* (Acaulosporaceae), and *Ambispora* sp. (Ambisporaceae), respectively, as potential SL fungi missed in this study. 019_Rhz was common between the two slopes, and 278_Rhz was specific to the landward habitat. The three OTUs, 040_Rhz, 188_Aca, and 378_Unc, were specific to the seaward habitat (i.e., specific to *L. mollis*) (Fig. 1.8 and Supplementary material Table S1.3). On the other hand, it was also found that five of the 27 SL fungi in this study, 008_Rhz, 119_UnG, 206_Div, 318_Div, and 229_Par that are close relatives of *R. irregularis*, *Do. achra*, *Di. densissima*, *Di. sabulosa*, and *P. occultum*, respectively,

occurred in the previous trap cultures and thus are likely rapid regenerators. These observations support the possibilities that i) there are potential SL fungi missed in this study, ii) in which some could be *L. mollis*-specific, and that iii) some of the SL fungi are potentially rapid regenerators.

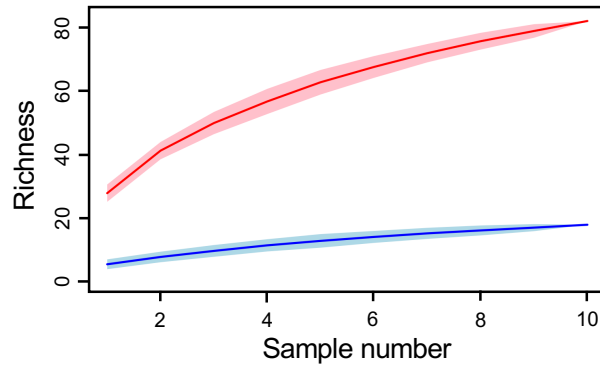


Fig. 1.3 Rarefaction analysis on AM fungal OTU richness in the trap cultures using the samples from five plots (two technical replication per plot) designated in each of the landward (red line) and seaward (blue line) slopes ($n = 10$) in Ishikari sand dunes based on the numbers of sample, in which 95% confident intervals are indicated with light colors.

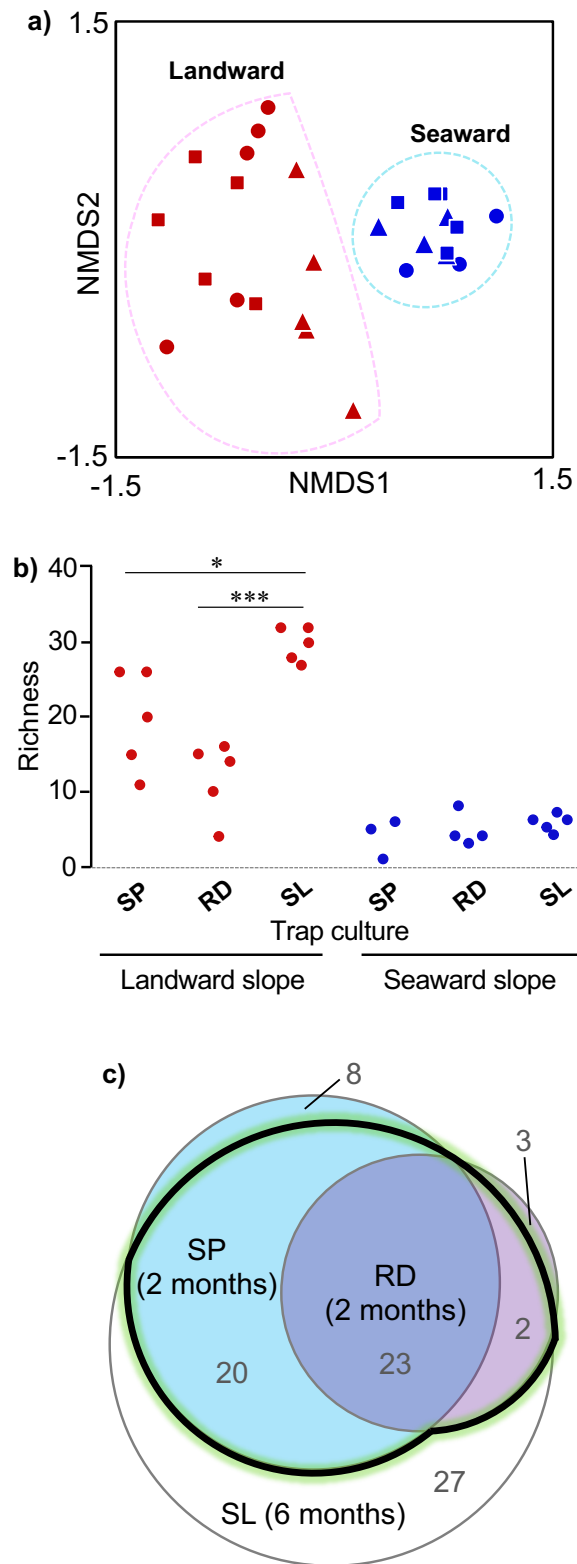


Fig. 1.4 Analysis of trap culture communities. Root-soil samples were collected from the landward and seaward slopes in Ishikari dunes, and *M. sinensis* seedlings were grown. a) Nonmetric multi-dimensional scaling of AM fungal communities in the trap cultures of

soil-propagule mediated regenerator (circles), root-direct regenerator (triangles), and slow regenerator (squares) raised from the landward and seaward samples. Bray–Curtis dissimilarity index was applied as a distance metric. Dimensions, 2; Stress=0.141. b) AM fungal OTU richness in the trap cultures of soil-propagule mediated regenerator (SP), root-direct regenerator (RD), and slow regenerator (SL) raised from the landward and seaward samples. Welch's *t*-test: *, $P < 0.05$; ***, $P < 0.001$. c) Venn diagram of AM fungal OTUs occurred in the trap cultures. Abbreviations are the same as above. The OTU numbers in each area are indicated. The common OTUs that occurred between the SL cultures and either of the SP or RD cultures are highlighted with a bold black line.

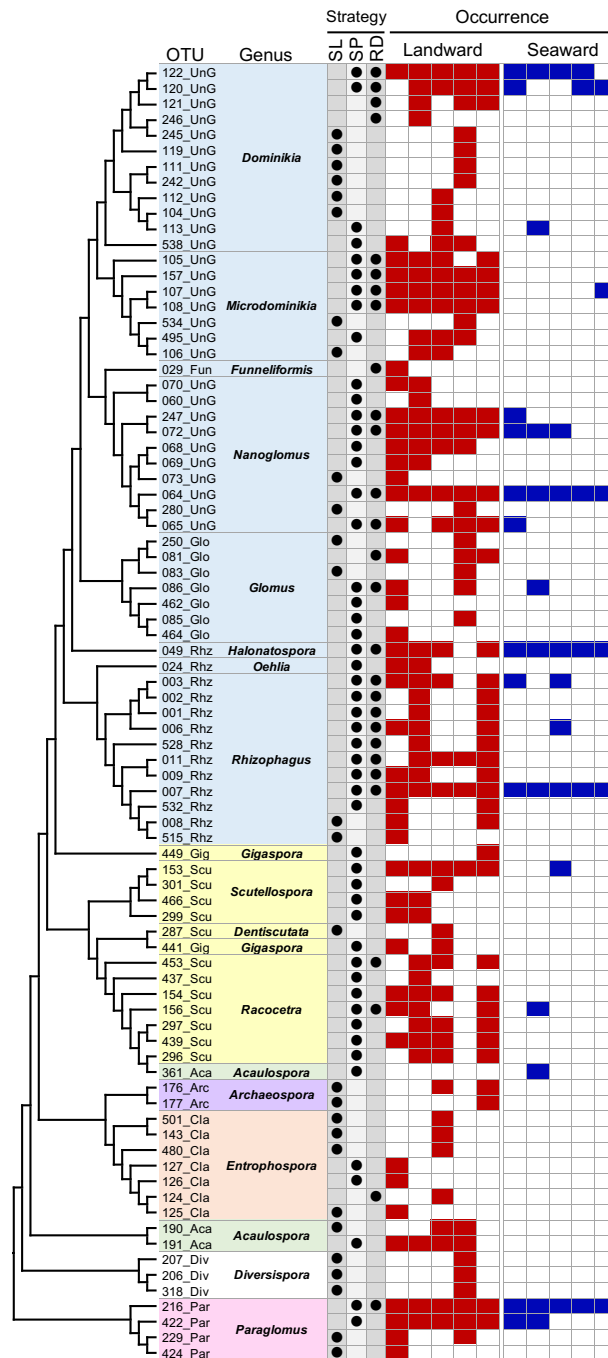


Fig. 1.5 Taxonomic positions and assigned strategy of all AM fungal OTUs detected in this study and their occurrences in the five plots in the landward and seaward slopes. The disturbance-tolerance strategies, soil-propagule mediated regenerator (SP), root-direct regenerator (RD), and slow regenerator (SL), were assigned to each OTUs based on their occurrence patterns in the trap culture experiment (Supplementary material table S1.2). The OTUs were aligned with MAFFT, and the maximum likelihood tree (left) was inferred with raxmlGUI. Family/genera are: Glomeraceae/ *Dominikia*, *Microdominikia*,

Funneliformis, *Nanoglomus*, *Glomus*, *Halonatospora*, *Oehlia*, and *Rhizophagus*;
Gigasporaceae/ *Gigaspora*, *Scutellospora*, *Dentiscutata*, and *Racocetra*;
Acaulosporaceae/ *Acaulospora*; Archaeosporaceae/ *Archaeospora*; Entrophosporaceae/
Entrophospora; Diversisporaceae/ *Diversispora*; Paraglomeraceae/ *Paraglomus*.

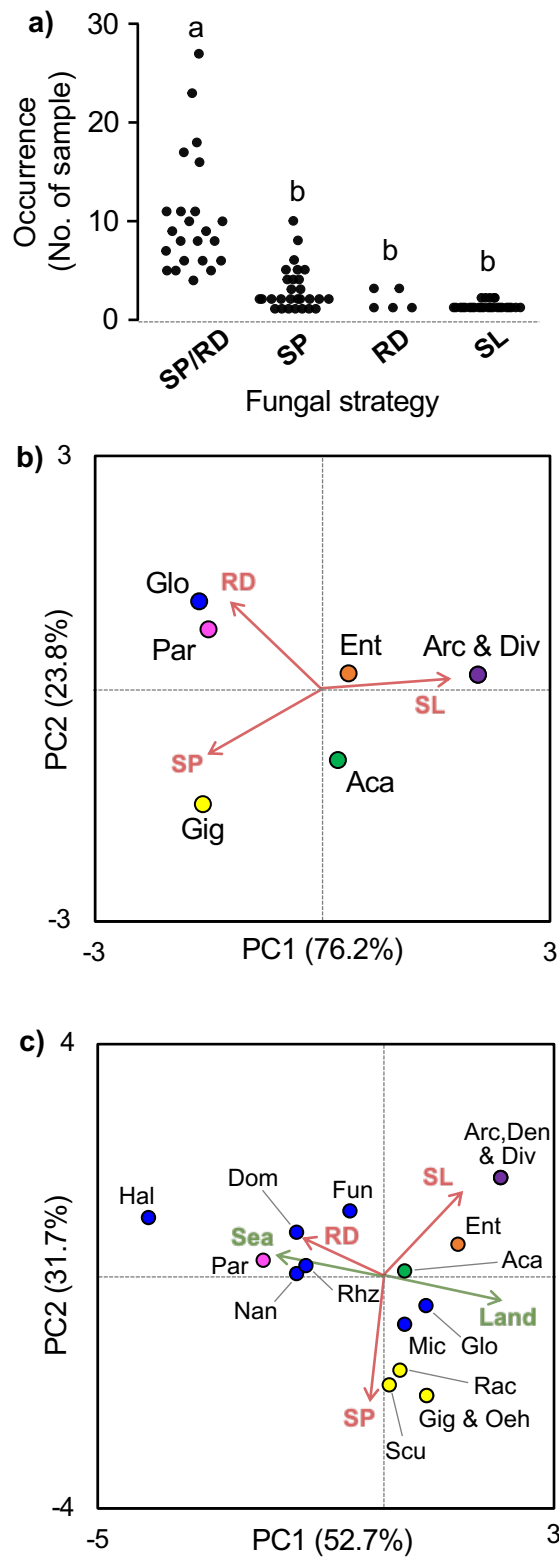


Fig. 1.6 a) Prevalence of AM fungal strategies for disturbance-tolerance in Ishikari dune ecosystem. Occurrence (numbers of samples) of the OTUs that have either of the soil-propagule mediated regenerator/root-direct regenerator (SP/RD), SP, RD, or slow

regenerator (SL) strategies were plotted (each dot represents each OTU). Different letters indicate significant difference (Welch's *t*-test, $P < 0.05$). b and c) Principal component analysis on the preferences for the SP, RD, and SL strategies at the family (b) and genus (c) levels. Occurrence (numbers of sample) of the OTU that employ either of the strategies were summed within the same families (a) or genera (b), normalized, and subjected to the analysis. In the genus level analysis, their occurrence data in the landward (Land) and seaward (Sea) samples were also included. Abbreviations of the families/ genera are: Aca, Acaulosporaceae/ *Acaulospora*; Arc, Archaeosporaceae/ *Archaeospora*; Den, *Dentiscutata*; Div, Diversisporaceae/ *Diversispora*; Dom, *Dominikia*; Ent, Entrophosporaceae/ *Entrophospora*; Fun, *Funneliformis*; Gig, Gigasporaceae/ *Gigaspora*; Glo, Glomeraceae/ *Glomus*; Hal, *Halonatospora*; Mic, *Microdominika*; Nan, *Nanoglomus*; Oeh, *Oehlia*; Par, Paraglomeraceae/ *Paraglomus*; Rac, *Racocetra*; Rhz, *Rhizophagus*; Scu, *Scutellospora*.

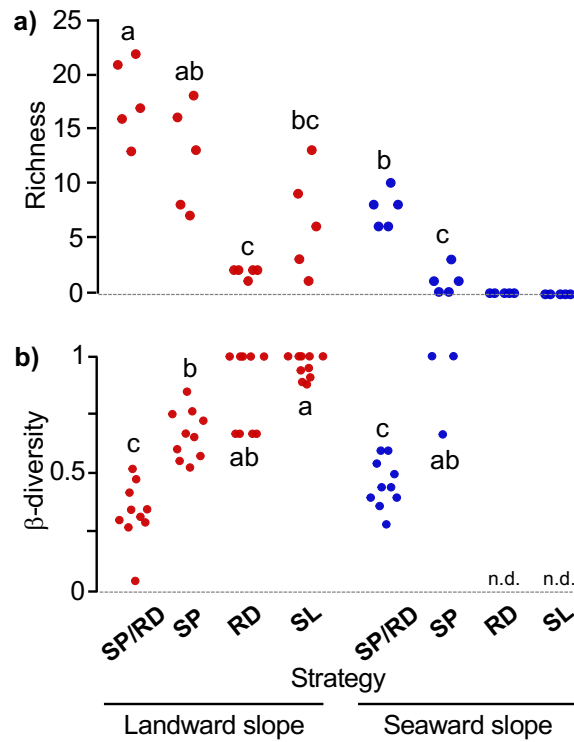


Fig. 1.7 Richness (a) and β -diversity (b) of AM fungal OTUs that have either of the disturbance-tolerance strategies, soil propagule-mediated/root-direct regenerator (SP/RD), SP, RD, or slow regenerators (SL). Richness represents the numbers of OTU. β -diversity represents Jaccard dissimilarity indices calculated by pairwise comparison between all combinations of the samples from different plots and was calculated only for the strategy categories in which the OTU occurred three or more plots (n.d., not determined). Welch's *t*-test, followed by Bonferroni corrections, were applied. Different letters indicate significant difference at $P < 0.05$.

