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Title: Disturbance tolerance of arbuscular mycorrhizal fungi: characterization of life-history strategies along a disturbance gradient in a coastal dune ecosystem

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1 **Abstract**

3 Purpose

4 Arbuscular mycorrhizal fungi that regenerate rapidly after disturbance play a significant role in
5 vegetation resilience. We employed the fungi inhabiting a coastal dune ecosystem to characterize
6 their strategies for survival and regeneration after disturbance.

7 Methods

8 Root-soil samples were collected from the seaward and landward slopes that differ in disturbance
9 severity. The native grass *Miscanthus sinensis* (donor plants) were grown in the soil samples for
10 two months, and from a half of the plants, disturbance-tolerant fungi that regenerate from spores
11 and extraradical hyphae (soil propagule-mediated regenerators, SP) and those that regenerate from
12 the colonized roots (root-direct regenerators, RD) were trapped separately with new seedlings
13 (assessment plants). The other half of the donor plants were further grown without destruction for
14 four months together with assessment plants, during which the fungi trapped by the plants were
15 categorized as disturbance-sensitive slow regenerators (SL). DNA was extracted from the
16 assessment plants for identification based on LSU rDNA sequences.

17 Results

18 All the fungi that occurred widely in the seaward samples belonged to the Glomeraceae and
19 Paraglomeraceae and preferred the RD strategy to the SP strategy. In the landward samples diverse
20 taxa that employed diverse strategies occurred, and among them, Gigasporaceae fungi showed a
21 distinct preference for the SP strategy.

22 Conclusion

23 These observations suggest that not only soil-borne propagules but also colonized roots play a key
24 role in the rapid regeneration of the fungi after disturbance, providing a new insight into the life-
25 history strategies of AM fungi in frequently disturbed environments.

26

27 **Key word:** arbuscular mycorrhizal fungi, coastal dunes, disturbance-tolerance, life-history strategy,
28 soil disturbance.

29

Introduction

Soil disturbance occurs through anthropogenic or natural processes, the latter, e.g., via volcanic ash fall (Crisafulli et al. 2015; del Moral 2010), landslide in steep slopes (Hung et al. 2014; Van Eynde et al. 2017), wildfire (Hosseini et al. 2016; Neary 2019), and sand movement by wind and tidal waves in coastal dunes (Delgado-Fernandez and Davidson-Arnott 2011). Such disturbances could cause 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 association 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.

In frequently disturbed ecosystems rapid regeneration of hyphal networks would be an important strategy for survival after disturbance. Chagnon et al. (2013) proposed a concept of AM fungal life-history strategy 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 fungi (C) 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., Gigasporaceae). Stress-tolerator fungi (S) can tolerate abiotic stresses, such as high-soil acidity and low temperature, have long-lived mycelia, and grow slowly (e.g., Acaulosporaceae). Ruderal fungi (R) 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., 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, Claroideoglomeraceae (=Entrophosporaceae), 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 the fungi to soil disturbance would be predictable at the family level. For example, Glomeraceae and Paraglomeraceae fungi, rhizophilic fungi that have larger biomass in the roots, could regenerate rapidly (i.e., rapid regenerators) not only via spores, but also from intraradical hyphae. Whereas Gigasporaceae and Diversisporaceae fungi would rather be susceptible to soil disturbance, that is, slow regenerators that require longer time for re-establishment, and regenerate mainly from soil-borne propagules, including spores and extraradical hyphae, as edaphophilic fungi have larger biomass in the soil.

Ishikari coastal dunes, Japan, show typical zonal distribution of vegetation along a soil-disturbance gradient; 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 fungi in the seaward slopes and

the presence of disturbance-sensitive fungi that disappeared after disturbance in the landward slopes through the comparative analysis of the community members before (field roots) and after soil disturbance (trap culture roots). But life-history strategies of these fungi with respect to regeneration after disturbance were not directly assessed and thus are experimentally characterized in this study.

We defined rapid and slow regenerators 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. In this study, 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.

Material and Methods

102 Sampling sites

103

104 A sampling area of $100 \times 1,000$ m was defined along Ishikari coastal dune, Hokkaido, Japan
105 (Supplemental material Table S1 and Figs. 1a and b). This ecosystem belongs to the subarctic zone
106 with the annual mean temperature and rainfall being 8.3°C and 651.0 mm, respectively. The primary
107 dunes are 2 – 6 m in height and located about 50 – 100 m inland from the coastal line. Vegetation in the
108 dune has been described in Kawahara and Ezawa (2013). Briefly, the landward slopes of the primary
109 dune are 80 – 150 m in width and largely dominated by the C4 perennial grass *Miscanthus sinensis*
110 Andersson (Fig. 1c). In addition, *Rosa rugosa* Thunb. and *Lathyrus japonicus* Willd. are also patchily
111 distributed. The seaward slopes are 40 – 70 m in width and dominated by *Leymus mollis* (Trin. ex
112 Spreng.) Pilg. (Fig. 1d). *Calystegia soldanella* (L.) Riöm. et Schult, *Arabis stelleri* var. *japonica* (A.
113 Gray) Fr. Schm., *Glehnia littoralis* F. Schmidt ex Miq., *Carex kobomugi* Ohwi., *Linaria japonica* Miq.,
114 and *Ixeris repens* (L.) A. Gray are also patchily distributed in the slopes. The habitats of *M. sinensis*
115 and *L. mollis* are clearly segregated between the slopes, and this typical zonal distribution is observed
116 more than 5 km along the coastal line. The topsoil layer of the seaward slopes is constantly disturbed
117 by wind thus unstable. Whereas the thick root system of *M. sinensis* stabilized the landward slopes, and
118 thus an organic layer (3 – 5 cm) originated from *M. sinensis* litter has been developed. The soil
119 chemical properties in the two slopes are described in Kawahara and Ezawa (2013). Briefly, sodium
120 concentration in the root-zone soils was not significantly different between the seaward and landward
121 slopes (140 and 128 mg-Na kg^{-1} , respectively, on average), but organic carbon (1.4 and 5.1 g-C kg^{-1} ,
122 respectively, on average) and total nitrogen (0.18 and 0.4 g-N kg^{-1} , respectively, on average) contents
123 were significantly different between the two slopes.

124

125 Soil sampling

126

127 Five sampling plots were designated at 200 m intervals along the coastal line in each of the seaward
128 and landward slopes (