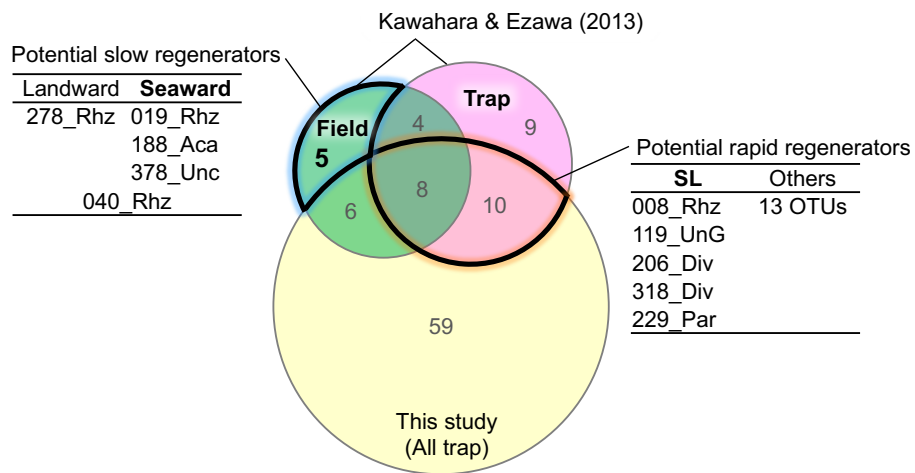


Fig. 1.8 Venn diagram of AM fungal OTUs occurred in this study (light yellow) and Kawahara and Ezawa (2013) in which the root samples from the field (green) and trap culture experiment (magenta) were analyzed. The numbers of OTU are indicated. The five OTUs that were detected uniquely in the field roots collected from the landward (*M. sinensis*) and seaward (*L. mollis*) slopes are highlighted with a bold black line as potential slow regenerators missed in this study. The common OTUs that occurred in the previous trap culture and this study were also highlighted with a bold black line, in which five potential rapid regenerator OTUs that were assigned to slow regenerators in this study but occurred in the previous trap cultures are included.

1.4 Discussion

This study provides experimental support for the previous study (Kawahara and Ezawa 2013) through characterizing the AM fungal life-history strategies for regeneration after disturbance. Soil-borne propagules and colonized roots play a major role in the rapid regeneration of AM fungi inhabiting a frequently disturbed ecosystem, supporting the first hypothesis. Intriguingly, many SP strategist fungi were also capable of regenerating from the colonized roots, that is, they are SP/RD strategists. The extension of growth period for the trap cultures assisted the appearance of new OTUs that required longer time to regenerate after disturbance; they are designated as disturbance-sensitive SL fungi, supporting the second hypothesis. The majority of the rapid regenerators belong to the families Glomeraceae and Paraglomeraceae, rhizophilic/ruderal fungi, but unexpectedly, a number of SP fungi were also found in the Gigasporaceae that has been characterized as the family of edaphophilic/competitor fungi. Similarly, SL fungi were distributed not only in the Gigasporaceae and Diversisporaceae but also across all the families occurred in this study. Accordingly, the third hypothesis was only partially supported.

The dual strategy (SP and RD) for rapid regeneration would be the nature of the rhizophilic fungi. The production of abundant spore (i.e., soil-propagule) is a prevalent trait in the Glomeraceae (Chagnon et al. 2013; Klironomos and Hart 2002) and probably in the Paraglomeraceae. In fact, 34 out of the 51 SP fungi belong to these families. Whereas the RD strategy was also prevalent in the two families; 25 of the 28 RD fungi belong to the families. Furthermore, the five dominant OTUs in the genera *Halonatospora*, *Dominikia*, *Nanoglomus*, *Rhizophagus*, and *Paraglomus* in these families showed broad distributions across the habitats and higher preferences to the RD strategy (i.e., higher

occurrences in the RD treatment), suggesting that the RD strategy is more important than the SP strategy for the survival in severely/ frequently disturbed environments.

For rapid regeneration after disturbance, various strategies known as the ruderal traits (Chagnon et al. 2013) may function together. SP fungi may have short life cycles (Declerck et al. 2001) thus could produce spore more rapidly than those that have longer life cycles. The efficient mechanisms for bridging/healing of fragmented extraradical hyphae (de la Providencia et al. 2007) would sustain not only survival of detached extraradical hyphae but also their inoculum potential in the soil. Rapid hyphal growth (Avio et al. 2006; Hart and Reader 2005) is a quite important trait for SP and RD fungi for rapid colonization and reconstruction of hyphal networks. It was unexpected, however, that most of the rapid regenerators (SP/RD, SP, and RD fungi) remained colonized in the SL trap cultures (i.e., six months after disturbance), which implies that their life cycles are not as short as considered and that they are not necessarily less competitive against competitor fungi. Further study is necessary to elucidate the involvement of these "typical ruderal traits", which were not considered in this study, in the disturbance tolerance of the fungi.

It was hypothesized that the members in the Gigasporaceae would be SL fungi because they colonize roots mainly via spores, rarely via the colonized roots, and produce spores at later stages of their life cycles (Biermann and Linderman 1983; Jasper et al. 1993; Klironomos and Hart 2002; Oehl et al. 2009), which are typical of the competitor traits (Chagnon et al. 2013). In line with these studies, the Gigasporaceae showed a strong preference to the SP strategy, confirming that their intraradical hyphae may not be major inoculum source in the soil. Whereas the occurrence of the Gigasporaceae OTUs in the SP trap cultures implies that they are capable of regenerating rapidly via extraradical hyphae detached from the roots (de la Providencia et al. 2007; Jasper et al. 1993) and/or via mature

spores produced within two months. Their preferences for the landward habitat, however, suggest that their propagules in the soil are susceptible to severe/frequent disturbance. For further characterization of the life-history strategies of Gigasporaceae fungi, the inoculum potential of extraradical hyphae and timing of their sporulation need to be evaluated by comparing those inhabiting disturbed and undisturbed ecosystems.

β -diversity of the SL fungal community was higher than that of the communities of the SP/RD and SP strategists in the same habitat, reflecting that most of the SL fungi were unique to each plot. This patchy distribution of the SL fungi suggests that stochastic processes, rather than environmental factors such as disturbance severity, may play an important role in structuring the community (e.g., Dumbrell et al. 2010; Lekberg et al. 2012). This could be interpreted by the reproductive strategy of competitor fungi; they generally invest more in vegetative growth than in reproductive growth (Chagnon et al. 2013), constructing dense hyphal networks through which they explore new hosts, and thus their dispersal ability may not be so high as the SP (ruderal) strategists that are capable of dispersing over long distances. It is considered, due to these reasons, that the SL fungi showed stochastic (i.e., heterogeneous) distribution, compared with those that have high-dispersal ability.

The possibility that the fungi that occur in trap cultures are inherently disturbance tolerant is considered. The SL fungi were distributed across diverse families, including the members of the ruderal families Glomeraceae and Paraglomeraceae, suggesting that not all the SL fungi are competitor strategists, but potentially ruderal strategists. They might not be able to establish within two months, probably due to the rapid occupation of the niche by the dominant rapid regenerators that produced abundant propagules in the soil. This observation suggests that our approach has limitations for detecting SL fungi (i.e.,

disturbance-sensitive fungi), and more detailed characterization, e.g., spore productivity and resilience of hyphal networks, is necessary in the future.

The absence of SL fungi in the seaward slopes is supported by the previous findings that the AM fungal community in the slopes was robust against soil disturbance, that is, most fungi in the habitat were disturbance tolerant (Kawahara and Ezawa 2013). However, biases in the community compositions caused by the destructive collection of the samples, as well as by using the non-native host in the trap cultures, cannot be excluded. Among the three OTUs that occurred uniquely in the seaward field roots in the previous study, the two OTUs, 188_Aca (*Ac. scrobiculata*) and 378_Unc (*Ambispora* sp.) that belong to the families of "ancestral" fungi (Weber et al. 2019), are more likely to be SL fungi than the *Rhizophagus* OTU and potentially *L. mollis*-specific. Similar to the proposed mechanism underlying the occurrence of potential ruderal fungi in the SL trap cultures, the presence of the massive amounts of propagules of the dominant rapid regenerators, which had continuously been supplied from the other side of the slopes to the habitat, was likely to overshadow SL fungi in the habitat. For more detailed studies, the improvement of isolation (detection) method for disturbance-sensitive fungi is necessary, although it may not be easy due to the coexistence of rapid regenerators.

The mesh-separated compartment culture system successfully differentiated AM fungal strategies responsible for regeneration after disturbance. To characterize soil-borne propagules of the fungi, the destructive method (i.e., wet sieving) has generally been employed in previous studies (e.g., Klironomos and Hart 2002; Varela-Cervero et al. 2016). It is likely, however, that the isolation of spore and hyphae by the destructive manners would lead to underestimation of inoculum potential via damaging/losing the propagules. The compartment culture system had originally been established three decades ago to assess the impact of destruction of extraradical hyphae on their inoculum potential

(Jasper et al. 1989) and has recently been applied to physiological studies on fungal nutrient uptake (e.g., Hodge & Fitter 2010; Kikuchi et al. 2016). The present study demonstrated that the application of this technique enables us to assess the potential of extraradical mycelia in a non-destructive manner, in which extra-radical mycelia were separated from the mycorrhizal roots simply by removing the mesh bag, minimizing the damages of the propagules. This culture system, however, cannot separate spores and extraradical hyphae that may differ in inoculum potential. Given that spores are likely to survive a longer period than detached hyphae, further experiments that take into account a time factor are necessary to assess the significance of these two types of inocula in regeneration after disturbance.

Chapter 2

Selection of AM fungal partners by juvenile C₄ grass under low-light conditions

2.1 Introduction

In coastal sand dune ecosystems, soil is constantly disturbed by wind and tide, and vegetation often distributes zonally along coastlines due to steep gradients of soil disturbance and salinity from the seaward to landward slopes of dunes (Wilson and Sykes 1999; Moreno-Casasola 1986). The seaward slopes are severely disturbed, in which only limited plant species that are specifically adapted to the environment inhabit, whereas severity of disturbance is lower in the landward slopes where species-rich coastal grassland develops (e.g., Kawahara and Ezawa 2013). Frequent disturbance acts as selection pressure not only for plants but also for arbuscular mycorrhizal (AM) fungi. AM fungi form symbiotic associations with most land plants, deliver mineral nutrients to the host (Smith and Read 2008) and play a significant role in the establishment and maintenance of vegetation in harsh environments (Corkidi and Rincón 1997; Fujiyoshi et al. 2006; Maki et al. 2008). Life-history strategies of AM fungi have recently been characterized along a disturbance gradient in Ishikari coastal sand dune ecosystem, Japan (Chapter 1) according to the C-S-R framework applied to AM fungi (Chagnon et al. 2013). In the seaward slopes typical "ruderal fungi" that are capable of regenerating rapidly after disturbance from soil propagules and colonized roots were enriched, and a majority of this type of fungi belong to the Glomeraceae in the order Glomerales and Gigasporaceae (Gigasporales) (Chapter 1). Whereas in the landward slopes not only ruderal fungi but also disturbance-sensitive "competitor fungi" that explore a new host mainly through hyphal networks in the soil, namely network competitor (NC) fungi in this chapter, coexist. Many of the competitor

fungi belong to the Entrophosporaceae (Entrophosporales), Diversisporaceae (Diversisporales), and Archaeosporaceae (Archaeosporales) (Chapter 1).

In coastal grasslands it is likely that not only disturbance severity but also carbon (C) cost of AM fungi are crucial factors for the successful establishment of juvenile plants, particularly under the canopy of adult plants that shade the juvenile plants and thus restrict their photosynthetic activity. Light availability has a significant impact on mycorrhizal benefits (Konvalinková and Jansa 2016). Phosphorus uptake through the mycorrhizal pathway rapidly declines by short-term shading without apparent decreases in fungal colonization, and long-term shading often results in negative host growth response to mycorrhiza (Konvalinková et al. 2015), implying that C cost for mycorrhizal colonization exceeds the benefits under the shade. Selection of low-cost AM fungal partners, therefore, is likely a critical issue for the juvenile plants, but information about the impact of light availability on the selection of AM fungal partners is limited.

C cost of AM fungi may differ among those that have different life-history strategies. NC fungi are likely advantageous for juvenile plants because C cost for constructing intraradical hyphae in a new host, at least at the initial stage of colonization, could be covered by the original (adult) host (Nakano-Hylander and Olsson 2007). Furthermore, the association with NC fungi may enable the new host to share the hyphal networks constructed by the original host through which the plants can readily take up nutrients. In contrast, ruderal fungi that colonize new hosts mainly via soil propagules, namely soil-propagule-mediated colonizer (SP) in this study, may cost more than NC fungi. This is because SP fungi have limited C storage in the spores, and thus the new host should cover most of the cost for the establishment of intraradical and extraradical hyphal networks.

The landward slopes of the primary dunes in Ishikari coastal ecosystem are dominated by the C₄ perennial grass *M. sinensis* that grows over 2 m height (Kawahara and Ezawa 2013). *M. sinensis* juvenile plants (seedlings), therefore, should survive under the canopy of the adult plants. In this chapter, the significance of the life-history strategies of AM fungi in the partner selection of the juvenile plants under low-light availability was assessed, addressing the following hypotheses; 1) *M. sinensis* seedlings preferentially associate with NC fungi under low-light conditions, which is accompanied by 2) decreases in the occurrence of the SP fungi in the orders Glomerales and Gigasporales and increases in the occurrence of the NC fungi in the orders Entrophosporales, Diversisporales, and Archaeosporales. To test these hypotheses, a greenhouse experiment with or without shading to mimic the growth conditions of *M. sinensis* juvenile plants in the field was conducted, employing the nylon-mesh-separated compartment culture to identify the NC and SP strategist fungi.

2.2 Materials and methods

Sampling site and soil sampling

In June 2022, soil sampling was conducted in the landward slopes of Ishikari coastal dunes, Hokkaido, Japan (43°14'N, 141°20'E), of which vegetation was described in Kawahara and Ezawa (2013) and Chapter 1. Three sampling squares of 1-m-quadrat were designated at 100 m intervals along the coastal line, and six pairs of a root-soil-core sample with or without an aboveground part of *M. sinensis* plant were collected from each of them by stainless-steel core samplers [5 × 5 cm (diam/ height), 100 mL in vol]. The aboveground part was cut at 5 cm above the ground level before collecting by the sampler, which were designated as NC inocula. Whereas the samples without a living plant were sieved on a 2-mm-stainless mesh to destruct AM fungal hyphal networks before trap culture and termed as SP inocula. Twenty kilograms of sand was also collected from the seaward slope of the dunes where no vegetation was present and autoclaved for 2 h as a base medium for trap culture.

Soil trap cultures

As a main experiment, the NC inoculum was transferred to a cylinder-shaped 37- μ m-nylon mesh bag [5 × 5 cm (diam/ height)] inserted in a 400-mL plastic pot, and then the pot was filled with a mixture of the SP inoculum (100 mL) and base medium (200 mL) (Fig. 2.1). The nylon mesh allows AM fungal hyphae, but not plant roots, to pass through. As a supporting experiment, two treatments were set up to assign either of the two life-history strategies NC and SP to the fungi detected in the main experiment as follows. As NC treatment, the NC inoculum was transferred to the mesh bag, and the pot was filled with a mixture of an autoclaved SP inoculum and the base medium, whereas as a SP treatment, four-week-old *M. sinensis* seedling was transplanted in an autoclaved NC inoculum (the

living plant was removed prior to autoclaving) in the mesh bag, and the pot was filled with a mixture of the SP inoculum and base medium. Then *M. sinensis* seeds (Snow Brand Seed Co., Ltd., Sapporo) were sown outside the mesh bag as test plants, covered with a thin layer of autoclaved sand, and grown in a greenhouse either under natural light or under a plastic net that reduces natural light by 80% (n = 3). The seedlings were grown with tap water for the first month and with liquid fertilizer made of Peters Professional 25-5-20 (ICL, St. Louis, MO) at 50 μ M phosphate once a week thereafter.

After two months, the roots of the test plants were collected, processed, and stored at -80°C for DNA extraction as described in Chapter 1.

Molecular identification and strategy assignment

DNA was extracted from the frozen roots, and the LSU rDNA was amplified and sequenced as described in Chapter 1. The sequence reads were subjected to BLASTn searches against the fungal LSU rDNA database consisted of 82,769 operational taxonomic units (OTUs) of fungi, including 687 OTUs of AM fungi (<http://amfungi.kazusa.or.jp/>) and assigned to the OTUs as described in Chapter 1. Details of the phylogenetic positions of the 687 OTUs are indicated in the maximum likelihood tree of am03 release (ML-tree of am03) at <http://amfungi.kazusa.or.jp/>.

The life-history strategies of the AM fungal OTUs detected in the main experiment were assigned with reference to the supporting experiment and previous studies as follows. All the OTUs that occurred in the SP treatment in the supporting experiment, those that were categorized as SP or SP/RD fungi in Chapter 1, and those that occurred in the trap cultures in Kawahara and Ezawa (2013) were assigned to SP strategists, irrespective of the occurrence in the NC treatment. Only the OTUs that occurred "uniquely" in the NC treatment and those that were categorized as SL fungi in Chapter 1 were assigned to NC

strategists. The OTUs that occurred only in the main experiment, but neither in the supporting experiment nor in the previous studies were left unassigned. The sequences obtained in Chapter 1 and Kawahara and Ezawa (2013) were subjected to BLASTn searches and re-assigned to the latest 687 AM fungal OTUs as above prior to the strategy assignment.

Statistical analysis

All read count data were transformed to presence-absence data prior to analysis, and numbers of the sample in which the OTUs occurred were designated as abundance data for abundance-based analyses. Welch's *t*-test and two-way ANOVA followed by Tukey's post hoc test were performed on the R 4.3.1 platform (R Core Team 2023). Scatter plots were drawn with the dabestr package on the R platform (Ho et al. 2019). Similarity-difference-replacement (SDR) simplex analysis for abundance data was performed to explore OTU distribution patterns between the light and shaded conditions using the SDR-abunSimplex program (Podani et al. 2013) (<http://podani.web.elte.hu/SYN2000.html>). Light-shade preference index of the *M. sinensis* seedlings (test plants) for the OTUs were defined by the following equation:

$$\text{Light-shade preference index} = \text{OC}_{\text{shade}} - \text{OC}_{\text{light}}$$

where OC_{shade} and OC_{light} represent the occurrence of OTU, that is, the numbers of samples in which the OTU occurred, under the shaded and light conditions, respectively. Positive and negative values of the index imply that the plants associate with the OTU more preferentially under the shaded and light conditions, respectively.

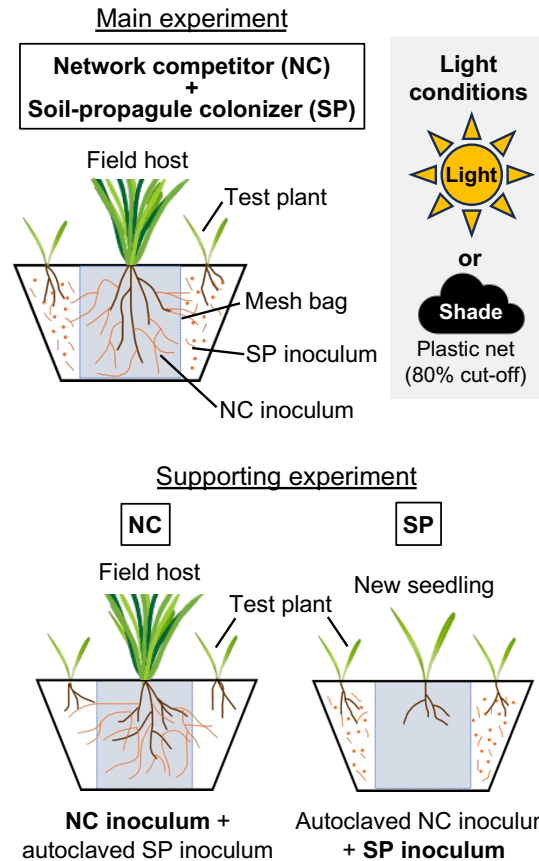


Fig. 2.1 Design of main and supporting experiments to define the two life-history strategies, network competitors (NC) and soil-propagule-mediated colonizers (SP), of AM fungi. In the main experiment, the intact root-soil core (NC inoculum) that contains a *Miscanthus sinensis*-field plant was transferred to a cylinder-shaped nylon mesh bag and inserted in a 400-mL plastic pot, and then the pot was filled with a mixture of the sieved root-zone soil (SP inoculum) and base medium. In the NC treatment of supporting experiment, the NC inoculum was surrounded by an autoclaved SP inoculum and base medium, while in the SP treatment an autoclaved NC inoculum in which a four-week-old *M. sinensis* seedling was transplanted after removing the field plant was surrounded by a mixture of the SP inoculum and base medium. Then *M. sinensis* seeds were sown outside the mesh bag as test plants, and the seedlings were grown either under the normal light or under shaded (20% of normal light) conditions for two months in a greenhouse. All AM fungi detected in the test plants of the SP treatment of the supporting experiment were defined as SP strategists, and those that occurred uniquely in the NC treatment were defined as NC strategists. Strategies of the fungi that occurred uniquely in the main experiment were assigned in reference to Kawahara and Ezawa (2013) and Chapter 1.

2.3 Results

Assignment of OTU and life-history strategy

Sequencing of the PCR products generated 23,334 – 38,802 reads per sample (29,396 reads on average). Among them, 6.2 % - 98.6 % (75.4 % on average) were assigned to 92 AM fungal OTUs (Supplementary material table S2.1). In the main experiment 50 and 44 OTUs were detected in the light and shaded conditions, respectively, in which 21 OTUs are shared between them. In the supporting experiment 23 and 28 OTUs were detected in the NC and SP treatments, respectively, under the light conditions, whereas 34 and 27 OTUs were detected in the NC and SP treatments, respectively, under the shaded conditions. Based on the occurrence of the OTUs in the supporting experiment and the previous studies, 64 and 18 OTUs could be assigned to SP and NC strategists, respectively, but 10 OTUs were left unassigned (Supplementary material table S2.2).

Impact of shading on plant preference for AM fungal life-history strategies

In the main experiment most OTUs associated with the plants were SP strategists (Fig. 2.2a). OTU richness of the whole communities (i.e., all strategists), NC fungi, SP fungi, and strategy-unassigned fungi was not different between the light and shaded conditions. Relative abundances of the NC, SP, and unassigned fungal OTUs, that is, their percentages in the total OTU numbers in each sample, were also not different between the light and shaded conditions (Fig. 2.3). Genus richness, however, was significantly higher in the whole communities under the shaded conditions, which was due to the higher genus richness of the SP fungi under the conditions ($P < 0.01$) (Fig. 2.2b).

SDR-simplex analysis at the OTU level showed that richness agreement (sum of similarity and replacement) accounted for 98.0 % of the OTU distribution pattern in which replacement consisted of 73.1%, implying that many of OTUs were replaced between the

light and shaded conditions without changing richness (Table 2.1). In contrast, the genus level analysis indicated that replacement explained only 19.3% of the distribution pattern, but instead, nestedness (sum of similarity and difference) accounted for 80.7%, implying that the genus-poor communities (i.e., those under the light conditions) were a subset of the genus-rich communities (i.e., those under the shaded conditions). The same analysis was performed on the combined dataset of the main and supporting experiments, in which similar trends were observed; richness agreement accounted for 95.7% in which replacement consisted of 55.5% at the OTU level, whereas, at the genus level, nestedness accounted for 82.9% of the pattern (Table 2.2).

Phylogenetic distribution with respect to light-shade preference index

The OTU replacement, which was accompanied by the increases in the genus richness of SP strategists with nested structure, led us to analyze phylogenetic distribution of the OTUs with respect to the light-shade preference index. At the genus level, the index of the OTUs belong to the genera *Nanoglomus* and *Paraglomus* (most are SP strategists), as well as those belong to *Rhizophagus* and *Acaulospora* (both SP and NC fungi), varied from negative to positive values (Fig. 2.4). Most of the OTUs (mainly SP strategists) in *Microdominikia* and *Racocetra* showed negative values, while all OTUs in *Glomus* (SP fungi), *Complexispora* (both SP and NC fungi), and *Entrophospora* (both SP and NC fungi) showed positive values. Genus-level-averages of the index values of the OTUs that occurred in three or more samples are: *Microdominikia*, -2.00; *Racocetra*, -0.89; *Rhizophagus*, -0.55; *Acaulospora*, 0; *Nanoglomus*, 0.20; *Glomus*, 1.00; *Complexispora*, 1.00; *Paraglomus*, 1.25; *Entrophospora*, 1.50; *Dominikia*, 2.00; *Scutellospora*, 2.00 (Fig. 2.5). At the order level, the average index value of the members of Glomerales was negative (-0.19), which was mainly due to the negative average values of the *Rhizophagus*

OTUs that consist of 57% of all Glomerales OTUs (Fig. 2.4). Whereas all average values in the other orders were positive: Gigasporales, 0.67; Entrophosporales (only *Claroideoglossum*), 1.50; Diversisporales, 0.11; Paraglomerales (only *Paraglossum*), 1.25. As expected, the average index value of the non-Glomerales OTUs (0.57) was significantly higher than that of the Glomerales OTUs (Fig. 2.6). The average values of the SP, NC, and unassigned fungi in the Glomerales were negative, whereas those in the non-Glomerales were positive (Fig. 2.7a). Among them, the average of the Glomerales SP fungi was statistically lower than that of the non-Glomerales SP fungi. This is likely due to the fact that the most *Rhizophagus* SP fungi (i.e., 10 out of the 14 OTUs) showed negative values (Fig. 2.4 and Supplementary material table S2.1). The percentages of non-Glomerales OTUs were positively correlated with the index values ($R^2 = 0.702$, $P < 0.01$) (Fig. 2.7b), implying that the plants preferentially associated with non-Glomerales fungi under shaded conditions.

Table 2.1 SDR-simplex analysis on the OTU and genus distribution patterns between the light and shaded conditions in the main experiment

Taxon level	2D Simplex (%)			1D Simplex (%)		
	Similarity (S)	Difference (D)	Replacement (R)	Beta diversity (R+D)	Agreement (S+R)	Nestedness (S+D)
OTU	25.0	1.9	73.1	75.0	98.1	26.9
Genus	35.5	45.2	19.3	64.5	54.8	80.7

Table 2.2 SDR-simplex analysis on the OTU and genus distribution patterns of the combined dataset of all experiments between the light and shaded conditions

Taxon level	2D Simplex (%)			1D Simplex (%)		
	Similarity (S)	Difference (D)	Replacement (R)	Beta diversity (R+D)	Agreement (S+R)	Nestedness (S+D)
OTU	40.2	4.3	55.5	59.8	95.7	44.5
Genus	48.6	34.3	17.1	51.4	65.7	82.9

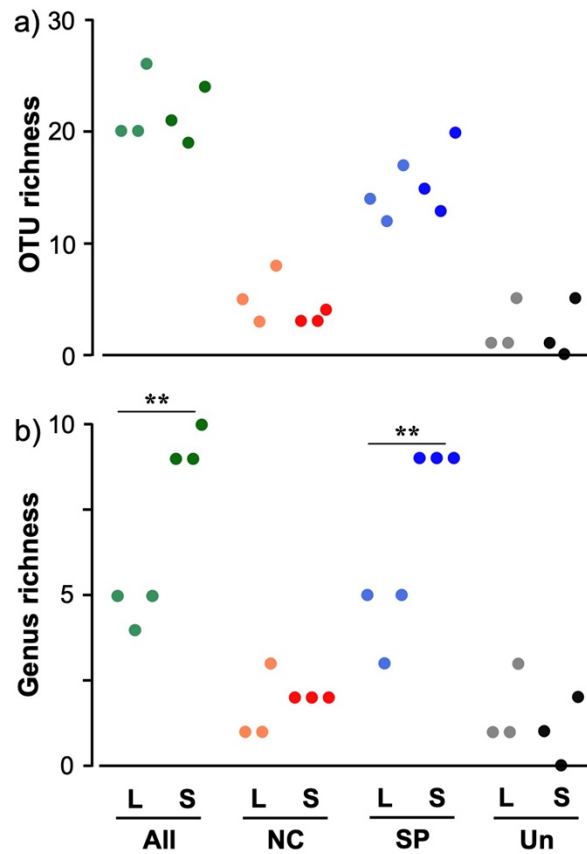


Fig. 2.2 AM fungal OTU (a) and genus (b) richness of all strategists (All), network-competitors (NC), soil-propagule-mediated colonizers (SP), and strategy-unassigned fungi (Un) under the light (L) and shaded (S) conditions in the main experiment. Welch's *t*-test was applied for the assessment of statistical significance: **, $P < 0.01$ ($n = 3$).

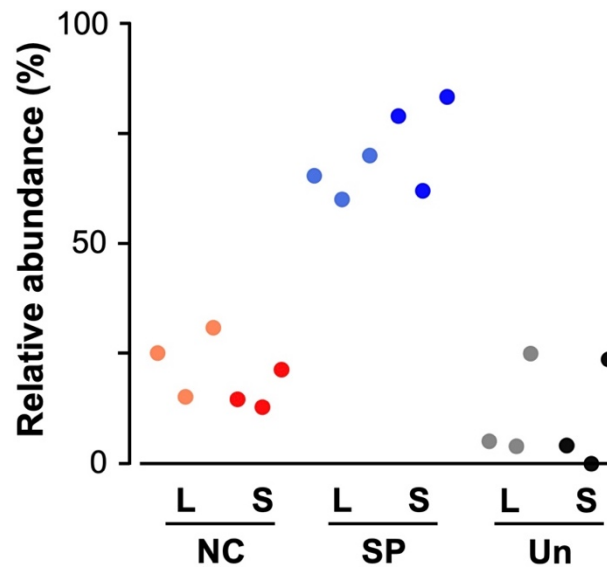


Fig. 2.3 Impact of light (L) and shading (S) on the relative abundance of network competitors (NC), soil-propagule-mediated colonizers (SP), and strategy-unassigned OTUs (Un). Relative abundance is the percentages of the numbers of NC, SP, and Un OTUs in total OTU number.

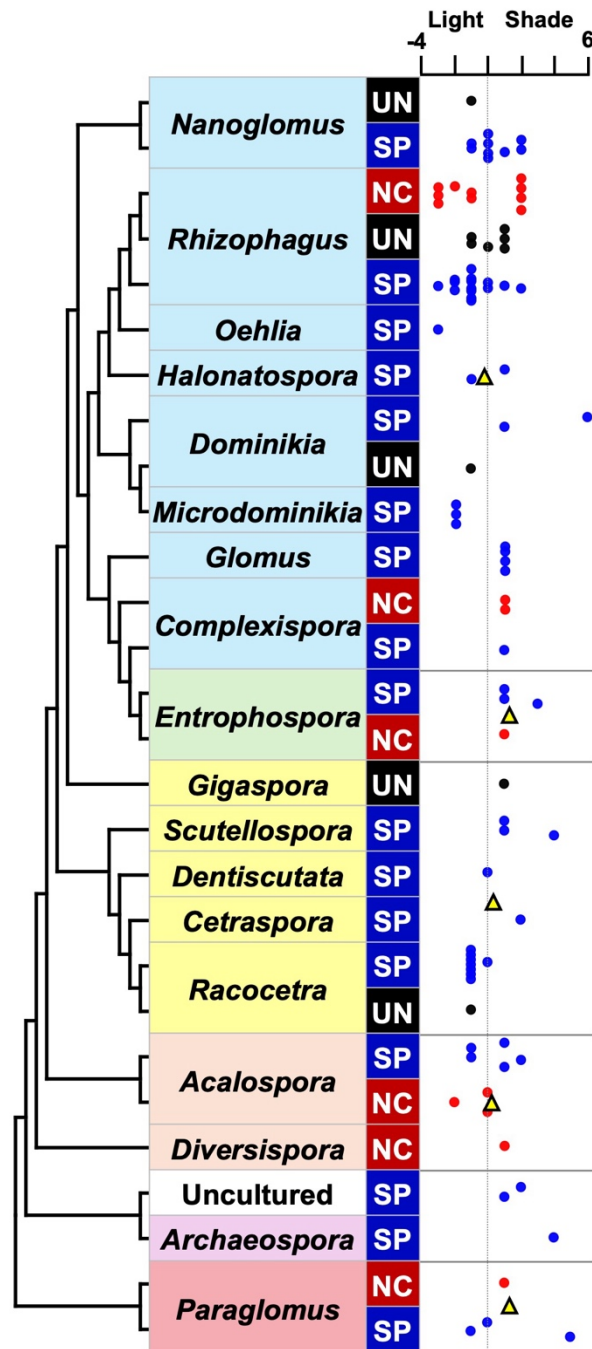


Fig. 2.4 Phylogenetic distribution of the AM fungal OTUs with which *M. sinensis* seedlings preferentially associated under the light and shaded conditions. The light-shade preference index of the OTUs was calculated by subtracting the occurrence of the OTU (number of the samples in which the OTU occurred) in the light treatment from that in the shade treatment in the combined dataset of the main and supporting experiments. For phylogenetic analysis, the OTUs are combined within the life-history strategies in each genus, and their taxonomic positions are inferred by the maximum likelihood tree

constructed using representative sequences of randomly chosen OTUs from each life-history strategy in each genus. *Nanoglomus*, *Rhizophagus*, *Oehlia*, *Halonatospora*, *Dominikia*, *Microdominikia*, *Glomus*, and *Complexispora* belong to the order Glomerales; *Entrophospora* belong to the Entrophosporales; *Gigaspora*, *Scutellospora*, *Racocetra*, *Cetraspora*, and *Dentiscutata* belong to the Gigasporales; *Acaulospora* and *Diversispora* belong to the Diversisporales; *Archaeospora* belong to the Archaeosporales; *Paraglomus* belong to the Paraglomerales. The taxonomic positions of the uncultured Glomeromycota OTUs (Uncultured) are uncertain. Triangles indicate averages of the index within each order.

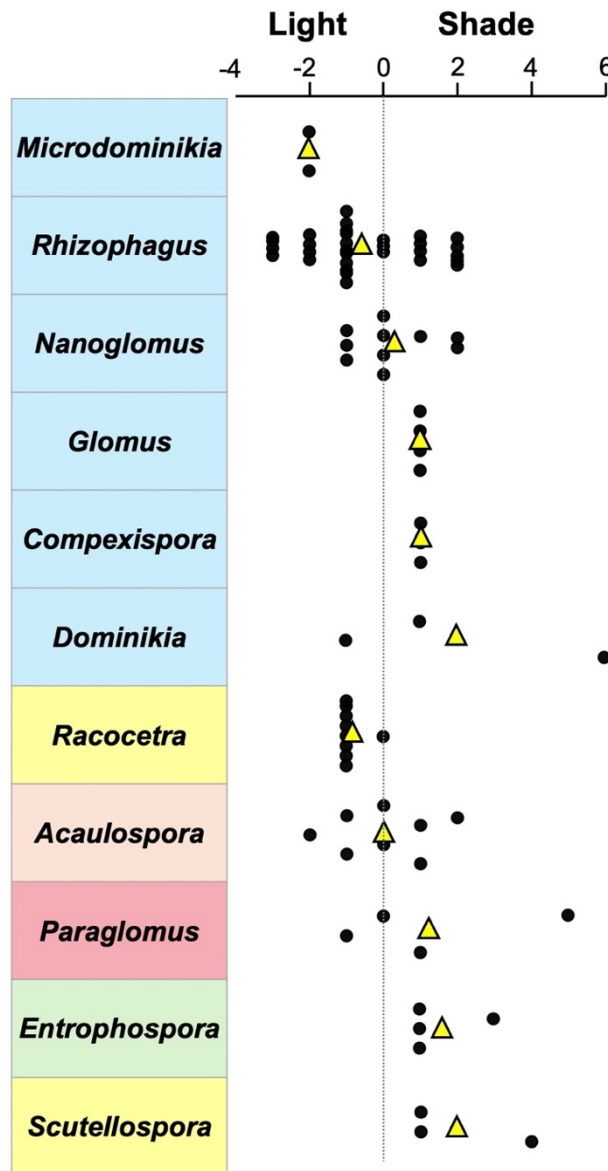


Fig. 2.5 Light-shade preference index of the OTUs belong to the genera *Microdominikia*, *Racocetra*, *Rhizophagus*, *Acaulospora*, *Nanoglomus*, *Glomus*, *Compexispora*, *Paraglomus*, *Entrophospora*, *Dominikia*, and *Scutellospora*, in which those that occurred three or more samples are presented. The light-shade preference index of the OTUs was calculated by subtracting the occurrence of the OTU (number of the samples in which the OTU occurred) in the light treatment from that in the shade treatment in the combined dataset of the main and supporting experiments. Triangles represent average values.

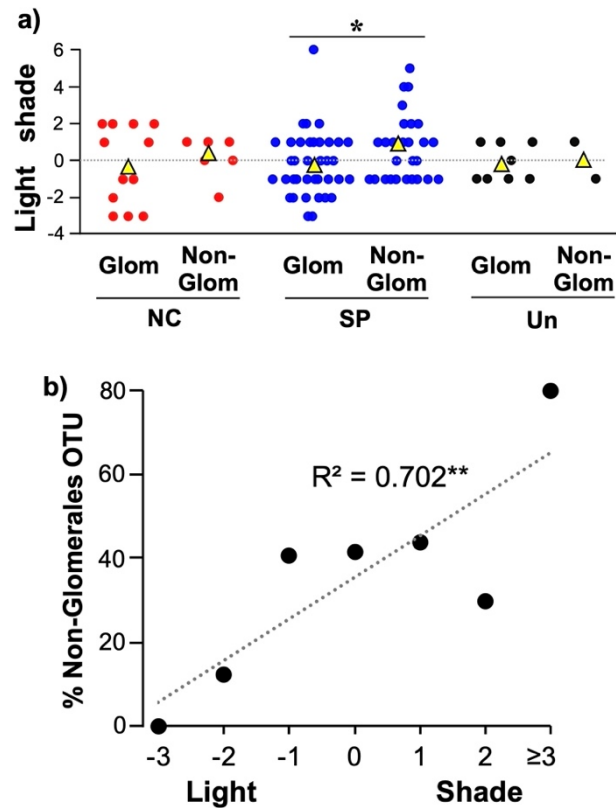


Fig. 2.7 Increases in the occurrence of non-Glomerales OTUs under the shaded conditions. a) Light-shade preference index of the network competitors (NC), soil-propagule-mediated colonizers (SP), and strategy-unassigned OTUs (Un) of Glomerales and non-Glomerales OTUs. The light-shade preference index was calculated by subtracting the occurrence of the OTU (number of the samples in which the OTU occurred) in the light treatment from that in the shade treatment in the combined dataset of the main and supporting experiments. Triangles represent average values. Welch's *t*-test was applied for the assessment of statistical significance: *, $P < 0.05$. b) Correlation of light-shade preference index and percentage non-Glomerales OTUs. Student's *t*-test was applied for the assessment of statistical significance: **, $P < 0.01$.

2.4 Discussion

This study demonstrated that SP strategist fungi play a significant role in partner selection by juvenile plants under different light availability. Unexpectedly, the impact of shading was evident only on SP fungi, but not on NC fungi, which is contrary to our first hypothesis. Shading increased the genus diversity of SP fungi without changing OTU richness, leading to nested structure at the genus level. Although the plant preferences for fungal partners generally varied at the OTU level, irrespective of their strategies, consistent preferences for particular genera were also observed; e.g., the members of *Glomus*, *Complexispora*, and *Entrophospora* were preferred under the shaded conditions, whereas those of *Microdominikia* and *Racocetra* were preferred under the light conditions. In general, the plants associated more with Glomerales fungi under the light conditions, but their occurrences were decreased under the shaded conditions with increasing the occurrences of non-Glomerales SP fungi, which partially supports our second hypothesis.

Shading did not affect the OTU richness of any of the strategists but increased the genus diversity of SP strategists by replacing with the OTUs from diverse genera, indicating that the plants selected fungal partners in response to light availability. So far, minimum information about the influence of shading on AM fungal community is available, in which shading had only limited impact not only on AM fungal richness but also on the community compositions (Koorem et al. 2017; Liu et al. 2014; Neuenkamp et al. 2021). For example, Liu et al. (2014) observed no apparent changes in richness by shading neither at the OTU level nor at higher taxonomic levels. These studies were conducted in meadows (Koorem et al. 2017; Liu et al. 2014) and forest understories (Neuenkamp et al. 2021) where soil disturbance is likely minimum. In the AM fungal communities in such stable ecosystems, a small number of competitive fungi, e.g., NC strategists, may occupy the niche and is likely robust against environmental perturbations,

e.g., host C deprivation by shading. In contrast, the coastal sand dune ecosystem employed in this study is frequently disturbed by wind and tide, and high-potential diversity of AM fungi is maintained, particularly in the landward slopes (Chapter 1; Kawahara and Ezawa, 2012) where the inocula were collected. It is likely, therefore, that this high-potential diversity, as well as the empty niche of the newly growing seedlings (test plants), allowed the plants to fine-tune the selection of the symbiotic partners.

There were no differences in the richness and relative abundance of SP and NC fungi between the light and shaded conditions. These results suggest that C cost of NC fungi is not necessarily lower than that of SP fungi for juvenile plants, or that C cost for the early stage of colonization, probably before forming arbuscules where nutrient exchange between the symbionts occurs (Lanfranco et al. 2018), is mostly covered by the fungi. The main propagule of SP fungi is likely to be spores (Chapter 1), and about 20% of spore biomass consists of neutral lipid (Olsson and Johansen 2000) that is consumed during germination (Gaspar et al. 1994) and probably also during the early stage of colonization. It is likely that this energy source in spores provides competitiveness of SP fungi, comparable to that of NC fungi.

OTU selection by the juvenile plants in response to light availability was evident in the SP fungal communities, but not in the NC fungal communities, suggesting that cost among SP fungi is more variable than that among NC fungi. The higher OTU richness of SP fungi in the roots suggests that potential diversity was much higher in the SP fungal communities than in the NC fungal communities in the ecosystem, as supported by the previous study (Chapter 1), and this would provide greater opportunities for exploring lower-cost SP fungi. Therefore, replacing high-cost SP fungi with lower-cost ones is likely more efficient than seeking lower-cost NC fungi for the plants to maintain the symbiotic function under the low-light conditions. One might assume that C deficiency occurred in

the NC fungi due to the restriction of photosynthesis in the donor plants under the shaded conditions and thus reduced inoculum potential of their extraradical hyphal networks. For example, C availability directly affects the growth of extraradical hyphae (Olsson et al. 2014). I consider, however, that this possibility is unlikely because apparent decreases in the OTU richness and relative abundance by shading was not observed in the NC communities, implying that the inoculum potential of NC fungi was comparable to that of SP fungi, irrespective of light availability. But this issue should be addressed in future studies for confirmation.

The plants generally preferred the non-Glomerales SP fungi under the shaded conditions, although there were some exceptions. This was typically characterized by the replacement of several *Rhizophagus* OTUs and all *Microdominikia* OTUs with those in the rather minor genera, e.g., *Entrophospora* (Entrophosporales), *Scutellospora* and *Cetraspora* (Gigasporales), and *Archaeospora* (Archaeosporales), leading to higher genus diversity. These observations suggest not only that C cost varies within the major genus *Rhizophagus*, even among those that have the same life-history strategy, but also that C cost of the fungi in the minor taxa is lower than that of Glomerales fungi. Glomerales fungi have been characterized as those that grow rapidly and allocate more biomass to intraradical mycelia, compared to Gigasporales fungi that grow slowly and allocate more biomass to extraradical mycelia in the soil (Hart and Reader 2002a). Glomerales fungi promote host growth greater than Gigasporales and Acaulosporales fungi in general, although significant intra-species, -genus, and -family variations in host growth response were observed (Hart and Reader 2002b). Similarly, Koch et al. (2017) observed highly variable host growth responses to different families, genera, species, and even isolates of the same species of the fungi, although fungal morphology, that is, biomass allocation to intra- and extra-radical mycelia, was conserved at the family level (Koch et al. 2017).

These studies imply that the biomass allocation patterns of AM fungi are not correlated with host growth responses. Although information about the morphology and host growth responses of such minor taxa is limited, I propose the following two mechanisms underlying the replacement of SP fungi in response to light availability; i) the Glomerales fungi are more sensitive to C availability and thus more competitive, that is, capable of colonizing more rapidly, under C-rich conditions than those in the minor taxa, but less competitive under C-deprived conditions, and/or ii) cost-benefit ratios of the fungi in the minor taxa are lower than those of the Glomerales fungi, even though their absolute benefits are smaller. Further characterization of the colonization strategies and host growth responses of such minor taxa, together with the well-characterized generalist fungi *Rhizophagus* spp., is necessary.

General discussion

In this study, Ishikari sand dunes were employed as a model ecosystem, and the significance of life-history strategies of AM fungi in a frequently disturbed ecosystem was evaluated. In Chapter 1, the life-history strategies of disturbance-tolerant and -sensitive AM fungi were experimentally characterized by taking into account the time factor necessary for regeneration after disturbance. In Chapter 2, the role of the life-history strategies in the partner selection of the juvenile plants under low-light conditions was assessed by mimicking the growth conditions of *M. sinensis* juvenile plants in the field. It is noteworthy that preferential life-history strategies are differentiated at the family (order) levels. The Glomeraceae (Glomerales) and Paraglomeraceae (Paraglomerales) fungi showed preferences for the RD strategy in addition to the SP strategy, whereas the Gigasporaceae (Gigasporales) fungi are mostly SP strategists and generally unable to regenerate from colonized roots (Chapter 1). The fact that the distribution of Gigasporales fungi was limited in the landward slopes, however, suggests that the RD strategy is more advantageous for survival in severely and frequently disturbed environments. Glomerales fungi that employ the SP/RD strategies are most prevalent in the ecosystem and play major roles not only in the rapid regeneration of the association but also in the establishment of *M. sinensis* juvenile plants, but their C cost or cost-benefit ratios seem higher than non-Glomerales fungi (Chapter 2), which is likely due to their life-history strategies. A majority of Glomerales fungi are rapid regenerators and employ the SP/RD strategies (i.e., dual strategy), but in non-Glomerales only a few fungi employ the dual strategy (Chapter 1). This suggests that Glomerales fungi invest more in propagule production, enabling more rapid regeneration after disturbance, but are more costly for the host. In conclusion, these studies provide a new insight into the life-history strategies of AM fungi in the frequently

disturbed ecosystems and will contribute to the practical application of AM fungi for the restoration of post-disturbed ecosystems.

Summary

Soil disturbance occurs through anthropogenic or natural processes and causes serious environmental issues. Arbuscular mycorrhizal (AM) fungi form symbiotic associations with most land plants, and soil disturbance has a serious impact on AM symbiosis by destructing extraradical hyphal networks in the soil. AM fungi that regenerate rapidly after disturbance may play a significant role in vegetation resilience. In this study, the significance of life-history strategies of AM fungi was assessed, employing a coastal sand dune ecosystem where the severity of disturbance is differentiated between the seaward and landward slopes of the dunes.

To characterize the life-history strategies of AM fungi along a disturbance gradient of the dunes, root-soil samples were collected from the seaward and landward slopes, and the native grass *Miscanthus sinensis* (donor plants) were grown in the soil samples. From a half of the plants, disturbance-tolerant fungi that regenerate from spores and extraradical hyphae (soil propagule-mediated regenerators, SP) and those that regenerate from the colonized roots (root-direct regenerators, RD) were trapped separately with new seedlings (assessment plants). The other half of the donor plants were further grown without destruction together with assessment plants, during which the fungi trapped by the plants were categorized as disturbance-sensitive slow regenerators (SL). DNA was extracted from the roots of assessment plants for identification based on LSU rDNA sequences. All the fungi that occurred widely in the seaward samples belonged to the Glomeraceae and Paraglomeraceae and preferred the RD strategy to the SP strategy. In the landward samples diverse taxa with diverse strategies occurred, and among them, Gigasporaceae fungi showed a distinct preference for the SP strategy. These observations suggest that not only SP but also RD strategies play a key role in the rapid regeneration of the fungi after disturbance.

To assess the significance of the two-contrasting life-history strategies, the fungi that explore a new host via spores and hyphal fragments (soil propagules, SP) and hyphal networks (network competitors, NC), in the partner selection of juvenile plants growing under the canopy of adult plants, a trap culture experiment was conducted in the presence and absence of shade. Intact root-soil-core samples (NC inocula) and root-zone-soil samples (sieved before inoculation, SP inocula) were collected from the landward slopes, and *M. sinensis* seedlings were grown with or without shading in the presence of both inocula (NC+SP, main experiment) and also grown either with the NC or SP inocula to assign the strategies of the fungi occurred in the main experiment. DNA was extracted from the roots for identification based on LSU rDNA sequences. Shading increased the genus richness of SP fungi, but not that of NC fungi, maintaining species richness. Similarity-difference-replacement analysis revealed that replacement was a dominant pattern at the species level, while nestedness was a dominant pattern at the genus level, indicating that the SP fungi that occurred under the light conditions, mainly *Rhizophagus* spp., were replaced with those from more diverse genera under the shaded conditions. These observations suggest that fungal life-history strategies affect plant partner selection under different light availability and, further, that cost of SP fungi is not necessarily higher than that of NC fungi.

Acknowledgements

First and foremost, praise is merely to Allah SWT for His grace and blessing.

Sincere grateful goes to my supervisor, Assoc. Prof. Tatsuhiro Ezawa, for his advice and guidance in conducting research and thesis writing. His passion in research and supervising strategies shape my perspective in understanding bioscience and global agriculture problems. I also extend my thanks to Prof. Yo Toma and Prof. Yoichi Kamagata for reviewing this thesis manuscript. I am also thankful to MEXT scholarship for providing funding for my five years study in Japan.

I also thankful for the members of Rhizosphere Control Laboratory and all friends living in Sapporo for meaningful discussion, helps, and encouragement in the laboratory and daily life.

I thank my parents (Waljito and Budi Rahayu) for their financial and moral support during my 23 years academic journey until this doctoral stage. Lastly, I thank my husband (Muh Imam Nurhidayat), my baby sister (Rafiqah Zahratunnisa), also my extended family and friends for their endless support in this journey.

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Supplementary material

Table S1.1 Raw read count of blastn searches against the LSU rDNA database consisted of 82,606 fungal operational taxonomic units (OTUs)

OTU	SP_L1	SP_L2	SP_L3	SP_L4	SP_L5	RD_L1	RD_L2	RD_L3	RD_L4	RD_L5	SL_L1	SL_L2	SL_L3	SL_L4	SL_L5
001_Rhz_AB369921	0	4	68	0	0	0	15	101	0	0	0	80	54	0	0
002_Rhz_HQ895800	0	0	2	0	0	0	1	5	0	0	0	6	2	0	0
003_Rhz_AB640749	0	1503	8383	0	3	0	4559	13471	78	0	0	9065	6081	0	0
006_Rhz_FM865608	0	5	26	0	0	0	3	29	0	0	0	18	14	0	16
007_Rhz_AB369745	1	8977	22924	105	0	36	24314	46137	337	21	10	22360	16739	5	25566
008_Rhz_AB640745	0	0	0	0	0	0	0	0	0	0	0	0	2	0	5939
009_Rhz_FM992381	0	0	3	0	0	0	0	1	0	0	0	2	2	0	1
011_Rhz_AB643805	0	1728	5261	4	0	0	5634	10054	69	0	1	5134	3659	1	0
024_Rhz_LC014178	0	3	0	0	0	0	0	0	0	0	0	5	0	0	1
029_Fun_AB812588	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
049_Rhz_AB561092	0	0	2	2489	34873	0	1383	183	41663	48233	0	2	32103	24191	0
060_UnG_LC176583	0	11	0	0	0	0	0	0	0	0	0	0	0	0	0
064_UnG_AB561101	0	0	295	0	3848	0	0	11	25714	29534	39614	94	22619	7743	2317
065_UnG_AB750741	0	0	0	0	1	0	0	0	5	4	29	0	16	10	6
068_UnG_AB369764	0	6058	0	0	0	0	0	0	0	0	2	0	0	5	5078
069_UnG_AB369759	0	2	0	0	0	0	0	0	0	0	0	0	0	0	2
070_UnG_HE794042	0	27	0	0	0	0	0	0	0	0	0	0	0	0	32
072_UnG_AB643635	0	9590	0	0	0	0	0	0	0	1	9	0	7	6	17389
073_UnG_AB369762	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3
081_Glo_AB710220	0	0	0	0	0	0	0	0	0	1	103	0	78	0	0
083_Glo_HE775339	0	0	0	0	0	0	0	0	0	0	45	0	0	0	0
085_Glo_AB840271	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
086_Glo_AB665502	828	0	0	0	8161	0	0	0	0	32	0	0	0	0	0
104_UnG_LC014191	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
105_UnG_AB640742	0	0	0	811	0	0	0	0	0	143	0	2	5	3866	0
106_UnG_LC176561	0	0	0	0	0	0	0	0	0	0	0	7	0	2	0
107_UnG_AB369767	11344	0	3	4028	8508	0	0	0	0	301	895	870	0	608	66
108_UnG_AB561107	89	0	4	284	363	0	0	0	0	3	263	744	0	838	75
111_UnG_LC176588	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
112_UnG_HE794043	0	0	0	0	0	0	0	0	0	0	0	0	0	84	0
113_UnG_AB547177	0	0	0	0	0	0	0	0	0	0	0	0	0	47	0
119_UnG_AB561104	0	0	0	0	0	0	0	0	0	0	6	0	0	0	0
120_UnG_AB369765	16884	0	356	0	0	11576	1	4961	11	0	12218	0	61	0	0
121_UnG_HE775338	0	0	0	0	0	0	1	6	0	0	23	0	0	0	0
122_UnG_JF439191	13169	0	12	0	1	20998	12579	2039	303	51	5069	236	2937	213	23
124_Cla_AB561129	0	0	0	0	0	0	0	0	44	0	0	0	0	0	0

Table S1.1 Continued

OTU	SP_S1	SP_S2	SP_S3	SP_S4	SP_S5	RD_S1	RD_S2	RD_S3	RD_S4	RD_S5	SL_S1	SL_S2	SL_S3	SL_S4	SL_S5
001_Rhz_AB369921	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
002_Rhz_HQ895800	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
003_Rhz_AB640749	0	1	0	0	0	1	0	0	0	0	0	1	0	0	0
006_Rhz_FM865608	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
007_Rhz_AB369745	0	0	429	0	0	898	197	1	256	0	0	144	12	2	511
008_Rhz_AB640745	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
009_Rhz_FM992381	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
011_Rhz_AB643805	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
024_Rhz_LC014178	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
029_Fun_AB812588	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
049_Rhz_AB561092	0	251	0	0	0	1437	820	225	0	0	1	0	1	2	3
060_UnG_LC176583	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
064_UnG_AB561101	0	1	0	0	0	6259	0	0	0	0	361	0	1	1201	240
065_UnG_AB750741	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0
068_UnG_AB369764	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
069_UnG_AB369759	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
070_UnG_HE794042	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
072_UnG_AB643635	0	499	1	0	0	1	0	0	0	0	0	0	0	0	0
073_UnG_AB369762	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
081_Glo_AB710220	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
083_Glo_HE775339	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
085_Glo_AB840271	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
086_Glo_AB665502	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
104_UnG_LC014191	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
105_UnG_AB640742	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
106_UnG_LC176561	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
107_UnG_AB369767	0	0	0	0	0	0	0	0	0	0	0	0	0	0	432
108_UnG_AB561107	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
111_UnG_LC176588	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
112_UnG_HE794043	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
113_UnG_AB547177	0	0	57	0	0	0	0	0	0	0	0	0	0	0	0
119_UnG_AB561104	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
120_UnG_AB369765	0	0	0	0	0	0	0	0	1	0	1	0	0	0	1
121_UnG_HE775338	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
122_UnG_JF439191	0	491	0	0	0	0	0	2	1	0	1	0	0	0	0
124_Cla_AB561129	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table S1.1 Continued

OTU	SP_L1	SP_L2	SP_L3	SP_L4	SP_L5	RD_L1	RD_L2	RD_L3	RD_L4	RD_L5	SL_L1	SL_L2	SL_L3	SL_L4	SL_L5
125_Cla_AB812617	0	0	0	0	0	0	0	0	0	0	0	0	0	0	248
126_Cla_AB812616	0	0	0	0	29	0	0	0	0	0	0	0	0	0	70
127_Cla_AB812614	0	0	0	0	7	0	0	0	0	0	0	0	0	0	59
143_Cla_AB935545	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0
153_Scu_AB561124	0	7800	64	2382	0	0	0	0	0	0	1	0	14	7	2851
154_Scu_FN547618	0	1411	48	0	0	0	0	0	0	0	0	901	35	25	10
156_Scu_FR750142	0	17356	4522	0	1	0	0	1	0	0	0	26992	1	0	0
157_UnG_AB665510	19960	18	10	7099	14255	0	1	0	0	444	9642	2820	0	2573	570
176_Arc_JX848923	0	0	0	0	0	0	0	0	0	0	0	0	937	5	0
177_Arc_JX848776	0	0	0	0	0	0	0	0	0	0	0	0	8	0	0
190_Aca_AY639328	0	0	0	0	0	0	0	0	0	0	14	0	0	24	0
191_Aca_AB610835	0	0	0	0	13	0	0	0	0	0	3575	1	0	3786	0
206_Div_AB640737	0	0	0	0	0	0	0	0	0	0	660	0	0	0	0
207_Div_JF439144	0	0	0	0	0	0	0	0	0	0	346	0	0	0	0
216_Par_AB610837	542	12	1245	57	54	3	140	1308	98	81	105	84	71	1177	6
229_Par_AB547188	0	0	0	0	0	0	0	0	0	0	128	0	0	0	4391
242_UnG_JQ218218	0	0	0	0	0	0	0	0	0	0	3593	0	0	0	0
245_UnG_JX043231	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
246_UnG_JF717517	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0
247_UnG_HE775292	0	7835	0	0	14	0	0	0	0	12	21	0	6	17	184
250_Glo_HE775291	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0
280_UnG_AB750742	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
287_Scu_HE962462	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
296_Scu_FM876834	0	532	434	0	0	0	0	0	0	0	0	1008	58	31	0
297_Scu_JN867206	0	6443	1425	0	0	0	0	0	0	0	0	8746	1	1	0
299_Scu_GU322902	0	1	0	0	0	0	0	0	0	0	0	0	0	0	2
301_Scu_JF717509	0	0	0	10	0	0	0	0	0	0	0	0	0	0	0
318_Div_AB561119	0	0	0	0	0	0	0	0	0	0	1725	0	0	0	0
361_Aca_LC191603	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
422_Par_LC191651	8712	13	3027	5453	0	0	0	0	0	0	4326	1492	0	1843	2283
424_Par_LC191653	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
437_Scu_LC416090	0	236	0	0	0	0	0	0	0	0	0	689	0	0	0
439_Scu_LC416092	0	15	1	9	1	0	0	0	0	0	0	6	0	0	0
441_Gig_LC416094	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0
449_Gig_LC416101	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0

Table S1.1 Continued

OTU	SP_S1	SP_S2	SP_S3	SP_S4	SP_S5	RD_S1	RD_S2	RD_S3	RD_S4	RD_S5	SL_S1	SL_S2	SL_S3	SL_S4	SL_S5
125_Cla_AB812617	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
126_Cla_AB812616	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
127_Cla_AB812614	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
143_Cla_AB935545	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
153_Scu_AB561124	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
154_Scu_FN547618	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
156_Scu_FR750142	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
157_UnG_AB665510	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
176_Arc_JX848923	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
177_Arc_JX848776	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
190_Aca_AY639328	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
191_Aca_AB610835	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
206_Div_AB640737	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
207_Div_JF439144	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
216_Par_AB610837	45987	38022	27876	0	0	55479	32043	40120	35616	0	83555	94350	108925	94845	95421
229_Par_AB547188	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
242_UnG_JQ218218	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
245_UnG_JX043231	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
246_UnG_JF717517	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
247_UnG_HE775292	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0
250_Glo_HE775291	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
280_UnG_AB750742	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
287_Scu_HE962462	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
296_Scu_FM876834	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
297_Scu_JN867206	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
299_Scu_GU322902	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
301_Scu_JF717509	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
318_Div_AB561119	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
361_Aca_LC191603	0	0	9	0	0	0	0	0	0	0	0	0	0	0	0
422_Par_LC191651	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0
424_Par_LC191653	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
437_Scu_LC416090	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
439_Scu_LC416092	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
441_Gig_LC416094	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
449_Gig_LC416101	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table S1.1 Continued

OTU	SP_L1	SP_L2	SP_L3	SP_L4	SP_L5	RD_L1	RD_L2	RD_L3	RD_L4	RD_L5	SL_L1	SL_L2	SL_L3	SL_L4	SL_L5
453_Scu_LC416104	0	3044	133	0	0	0	0	1	0	0	0	1597	81	68	0
462_Glo_LC416113	0	0	0	0	659	0	0	0	0	0	0	0	0	0	0
464_Glo_LC416115	0	0	0	0	5	0	0	0	0	0	0	0	0	0	0
466_Scu_LC416117	0	1	0	0	0	0	0	0	0	0	0	0	0	0	7
480_Cla_LC416130	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
495_UnG_LC416143	1	0	0	3	0	0	0	0	0	0	0	1	0	0	0
501_Cla_LC416190	0	0	0	0	0	0	0	0	0	0	0	0	0	11	0
515_Rhz_LC416158	0	0	0	0	0	0	0	0	0	0	0	0	0	0	365
528_Rhz_LC416191	0	6	12	0	0	0	22	31	0	0	0	9	7	0	0
532_Rhz_LC416173	0	0	1	0	0	0	0	0	0	0	0	0	0	0	18
534_UnG_LC416175	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
538_UnG_LC416177	0	0	0	1	2	0	0	0	0	0	16	0	0	1	0
AM fungi total	71531	72631	48262	22736	70799	32613	48656	78339	68322	78862	82445	82971	85598	47194	67579
Richness	11	26	26	15	20	4	14	16	10	15	32	28	27	32	30
% AMF read	96.1	86.9	61.2	23.4	98.9	99.3	79.9	97.3	72.1	100.0	97.8	96.3	99.7	44.6	82.9

Table S1.1 Continued

OTU	SP_S1	SP_S2	SP_S3	SP_S4	SP_S5	RD_S1	RD_S2	RD_S3	RD_S4	RD_S5	SL_S1	SL_S2	SL_S3	SL_S4	SL_S5
453_Scu_LC416104	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
462_Glo_LC416113	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
464_Glo_LC416115	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
466_Scu_LC416117	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
480_Cla_LC416130	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
495_UnG_LC416143	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
501_Cla_LC416190	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
515_Rhz_LC416158	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
528_Rhz_LC416191	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
532_Rhz_LC416173	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
534_UnG_LC416175	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
538_UnG_LC416177	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
AM fungi total	45987	39265	28372			64080	33060	40348	35874		83920	94497	108942	96050	96608
Richness	1	6	5			8	3	4	4		6	5	7	4	6
% AMF read	99.2	75.6	39.7			78.5	70.0	98.6	99.8		100.0	99.3	99.7	99.9	99.4

Table S1.2 Assignment of life-history strategy of the AM fungal OTUs.

OTU	Genus	Family	Assigned strategy	Occurrence				
				Trap culture			Habitat	
				SP	RD	SL	Land	Sea
001_Rhz_AB369921	<i>Rhizophagus</i>	Glomeraceae	Soil propagule-mediated/ root-direct	*	*	*	*	
002_Rhz_HQ895800	<i>Rhizophagus</i>	Glomeraceae	Soil propagule-mediated/ root-direct	*	*	*	*	
003_Rhz_AB640749	<i>Rhizophagus</i>	Glomeraceae	Soil propagule-mediated/ root-direct	*	*	*	*	*
006_Rhz_FM865608	<i>Rhizophagus</i>	Glomeraceae	Soil propagule-mediated/ root-direct	*	*	*	*	*
007_Rhz_AB369745	<i>Rhizophagus</i>	Glomeraceae	Soil propagule-mediated/ root-direct	*	*	*	*	*
008_Rhz_AB640745	<i>Rhizophagus</i>	Glomeraceae	Slow regenerator			*	*	
009_Rhz_FM992381	<i>Rhizophagus</i>	Glomeraceae	Soil propagule-mediated/ root-direct	*	*	*	*	
011_Rhz_AB643805	<i>Rhizophagus</i>	Glomeraceae	Soil propagule-mediated/ root-direct	*	*	*	*	
024_Rhz_LC014178	<i>Oehlia</i>	Glomeraceae	Soil propagule-mediated	*		*	*	
029_Fun_AB812588	<i>Funneliformis</i>	Glomeraceae	Root-direct		*		*	
049_Rhz_AB561092	<i>Halonatospora</i>	Glomeraceae	Soil propagule-mediated/ root-direct	*	*	*	*	*
060_UnG_LC176583	<i>Nanoglomus</i>	Glomeraceae	Soil propagule-mediated	*			*	
064_UnG_AB561101	<i>Nanoglomus</i>	Glomeraceae	Soil propagule-mediated/ root-direct	*	*	*	*	*
065_UnG_AB750741	<i>Nanoglomus</i>	Glomeraceae	Soil propagule-mediated/ root-direct	*	*	*	*	*
068_UnG_AB369764	<i>Nanoglomus</i>	Glomeraceae	Soil propagule-mediated	*		*	*	
069_UnG_AB369759	<i>Nanoglomus</i>	Glomeraceae	Soil propagule-mediated	*		*	*	
070_UnG_HE794042	<i>Nanoglomus</i>	Glomeraceae	Soil propagule-mediated	*		*	*	
072_UnG_AB643635	<i>Nanoglomus</i>	Glomeraceae	Soil propagule-mediated/ root-direct	*	*	*	*	*
073_UnG_AB369762	<i>Nanoglomus</i>	Glomeraceae	Slow regenerator			*	*	
081_Glo_AB710220	<i>Glomus</i>	Glomeraceae	Root-direct		*	*	*	
083_Glo_HE775339	<i>Glomus</i>	Glomeraceae	Slow regenerator			*	*	
085_Glo_AB840271	<i>Glomus</i>	Glomeraceae	Soil propagule-mediated	*			*	
086_Glo_AB665502	<i>Glomus</i>	Glomeraceae	Soil propagule-mediated/ root-direct	*	*	*	*	*
104_UnG_LC014191	<i>Dominikia</i>	Glomeraceae	Slow regenerator			*	*	
105_UnG_AB640742	<i>Microdominikia</i>	Glomeraceae	Soil propagule-mediated/ root-direct	*	*	*	*	
106_UnG_LC176561	<i>Microdominikia</i>	Glomeraceae	Slow regenerator			*	*	
107_UnG_AB369767	<i>Microdominikia</i>	Glomeraceae	Soil propagule-mediated/ root-direct	*	*	*	*	*
108_UnG_AB561107	<i>Microdominikia</i>	Glomeraceae	Soil propagule-mediated/ root-direct	*	*	*	*	
111_UnG_LC176588	<i>Dominikia</i>	Glomeraceae	Slow regenerator			*	*	

Table S1.2 continued

OTU	Genus	Family	Assigned strategy	Occurrence				
				Trap culture			Habitat	
				SP	RD	SL	Land	Sea
112_UnG_HE794043	<i>Dominikia</i>	Glomeraceae	Slow regenerator			*	*	
113_UnG_AB547177	<i>Dominikia</i>	Glomeraceae	Soil propagule-mediated	*		*	*	*
119_UnG_AB561104	<i>Dominikia</i>	Glomeraceae	Slow regenerator			*	*	
120_UnG_AB369765	<i>Dominikia</i>	Glomeraceae	Soil propagule-mediated/ root-direct	*	*	*	*	*
121_UnG_HE775338	<i>Dominikia</i>	Glomeraceae	Root-direct		*	*	*	
122_UnG_JF439191	<i>Dominikia</i>	Glomeraceae	Soil propagule-mediated/ root-direct	*	*	*	*	*
124_Cla_AB561129	<i>Entrophospora</i>	Entrophosporaceae	Root-direct		*		*	
125_Cla_AB812617	<i>Entrophospora</i>	Entrophosporaceae	Slow regenerator			*	*	
126_Cla_AB812616	<i>Entrophospora</i>	Entrophosporaceae	Soil propagule-mediated	*		*	*	
127_Cla_AB812614	<i>Entrophospora</i>	Entrophosporaceae	Soil propagule-mediated	*		*	*	
143_Cla_AB935545	<i>Entrophospora</i>	Entrophosporaceae	Slow regenerator			*	*	
153_Scu_AB561124	<i>Scutellospora</i>	Gigasporaceae	Soil propagule-mediated	*		*	*	*
154_Scu_FN547618	<i>Racocetra</i>	Gigasporaceae	Soil propagule-mediated	*		*	*	
156_Scu_FR750142	<i>Racocetra</i>	Gigasporaceae	Soil propagule-mediated/ root-direct	*	*	*	*	*
157_UnG_AB665510	<i>Microdominikia</i>	Glomeraceae	Soil propagule-mediated/ root-direct	*	*	*	*	
176_Arc_JX848923	<i>Archaeospora</i>	Arcaeosporaceae	Slow regenerator			*	*	
177_Arc_JX848776	<i>Archaeospora</i>	Arcaeosporaceae	Slow regenerator			*	*	
190_Aca_AY639328	<i>Acaulospora</i>	Acaurosporaceae	Slow regenerator			*	*	
191_Aca_AB610835	<i>Acaulospora</i>	Acaurosporaceae	Soil propagule-mediated	*		*	*	
206_Div_AB640737	<i>Diversispora</i>	Diversisporaceae	Slow regenerator			*	*	
207_Div_JF439144	<i>Diversispora</i>	Diversisporaceae	Slow regenerator			*	*	
216_Par_AB610837	<i>Paraglomus</i>	Paraglomeraceae	Soil propagule-mediated/ root-direct	*	*	*	*	*
229_Par_AB547188	<i>Paraglomus</i>	Paraglomeraceae	Slow regenerator			*	*	
242_UnG_JQ218218	<i>Dominikia</i>	Glomeraceae	Slow regenerator			*	*	
245_UnG_JX043231	<i>Dominikia</i>	Glomeraceae	Slow regenerator			*	*	
246_UnG_JF717517	<i>Dominikia</i>	Glomeraceae	Root-direct		*		*	
247_UnG_HE775292	<i>Nanoglomus</i>	Glomeraceae	Soil propagule-mediated/ root-direct	*	*	*	*	*
250_Glo_HE775291	<i>Glomus</i>	Glomeraceae	Slow regenerator			*	*	

Table S1.2 continued

OTU	Genus	Family	Assigned strategy	Occurrence				
				Trap culture			Habitat	
				SP	RD	SL	Land	Sea
280_UnG_AB750742	<i>Nanoglomus</i>	Glomeraceae	Slow regenerator			*	*	
287_Scu_HE962462	<i>Dentiscutata</i>	Gigasporaceae	Slow regenerator			*	*	
296_Scu_FM876834	<i>Racocetra</i>	Gigasporaceae	Soil propagule-mediated	*		*	*	
297_Scu_JN867206	<i>Racocetra</i>	Gigasporaceae	Soil propagule-mediated	*		*	*	
299_Scu_GU322902	<i>Scutellospora</i>	Gigasporaceae	Soil propagule-mediated	*		*	*	
301_Scu_JF717509	<i>Scutellospora</i>	Gigasporaceae	Soil propagule-mediated	*			*	
318_Div_AB561119	<i>Diversispora</i>	Diversisporaceae	Slow regenerator			*	*	
361_Aca_LC191603	<i>Acaulospora</i>	Acaulosporaceae	Soil propagule-mediated	*				*
422_Par_LC191651	<i>Paraglomus</i>	Paraglomeraceae	Soil propagule-mediated	*		*	*	*
424_Par_LC191653	<i>Paraglomus</i>	Paraglomeraceae	Slow regenerator			*	*	
437_Scu_LC416090	<i>Racocetra</i>	Gigasporaceae	Soil propagule-mediated	*		*	*	
439_Scu_LC416092	<i>Racocetra</i>	Gigasporaceae	Soil propagule-mediated	*		*	*	
441_Gig_LC416094	<i>Gigaspora</i>	Gigasporaceae	Soil propagule-mediated	*			*	
449_Gig_LC416101	<i>Gigaspora</i>	Gigasporaceae	Soil propagule-mediated	*			*	
453_Scu_LC416104	<i>Racocetra</i>	Gigasporaceae	Soil propagule-mediated/ root-direct	*	*	*	*	
462_Glo_LC416113	<i>Glomus</i>	Glomeraceae	Soil propagule-mediated	*			*	
464_Glo_LC416115	<i>Glomus</i>	Glomeraceae	Soil propagule-mediated	*			*	
466_Scu_LC416117	<i>Scutellospora</i>	Gigasporaceae	Soil propagule-mediated	*		*	*	
480_Cla_LC416130	<i>Entrophospora</i>	Entrophosporaceae	Slow regenerator			*	*	
495_UnG_LC416143	<i>Microdominikia</i>	Glomeraceae	Soil propagule-mediated	*		*	*	
501_Cla_LC416190	<i>Entrophospora</i>	Entrophosporaceae	Slow regenerator			*	*	
515_Rhz_LC416158	<i>Rhizophagus</i>	Glomeraceae	Slow regenerator			*	*	
528_Rhz_LC416191	<i>Rhizophagus</i>	Glomeraceae	Soil propagule-mediated/ root-direct	*	*	*	*	
532_Rhz_LC416173	<i>Rhizophagus</i>	Glomeraceae	Soil propagule-mediated	*		*	*	
534_UnG_LC416175	<i>Microdominikia</i>	Glomeraceae	Slow regenerator			*	*	
538_UnG_LC416177	<i>Dominikia</i>	Glomeraceae	Soil propagule-mediated	*		*	*	
Total OTU no.				51	28	72	82	18

*Asterisks indicate occurrence within each of the trap cultures and habitats.

Table S1.3 Summary of blast searches for the phylotypes in Kawahara and Ezawa (2013) against the 524 AM fungal operational taxonomic units (OTUs)

Phylotype	Clone	Accession	Habitat/origin	Source	Top hit OTU	% Identity	E value
Aca1	KB5-3	AB561120	Landward	Trap	183_Aca_AF389017	99.33	1.00E-152
Aca2	KB3-48	AB561121	Landward	Trap	361_Aca_LC191603	97.14	2.00E-150
Aca3	KB4-10	AB561122	Landward	Trap	191_Aca_AB610835	99.04	6.00E-160
Aca4	KAE9-1	AB561123	Seaward	Field	188_Aca_AB561123	100	8.00E-164
Cla1	08KB5-3	AB561128	Landward	Trap	145_Cla_AY639200	97.71	2.00E-170
Cla2	08KB4-14	AB561129	Landward	Trap	124_Cla_AB561129	100	2.00E-155
Div1	KA1-86	AB561115	Seaward	Trap	200_Div_JF439096	96.56	3.00E-163
Div2	KAE4-1	AB561116	Seaward	Field	206_Div_AB640737	98.85	1.00E-177
Div2	KA5-17	AB561117	Seaward	Trap	209_Div_AB561117	100	0
Div2	08KB4-21	AB640737	Landward	Trap	206_Div_AB640737	100	0
Div3	KBM6-2	AB561118	Landward	Field	204_Div_AB640743	99.43	0
Div3	08KA7-1	AB640743	Seaward	Trap	204_Div_AB640743	100	0
Div4	KB5-3-33	AB561119	Landward	Trap	318_Div_AB561119	100	2.00E-180
Gig1	KB3-2-43	AB561124	Landward	Trap	153_Scu_AB561124	100	8.00E-164
Gig2	KAE6-16	AB561125	Seaward	Field	154_Scu_FN547618	99.31	7.00E-149
Gig2	08KB6-47	AB561126	Landward	Trap	156_Scu_FR750142	97.59	2.00E-140
Glo1	KA1-64	AB561113	Seaward	Trap	034_Fun_FN547491	98.67	0
Glo1	KAE9-3	AB561114	Seaward	Field	034_Fun_FN547491	96.29	8.00E-174
Glo2	08KB9-3	AB640731	Landward	Trap	086_Glo_AB665502	96.24	5.00E-161
Glo3	KA1-55	AB561099	Seaward	Trap	069_UnG_AB369759	99.72	0
Glo3	KAE6-11	AB561100	Seaward	Field	073_UnG_AB369762	98.31	4.00E-177
Glo3	KB6-2-8	AB561101	Landward	Trap	064_UnG_AB561101	100	0
Glo3	KBM6-8	AB643635	Landward	Field	072_UnG_AB643635	100	0
Glo4	KBM9-8	AB561102	Landward	Field	066_UnG_AB561102	100	0
Glo4	08KB9-23	AB643636	Landward	Trap	066_UnG_AB561102	97.75	2.00E-174
Glo5	KAE4-19	AB561103	Seaward	Field	119_UnG_AB561104	98.29	2.00E-175
Glo5	KBM5-1	AB561104	Landward	Field	119_UnG_AB561104	100	0
Glo5	KA5-5	AB561105	Seaward	Trap	119_UnG_AB561104	98.86	8.00E-179
Glo5	KB4-22	AB561106	Landward	Trap	119_UnG_AB561104	98.86	8.00E-179
Glo6	KAE12-14	AB561107	Seaward	Field	108_UnG_AB561107	100	0
Glo6	KA6-24	AB561108	Seaward	Trap	107_UnG_AB369767	98.27	1.00E-172
Glo6	KBM12-5	AB561109	Landward	Field	157_UnG_AB665510	98.84	5.00E-176
Glo6	KB5-33	AB561110	Landward	Trap	157_UnG_AB665510	98.55	2.00E-174
Glo7	08KB5-33	AB561111	Landward	Trap	105_UnG_AB640742	99.42	8.00E-179
Glo7	KAE8-17	AB561112	Seaward	Field	105_UnG_AB640742	99.13	4.00E-177

Table S1.3

Phylotype	Clone	Accession	Habitat/origin	Source	Top hit OTU	% Identity	E value
Glo7	KA5-2-11	AB640742	Seaward	Trap	105_UnG_AB640742	100	0
Glo8	KBM3-26	AB561095	Landward	Field	007_Rhz_AB369745	97.17	2.00E-169
Glo8	KB6-21	AB561097	Landward	Trap	007_Rhz_AB369745	97.17	2.00E-169
Glo8	KA3-43	AB640738	Seaward	Trap	007_Rhz_AB369745	96.6	5.00E-166
Glo8	KAE3-10	AB640744	Seaward	Field	007_Rhz_AB369745	97.17	2.00E-169
Glo9	KB6-2-4	AB640732	Landward	Trap	008_Rhz_AB640745	99.67	2.00E-155
Glo9	08KA6-14	AB640739	Seaward	Trap	008_Rhz_AB640745	100	3.00E-157
Glo9	KAE2-27	AB640745	Seaward	Field	008_Rhz_AB640745	100	3.00E-157
Glo10	KAE3-3	AB643805	Seaward	Field	011_Rhz_AB643805	100	0
Glo11	KAE3-1	AB640746	Seaward	Field	003_Rhz_AB640749	100	0
Glo11	KBM6-5	AB640749	Landward	Field	003_Rhz_AB640749	100	0
Glo12	KAE10-12	AB640747	Seaward	Field	019_Rhz_AB640747	100	0
Glo13	08KA7-10	AB640740	Seaward	Trap	255_Rhz_AB640740	100	0
Glo13	KAE9-13	AB640748	Seaward	Field	040_Rhz_AB640750	99.72	0
Glo13	KBM2-28	AB640750	Landward	Field	040_Rhz_AB640750	100	0
Glo14	KBM2-5	AB561092	Landward	Field	049_Rhz_AB561092	100	0
Glo14	KA3-12	AB561093	Seaward	Trap	049_Rhz_AB561092	98.31	4.00E-177
Glo14	08KB9-22	AB561094	Landward	Trap	049_Rhz_AB561092	98.87	2.00E-180
Glo15	08KB4-33	AB640733	Landward	Trap	050_Rhz_AB640741	97.73	1.00E-172
Glo15	KA1-22	AB640741	Seaward	Trap	050_Rhz_AB640741	100	0
Glo16	KB5-3-9	AB640734	Landward	Trap	074_Rhz_AB640734	100	0
Glo17	KBM11-75	AB640751	Landward	Field	278_Rhz_AB640751	100	0
Par1	KAE9-4	AB561132	Seaward	Field	371_Par_LC191610	99.33	3.00E-152
Par2	KB3-27	AB561133	Landward	Trap	229_Par_AB547188	99.67	6.00E-155
Par2	KBM4-13	AB561134	Landward	Field	229_Par_AB547188	100	1.00E-156
Par2	KAE1-17	AB561135	Seaward	Field	229_Par_AB547188	100	1.00E-156
Par3	KB3-2-55	AB561136	Landward	Trap	216_Par_AB610837	97.64	3.00E-143
Par3	KA2-22	AB561137	Seaward	Trap	216_Par_AB610837	97.98	6.00E-145
Unc1	KAE5-15	AB561130	Seaward	Field	173_Unc_AB561131	100	9.00E-153
Unc1	KB3-2-29	AB561131	Landward	Trap	173_Unc_AB561131	100	9.00E-153
Unc2	KB3-2-18	AB640736	Landward	Trap	172_Unc_AB640736	100	3.00E-153

Table S2.1 Number of sequence read assigned to AM fungal operational taxonomic unit (OTU)

OTU	Order	Family	Genus	Closest species	Main experiment					
					Light			Shade		
					NCSP_1	NCSP_2	NCSP_3	NCSP_1	NCSP_2	NCSP_3
001_Rhz_AB369921	Glomerales	Glomeraceae	<i>Rhizophagus</i>	<i>Rhizophagus irregularis</i>	0	9954	0	76	109	0
002_Rhz_HQ895800	Glomerales	Glomeraceae	<i>Rhizophagus</i>	<i>Rhizophagus irregularis</i>	1	1818	0	366	5051	0
003_Rhz_AB640749	Glomerales	Glomeraceae	<i>Rhizophagus</i>	<i>Rhizophagus irregularis</i>	0	369	0	0	0	0
004_Rhz_AY541856	Glomerales	Glomeraceae	<i>Rhizophagus</i>	<i>Rhizophagus irregularis</i>	0	38	0	1	4	0
005_Rhz_LC191588	Glomerales	Glomeraceae	<i>Rhizophagus</i>	<i>Rhizophagus irregularis</i>	0	0	0	1	0	0
006_Rhz_FM865608	Glomerales	Glomeraceae	<i>Rhizophagus</i>	<i>Rhizophagus irregularis</i>	6	1472	0	2	25	0
007_Rhz_AB369745	Glomerales	Glomeraceae	<i>Rhizophagus</i>	<i>Rhizophagus irregularis</i>	8332	1316	1240	11	2408	0
008_Rhz_AB640745	Glomerales	Glomeraceae	<i>Rhizophagus</i>	<i>Rhizophagus irregularis</i>	3525	0	250	0	0	0
009_Rhz_FM992381	Glomerales	Glomeraceae	<i>Rhizophagus</i>	<i>Rhizophagus irregularis</i>	2	1	0	0	1	0
010_Rhz_AY541855	Glomerales	Glomeraceae	<i>Rhizophagus</i>	<i>Rhizophagus irregularis</i>	0	0	0	0	0	0
011_Rhz_AB643805	Glomerales	Glomeraceae	<i>Rhizophagus</i>	<i>Rhizophagus irregularis</i>	47	0	0	0	0	0
012_Rhz_FM865604	Glomerales	Glomeraceae	<i>Rhizophagus</i>	<i>Rhizophagus irregularis</i>	0	0	0	0	0	0
013_Rhz_FR750132	Glomerales	Glomeraceae	<i>Rhizophagus</i>	<i>Rhizophagus irregularis</i>	0	0	0	0	0	0
014_Rhz_FM865598	Glomerales	Glomeraceae	<i>Rhizophagus</i>	<i>Rhizophagus irregularis</i>	0	0	0	0	0	0
024_Rhz_LC014178	Glomerales	Glomeraceae	<i>Oehlia</i>	<i>Oehlia diaphana</i>	1	2	0	0	0	0
040_Rhz_AB640750	Glomerales	Glomeraceae	<i>Rhizophagus</i>	<i>Rhizophagus irregularis</i>	207	0	0	0	0	0
049_Rhz_AB561092	Glomerales	Glomeraceae	<i>Rhizophagus</i>	<i>Rhizophagus irregularis</i>	22	0	0	0	0	11
060_UnG_LC176583	Glomerales	Glomeraceae	<i>Nanoglomus</i>		0	0	0	0	0	0
062_UnG_LC176566	Glomerales	Glomeraceae	<i>Nanoglomus</i>	<i>Nanoglomus plukenetia</i>	0	140	0	0	0	0
064_UnG_AB561101	Glomerales	Glomeraceae	<i>Nanoglomus</i>		0	252	0	0	0	0
065_UnG_AB750741	Glomerales	Glomeraceae	<i>Nanoglomus</i>		0	4	0	0	0	0
068_UnG_AB369764	Glomerales	Glomeraceae	<i>Nanoglomus</i>		0	0	0	0	26	525
069_UnG_AB369759	Glomerales	Glomeraceae	<i>Nanoglomus</i>		0	0	0	0	0	0
070_UnG_HE794042	Glomerales	Glomeraceae	<i>Nanoglomus</i>		0	0	0	0	0	0
072_UnG_AB643635	Glomerales	Glomeraceae	<i>Nanoglomus</i>		0	0	0	0	227	2069
081_Glo_AB710220	Glomerales	Glomeraceae	<i>Complexispora</i>	<i>Complexispora multistrata</i>	0	0	0	0	0	67

Table S2.1 Continued

OTU	Supporting experiment											
	NC inoculum						SP inoculum					
	Light			Shade			Light			Shade		
	NC_1	NC_2	NC_3	NC_1	NC_2	NC_3	SP_1	SP_2	SP_3	SP_1	SP_2	SP_3
001_Rhz_AB369921	0	0	0	0	0	0	0	26	0	0	0	5
002_Rhz_HQ895800	0	0	0	0	0	0	0	1	0	0	0	0
003_Rhz_AB640749	24	0	0	0	0	4	0	1520	0	0	0	302
004_Rhz_AY541856	0	0	0	9	0	0	0	0	0	0	0	0
005_Rhz_LC191588	0	0	0	0	0	0	0	0	0	0	0	0
006_Rhz_FM865608	2	0	4	0	0	0	0	0	0	0	0	0
007_Rhz_AB369745	229	0	12858	270	0	53	10	5579	74	10	2	1334
008_Rhz_AB640745	20	0	4149	90	0	0	0	0	0	0	0	0
009_Rhz_FM992381	0	0	0	0	0	0	0	0	0	0	0	0
010_Rhz_AY541855	0	0	0	0	0	0	0	1	0	0	0	0
011_Rhz_AB643805	0	0	0	0	0	0	0	1025	0	0	0	0
012_Rhz_FM865604	0	0	0	1656	0	3432	0	0	0	0	0	0
013_Rhz_FR750132	0	0	0	4624	0	8633	0	0	1	0	1	0
014_Rhz_FM865598	0	0	0	570	0	2567	0	0	0	0	0	0
024_Rhz_LC014178	0	0	0	0	0	0	0	2	0	0	0	0
040_Rhz_AB640750	0	0	0	0	0	0	0	0	0	0	0	0
049_Rhz_AB561092	0	0	0	0	0	0	0	0	165	0	1	0
060_UnG_LC176583	0	0	0	0	0	2	0	1	0	0	0	0
062_UnG_LC176566	0	0	0	0	0	0	0	0	0	0	0	0
064_UnG_AB561101	0	0	0	0	0	0	0	0	0	0	0	0
065_UnG_AB750741	0	0	0	0	0	0	0	0	0	0	0	0
068_UnG_AB369764	0	0	89	0	0	2014	0	0	31	0	299	0
069_UnG_AB369759	0	0	0	0	0	1	0	0	0	0	0	0
070_UnG_HE794042	0	0	0	0	0	3	0	0	0	0	3	0
072_UnG_AB643635	0	1	178	0	0	7855	0	2	92	0	1392	0
081_Glo_AB710220	0	0	0	0	0	0	0	0	19	0	1	0

Table S2.1 Continued

OTU	Life-history strategy	Light-shade index	Reference for strategy assignment		
			Kawahara and Ezawa (2013)		Accession no.
			Cahyaningtyas & Ezawa (2023)	Trap culture	
001_Rhz_AB369921	Soil-propagule colonizer	1	Soil propagule-mediated/ root-direct		AB369921
002_Rhz_HQ895800	Soil-propagule colonizer	-1	Soil propagule-mediated/ root-direct		HQ895800
003_Rhz_AB640749	Soil-propagule colonizer	-1	Soil propagule-mediated/ root-direct		AB640749
004_Rhz_AY541856	Network competitor	2			AY541856
005_Rhz_LC191588	Unassigned	1			LC191588
006_Rhz_FM865608	Network competitor	-2	Slow regenerator		FM865608
007_Rhz_AB369745	Soil-propagule colonizer	-1	Soil propagule-mediated/ root-dir	●	AB369745
008_Rhz_AB640745	Soil-propagule colonizer	-3	Slow regenerator	●	AB640745
009_Rhz_FM992381	Soil-propagule colonizer	-1	Soil propagule-mediated/ root-direct		FM992381
010_Rhz_AY541855	Soil-propagule colonizer	-1			AY541855
011_Rhz_AB643805	Soil-propagule colonizer	-2	Soil propagule-mediated/ root-direct		AB643805
012_Rhz_FM865604	Network competitor	2			FM865604
013_Rhz_FR750132	Soil-propagule colonizer	2			FR750132
014_Rhz_FM865598	Network competitor	2			FM865598
024_Rhz_LC014178	Soil-propagule colonizer	-3	Slow regenerator		LC014178
040_Rhz_AB640750	Unassigned	-1			AB640750
049_Rhz_AB561092	Soil-propagule colonizer	0	Soil propagule-mediated/ root-dir	●	AB561092
060_UnG_LC176583	Soil-propagule colonizer	0	Soil propagule-mediated		LC176583
062_UnG_LC176566	Unassigned	-1			LC176566
064_UnG_AB561101	Soil-propagule colonizer	-1	Soil propagule-mediated/ root-dir	●	AB561101
065_UnG_AB750741	Soil-propagule colonizer	-1	Soil propagule-mediated/ root-direct		AB750741
068_UnG_AB369764	Soil-propagule colonizer	2	Soil propagule-mediated/ root-direct		AB369764
069_UnG_AB369759	Soil-propagule colonizer	1	Soil propagule-mediated	●	AB369759
070_UnG_HE794042	Soil-propagule colonizer	2	Soil propagule-mediated		HE794042
072_UnG_AB643635	Soil-propagule colonizer	0	Soil propagule-mediated/ root-direct		AB643635
081_Glo_AB710220	Soil-propagule colonizer	1	Root-direct		AB710220

Table S2.1 Continued

OTU	Order	Family	Genus	Closest species	Main experiment					
					Light			Shade		
					NCSP_1	NCSP_2	NCSP_3	NCSP_1	NCSP_2	NCSP_3
083_Glo_HE775339	Glomerales	Glomeraceae	<i>Complexispora</i>	<i>Complexispora mediterranea</i>	0	0	0	0	0	101
086_Glo_AB665502	Glomerales	Glomeraceae	<i>Glomus</i>	<i>Glomus macrocarpum</i>	0	0	0	0	238	0
105_UnG_AB640742	Glomerales	Glomeraceae	<i>Nanoglomus</i>		1080	0	0	0	0	0
107_UnG_AB369767	Glomerales	Glomeraceae	<i>Microdominikia</i>	<i>Microdominikia litorea</i>	0	0	0	0	0	0
108_UnG_AB561107	Glomerales	Glomeraceae	<i>Microdominikia</i>	<i>Microdominikia litorea</i>	0	0	0	0	0	0
120_UnG_AB369765	Glomerales	Glomeraceae	<i>Dominikia</i>	<i>Dominikia aurea</i>	1	0	0	176	0	332
122_UnG_JF439191	Glomerales	Glomeraceae	<i>Dominikia</i>	<i>Dominikia aurea</i>	22	0	0	0	188	29
124_Cla_AB561129	Entrophosporales	Entrophosporaceae	<i>Entrophospora</i>		0	0	0	0	3	5185
125_Cla_AB812617	Entrophosporales	Entrophosporaceae	<i>Entrophospora</i>		0	0	0	0	347	0
126_Cla_AB812616	Entrophosporales	Entrophosporaceae	<i>Entrophospora</i>		0	0	0	0	94	0
153_Scu_AB561124	Gigasporales	Scutellosporaceae	<i>Scutellospora</i>	<i>Scutellospora dipurpurascens</i>	0	0	0	6353	1759	457
154_Scu_FN547618	Gigasporales	Racocetraceae	<i>Cetraspora</i>	<i>Cetraspora gilmorei</i>	0	0	0	11259	0	0
156_Scu_FR750142	Gigasporales	Racocetraceae	<i>Racocetra</i>	<i>Racocetra fulgida</i>	0	0	242	12	0	0
157_UnG_AB665510	Glomerales	Glomeraceae	<i>Microdominikia</i>	<i>Microdominikia litorea</i>	0	0	0	0	0	0
173_Unc_AB561131	Uncultured Glomeromycota				0	0	0	2612	11	0
176_Arc_JX848923	Archaeosporales	Archaeosporaceae	<i>Archaeospora</i>	<i>Archaeospora trappei</i>	0	0	0	14	0	0
190_Aca_AY639328	Diversisporales	Acalosporaceae	<i>Acalospora</i>	<i>Acalospora paulinae</i>	0	0	0	0	0	0
191_Aca_AB610835	Diversisporales	Acalosporaceae	<i>Acalospora</i>	<i>Acalospora paulinae</i>	0	0	0	0	10	0
195_Aca_AB369793	Diversisporales	Acalosporaceae	<i>Acalospora</i>	<i>Acaulospora morrowiae</i>	0	0	5012	1	0	2091
214_Par_AB812604	Paraglomerales	Paraglomelaceae	<i>Paraglomus</i>	<i>Paraglomus brasilianum</i>	0	0	1932	1	0	0
216_Par_AB610837	Paraglomerales	Paraglomelaceae	<i>Paraglomus</i>	<i>Paraglomus brasilianum</i>	0	0	0	0	0	0
229_Par_AB547188	Paraglomerales	Paraglomelaceae	<i>Paraglomus</i>	<i>Paraglomus occultum</i>	0	0	0	388	2801	190
230_Rhz_JN937313	Glomerales	Glomeraceae	<i>Rhizophagus</i>	<i>Rhizophagus irregularis</i>	0	3875	0	45	69	0
231_Rhz_FR750064	Glomerales	Glomeraceae	<i>Rhizophagus</i>	<i>Rhizophagus irregularis</i>	0	2	0	0	0	0
244_UnG_AB710223	Glomerales	Glomeraceae	<i>Dominikia</i>	<i>Dominikia iranica</i>	0	1	0	0	0	0

Table S2.1 Continued

OTU	Supporting experiment											
	NC inoculum						SP inoculum					
	Light			Shade			Light			Shade		
	NC_1	NC_2	NC_3	NC_1	NC_2	NC_3	SP_1	SP_2	SP_3	SP_1	SP_2	SP_3
083_Glo_HE775339	0	0	0	0	0	0	0	0	0	0	0	0
086_Glo_AB665502	0	0	0	0	0	0	0	0	0	0	0	0
105_UnG_AB640742	0	0	0	0	0	0	0	0	0	183	0	0
107_UnG_AB369767	0	0	0	0	0	0	149	26	0	0	0	0
108_UnG_AB561107	0	0	0	0	0	0	180	23	0	0	0	0
120_UnG_AB369765	0	0	0	6	9	17	0	0	0	0	8	144
122_UnG_JF439191	42	0	0	3	0	0	11	0	0	0	1	0
124_Cla_AB561129	0	0	0	0	0	0	0	4	0	0	3057	14446
125_Cla_AB812617	0	0	0	0	0	0	0	0	0	0	0	0
126_Cla_AB812616	0	0	0	0	0	0	0	0	0	0	0	0
153_Scu_AB561124	0	0	0	0	0	32	0	0	0	0	0	0
154_Scu_FN547618	0	0	0	0	0	0	0	0	0	2	0	0
156_Scu_FR750142	0	0	0	0	0	0	0	0	0	0	0	0
157_UnG_AB665510	0	0	0	0	0	0	1355	94	0	0	0	0
173_Unc_AB561131	0	0	0	0	0	0	0	0	0	0	0	0
176_Arc_JX848923	0	0	0	0	0	0	0	0	0	3078	4	2
190_Aca_AY639328	0	0	0	0	0	0	0	0	0	0	0	1
191_Aca_AB610835	0	0	0	0	0	0	0	3	0	0	0	1277
195_Aca_AB369793	4763	3706	0	1121	6096	0	0	0	0	3	0	0
214_Par_AB812604	0	16432	0	31	0	12	0	0	0	0	0	0
216_Par_AB610837	13	0	2	0	0	0	0	0	0	1033	0	0
229_Par_AB547188	0	0	0	0	19	208	0	236	0	0	20321	0
230_Rhz_JN937313	0	0	0	0	0	0	0	0	0	0	0	0
231_Rhz_FR750064	0	0	0	0	0	0	0	0	0	0	0	0
244_UnG_AB710223	0	0	0	0	0	0	0	0	0	0	0	0

Table S2.1 Continued

OTU	Life-history strategy	Light-shade index	Reference for strategy assignment		
			Cahyaningtyas & Ezawa (2023)	Trap culture	Accession no.
083_Glo_HE775339	Network competitor	1	Slow regenerator		HE775339
086_Glo_AB665502	Soil-propagule colonizer	1	Soil propagule-mediated/ root-dir	●	AB665502
105_UnG_AB640742	Soil-propagule colonizer	0	Soil propagule-mediated/ root-dir	●	AB640742
107_UnG_AB369767	Soil-propagule colonizer	-2	Soil propagule-mediated/ root-dir	●	AB369767
108_UnG_AB561107	Soil-propagule colonizer	-2	Soil propagule-mediated/ root-direct		AB561107
120_UnG_AB369765	Soil-propagule colonizer	6	Soil propagule-mediated/ root-direct		AB369765
122_UnG_JF439191	Soil-propagule colonizer	1	Soil propagule-mediated/ root-direct		JF439191
124_Cla_AB561129	Soil-propagule colonizer	3	Root-direct	●	AB561129
125_Cla_AB812617	Network competitor	1	Slow regenerator		AB812617
126_Cla_AB812616	Soil-propagule colonizer	1	Soil propagule-mediated		AB812616
153_Scu_AB561124	Soil-propagule colonizer	4	Soil propagule-mediated	●	AB561124
154_Scu_FN547618	Soil-propagule colonizer	2	Soil propagule-mediated		FN547618
156_Scu_FR750142	Soil-propagule colonizer	0	Soil propagule-mediated	●	FR750142
157_UnG_AB665510	Soil-propagule colonizer	-2	Soil propagule-mediated/ root-dir	●	AB665510
173_Unc_AB561131	Soil-propagule colonizer	2		●	AB561131
176_Arc_JX848923	Soil-propagule colonizer	4	Slow regenerator		JX848923
190_Aca_AY639328	Soil-propagule colonizer	1			AY639328
191_Aca_AB610835	Soil-propagule colonizer	1	Soil propagule-mediated	●	AB610835
195_Aca_AB369793	Soil-propagule colonizer	2			AB369793
214_Par_AB812604	Network competitor	1			AB812604
216_Par_AB610837	Soil-propagule colonizer	-1	Soil propagule-mediated/ root-dir	●	AB610837
229_Par_AB547188	Soil-propagule colonizer	5	Slow regenerator	●	AB547188
230_Rhz_JN937313	Unassigned	1			JN937313
231_Rhz_FR750064	Unassigned	-1			FR750064
244_UnG_AB710223	Unassigned	-1			AB710223

Table S2.1 Continued

OTU	Order	Family	Genus	Closest species	Main experiment					
					Light			Shade		
					NCSP_1	NCSP_2	NCSP_3	NCSP_1	NCSP_2	NCSP_3
247_UnG_HE775292	Glomerales	Glomeraceae	<i>Nanoglomus</i>		0	0	0	0	0	0
250_Glo_HE775291	Glomerales	Glomeraceae	<i>Complexispora</i>		0	0	0	0	0	1
255_Rhz_AB640740	Glomerales	Glomeraceae	<i>Rhizophagus</i>	<i>Rhizophagus irregularis</i>	34	0	0	0	0	0
297_Scu_JN867206	Gigasporales	Racocetraceae	<i>Racocetra</i>	<i>Racocetra verrucosa</i>	0	0	1	0	0	0
299_Scu_GU322902	Gigasporales	Scutellosporaceae	<i>Scutellospora</i>	<i>Scutellospora spinosissima</i>	0	0	0	0	0	1
301_Scu_JF717509	Gigasporales	Scutellosporaceae	<i>Scutellospora</i>	<i>Scutellospora dipurpurascens</i>	0	0	0	0	0	0
318_Div_AB561119	Diversisporales	Diversisporaceae	<i>Diversispora</i>	<i>Diversispora eburnea</i>	0	0	0	0	0	0
327_Aca_AB369790	Diversisporales	Acalosporaceae	<i>Acalospora</i>	<i>Acaulospora morrowiae</i>	0	0	183	0	0	5
358_Scu_LC191600	Gigasporales	Racocetraceae	<i>Racocetra</i>	<i>Racocetra coralloidea</i>	0	0	105	0	0	0
361_Aca_LC191603	Diversisporales	Acalosporaceae	<i>Acalospora</i>	<i>Acaulospora dilatata</i>	0	0	148	0	0	546
366_Scu_LC191606	Gigasporales	Racocetraceae	<i>Racocetra</i>	<i>Racocetra crispa</i>	0	0	342	0	0	0
372_Unc_LC191611	Uncultured Glomeromycota				0	0	0	0	0	0
373_Rhz_LC191612	Glomerales	Glomeraceae	<i>Rhizophagus</i>	<i>Rhizophagus irregularis</i>	873	99	31	0	0	0
374_Rhz_LC191613	Glomerales	Glomeraceae	<i>Rhizophagus</i>	<i>Rhizophagus irregularis</i>	3138	121	220	0	0	0
375_Rhz_LC191614	Glomerales	Glomeraceae	<i>Rhizophagus</i>	<i>Rhizophagus irregularis</i>	4	0	1	0	0	0
381_Rhz_LC191620	Glomerales	Glomeraceae	<i>Rhizophagus</i>	<i>Rhizophagus irregularis</i>	0	0	0	15	0	0
390_Rhz_LC191627	Glomerales	Glomeraceae	<i>Rhizophagus</i>	<i>Rhizophagus irregularis</i>	0	0	0	0	0	0
399_Scu_LC191634	Gigasporales	Dentiscutataceae	<i>Dentiscutata</i>	<i>Dentiscutata erythropus</i>	0	0	682	0	0	0
410_Rhz_LC191641	Glomerales	Glomeraceae	<i>Rhizophagus</i>	<i>Rhizophagus irregularis</i>	0	10	0	0	0	0
422_Par_LC191651	Paraglomerales	Paraglomelaceae	<i>Paraglomus</i>	<i>Paraglomus occultum</i>	0	0	2728	0	193	9511
427_Aca_LC191656	Diversisporales	Acalosporaceae	<i>Acalospora</i>	<i>Acaulospora longula</i>	0	0	7	0	0	0
439_Scu_LC416092	Gigasporales	Racocetraceae	<i>Racocetra</i>	<i>Racocetra crispa</i>	0	0	13	0	0	0
449_Gig_LC416101	Gigasporales	Gigasporaceae	<i>Gigaspora</i>		0	0	0	16	0	0
462_Glo_LC416113	Glomerales	Glomeraceae	<i>Glomus</i>		0	0	0	0	4	0
477_Cla_LC416127	Entrophosporales	Entrophosporaceae	<i>Entrophospora</i>		0	0	0	0	0	0

Table S2.1 Continued

OTU	Supporting experiment											
	NC inoculum						SP inoculum					
	Light			Shade			Light			Shade		
	NC_1	NC_2	NC_3	NC_1	NC_2	NC_3	SP_1	SP_2	SP_3	SP_1	SP_2	SP_3
247_UnG_HE775292	0	0	0	0	0	7	0	0	1	0	0	0
250_Glo_HE775291	0	0	0	0	0	0	0	0	0	0	0	0
255_Rhz_AB640740	0	0	0	0	0	0	0	0	0	0	0	0
297_Scu_JN867206	0	0	0	0	0	0	0	0	0	0	0	0
299_Scu_GU322902	0	0	0	0	0	0	0	0	0	0	0	0
301_Scu_JF717509	0	0	0	0	0	0	0	0	0	0	1	0
318_Div_AB561119	0	0	0	0	191	0	0	0	0	0	0	0
327_Aca_AB369790	281	204	0	39	1106	0	0	0	0	0	0	0
358_Scu_LC191600	0	0	0	0	0	0	0	0	0	0	0	0
361_Aca_LC191603	1356	811	0	217	0	0	0	0	0	0	0	0
366_Scu_LC191606	0	0	0	0	0	0	0	0	0	0	0	0
372_Unc_LC191611	0	0	0	0	0	0	0	0	0	1	0	0
373_Rhz_LC191612	326	0	3139	498	0	75	0	0	0	0	0	0
374_Rhz_LC191613	880	0	5052	1020	0	190	10	0	0	6	0	0
375_Rhz_LC191614	124	0	0	302	0	87	0	0	0	0	0	0
381_Rhz_LC191620	0	0	0	0	0	0	0	0	0	0	0	0
390_Rhz_LC191627	1	0	0	0	0	0	0	0	0	0	0	0
399_Scu_LC191634	0	0	0	0	2986	0	0	0	0	0	0	0
410_Rhz_LC191641	0	0	0	0	0	0	0	1	0	0	0	0
422_Par_LC191651	0	587	737	0	253	519	6	16232	26748	0	3143	1452
427_Aca_LC191656	12	11	0	0	19	0	0	0	0	0	0	0
439_Scu_LC416092	0	0	0	0	0	0	0	0	0	0	0	0
449_Gig_LC416101	0	0	0	0	0	0	0	0	0	0	0	0
462_Glo_LC416113	0	0	0	0	0	0	0	0	0	0	0	0
477_Cla_LC416127	0	0	0	0	0	0	0	0	0	0	0	10889

Table S2.1 Continued

OTU	Life-history strategy	Light-shade index	Reference for strategy assignment		
			Cahyaningtyas & Ezawa (2023)	Trap culture	Accession no.
247_UnG_HE775292	Soil-propagule colonizer	0	Soil propagule-mediated/ root-direct		HE775292
250_Glo_HE775291	Network competitor	1	Slow regenerator		HE775291
255_Rhz_AB640740	Soil-propagule colonizer	-1		●	AB640740
297_Scu_JN867206	Soil-propagule colonizer	-1	Soil propagule-mediated		JN867206
299_Scu_GU322902	Soil-propagule colonizer	1	Soil propagule-mediated		GU322902
301_Scu_JF717509	Soil-propagule colonizer	1			JF717509
318_Div_AB561119	Soil-propagule colonizer	1	Slow regenerator	●	AB561119
327_Aca_AB369790	Network competitor	0			AB369790
358_Scu_LC191600	Unassigned	-1			LC191600
361_Aca_LC191603	Soil-propagule colonizer	-1	Soil propagule-mediated		LC191603
366_Scu_LC191606	Soil-propagule colonizer	-1	Soil propagule-mediated		LC191606
372_Unc_LC191611	Soil-propagule colonizer	1			LC191611
373_Rhz_LC191612	Network competitor	-3	Slow regenerator		LC191612
374_Rhz_LC191613	Soil-propagule colonizer	-3	Slow regenerator		LC191613
375_Rhz_LC191614	Network competitor	-1			LC191614
381_Rhz_LC191620	Unassigned	1			LC191620
390_Rhz_LC191627	Network competitor	-1			LC191627
399_Scu_LC191634	Soil-propagule colonizer	0	Soil propagule-mediated		LC191634
410_Rhz_LC191641	Soil-propagule colonizer	-2	Soil propagule-mediated		LC191641
422_Par_LC191651	Soil-propagule colonizer	0	Soil propagule-mediated		LC191651
427_Aca_LC191656	Network competitor	-2			LC191656
439_Scu_LC416092	Soil-propagule colonizer	-1	Soil propagule-mediated		LC416092
449_Gig_LC416101	Unassigned	1			LC416101
462_Glo_LC416113	Soil-propagule colonizer	1	Soil propagule-mediated		LC416113
477_Cla_LC416127	Soil-propagule colonizer	1			LC416127

Table S2.1 Continued

OTU	Order	Family	Genus	Closest species	Main experiment					
					Light			Shade		
					NCSP_1	NCSP_2	NCSP_3	NCSP_1	NCSP_2	NCSP_3
515_Rhz_LC416158	Glomerales	Glomeraceae	<i>Rhizophagus</i>	<i>Rhizophagus irregularis</i>	134	0	2	0	0	0
528_Rhz_LC416191	Glomerales	Glomeraceae	<i>Rhizophagus</i>	<i>Rhizophagus irregularis</i>	3	6621	0	3115	12138	0
532_Rhz_LC416173	Glomerales	Glomeraceae	<i>Rhizophagus</i>	<i>Rhizophagus irregularis</i>	0	0	23	0	0	0
552_Aca_MT832212	Diversisporales	Acalosporaceae	<i>Acalospora</i>	<i>Acaulospora dilatata</i>	0	0	6147	0	0	3521
562_Aca_MT832211	Diversisporales	Acalosporaceae	<i>Acalospora</i>	<i>Acaulospora morrowiae</i>	0	0	316	0	0	9
641_Glo_FR750526	Glomerales	Glomeraceae	<i>Glomus</i>	<i>Glomus macrocarpum</i>	0	0	0	0	5	0
642_Glo_FR750539	Glomerales	Glomeraceae	<i>Glomus</i>	<i>Glomus macrocarpum</i>	0	0	0	0	293	0
644_Hal_MH560602	Glomerales	Glomeraceae	<i>Halonatospora</i>	<i>Halonatospora panshihalo</i>	1	0	0	0	0	0
645_Hal_MH560604	Glomerales	Glomeraceae	<i>Halonatospora</i>	<i>Halonatospora panshihalo</i>	0	0	0	0	0	7
679_Rac_FR750143	Gigasporales	Racocetraceae	<i>Racocetra</i>	<i>Racocetra fulgida</i>	0	0	2	0	0	0
680_Rac_MT832224	Gigasporales	Racocetraceae	<i>Racocetra</i>	<i>Racocetra fulgida</i>	0	0	7	0	0	0
681_Rac_GU385898	Gigasporales	Racocetraceae	<i>Racocetra</i>	<i>Racocetra tropicana</i>	0	0	1158	0	0	0
682_Rac_AY900507	Gigasporales	Racocetraceae	<i>Racocetra</i>	<i>Racocetra verrucosa</i>	0	0	784	0	0	0
689_Rhz_MT832188	Glomerales	Glomeraceae	<i>Rhizophagus</i>	<i>Rhizophagus irregularis</i>	0	0	0	0	0	0
691_Rhz_MT832194	Glomerales	Glomeraceae	<i>Rhizophagus</i>	<i>Rhizophagus irregularis</i>	2710	5087	192	48	198	0
692_Rhz_MT832195	Glomerales	Glomeraceae	<i>Rhizophagus</i>	<i>Rhizophagus irregularis</i>	0	11	0	1	0	0
Genus richness					5	4	5	10	9	9
OTU richness					20	20	26	21	24	19
Total reads					20143	31193	21768	24513	26202	24658

Table S2.1 Continued

OTU	Supporting experiment											
	NC inoculum						SP inoculum					
	Light			Shade			Light			Shade		
	NC_1	NC_2	NC_3	NC_1	NC_2	NC_3	SP_1	SP_2	SP_3	SP_1	SP_2	SP_3
515_Rhz_LC416158	27	0	7	36	0	0	0	0	0	0	0	0
528_Rhz_LC416191	0	0	0	0	0	0	0	10	0	0	0	0
532_Rhz_LC416173	35	0	2	11	0	0	0	0	0	0	0	0
552_Aca_MT832212	6789	4872	0	1753	13350	0	0	1	0	0	0	0
562_Aca_MT832211	29	16	0	7	1569	0	0	0	0	0	0	0
641_Glo_FR750526	0	0	0	0	0	0	0	0	0	0	0	0
642_Glo_FR750539	0	0	0	0	0	0	0	0	0	0	0	0
644_Hal_MH560602	0	0	0	0	0	0	0	0	0	0	0	0
645_Hal_MH560604	0	0	0	0	0	0	0	0	56	0	1	0
679_Rac_FR750143	0	0	0	0	0	0	0	0	0	0	0	0
680_Rac_MT832224	0	0	0	0	0	0	0	0	0	0	0	0
681_Rac_GU385898	0	0	0	0	0	0	0	0	0	0	0	0
682_Rac_AY900507	0	0	0	0	0	0	0	0	0	0	0	0
689_Rhz_MT832188	0	0	0	206	0	12	0	0	0	0	0	0
691_Rhz_MT832194	190	0	2618	523	0	44	0	165	0	1	0	15
692_Rhz_MT832195	0	0	0	0	0	0	0	0	0	0	0	0
Genus richness	4	2	3	4	5	5	4	7	5	7	9	6
OTU richness	19	9	12	21	10	21	7	20	9	9	15	11
Total reads	15143	26640	28835	12992	25598	25767	1721	24952	27187	4317	28235	29867

Table S2.1 Continued

OTU	Life-history strategy	Light-shade index	Reference for strategy assignment		
			Cahyaningtyas & Ezawa (2023)	Kawahara and Ezawa (2013) Trap culture	Accession no.
515_Rhz_LC416158	Network competitor	-3	Slow regenerator		LC416158
528_Rhz_LC416191	Soil-propagule colonizer	-1	Soil propagule-mediated/ root-direct		LC416191
532_Rhz_LC416173	Soil-propagule colonizer	-2	Soil propagule-mediated		LC416173
552_Aca_MT832212	Soil-propagule colonizer	-1			MT832212
562_Aca_MT832211	Network competitor	0			MT832211
641_Glo_FR750526	Soil-propagule colonizer	1	Soil propagule-mediated/ root-direct		FR750526
642_Glo_FR750539	Soil-propagule colonizer	1	Soil propagule-mediated		FR750539
644_Hal_MH560602	Soil-propagule colonizer	-1	Soil propagule-mediated/ root-direct		MH560602
645_Hal_MH560604	Soil-propagule colonizer	1	Soil propagule-mediated/ root-direct		MH560604
679_Rac_FR750143	Soil-propagule colonizer	-1	Soil propagule-mediated		FR750143
680_Rac_MT832224	Soil-propagule colonizer	-1	Soil propagule-mediated		MT832224
681_Rac_GU385898	Soil-propagule colonizer	-1	Soil propagule-mediated/ root-direct		GU385898
682_Rac_AY900507	Soil-propagule colonizer	-1	Soil propagule-mediated		AY900507
689_Rhz_MT832188	Network competitor	2			MT832188
691_Rhz_MT832194	Soil-propagule colonizer	0	Soil propagule-mediated/ root-direct		MT832194
692_Rhz_MT832195	Unassigned	0			MT832195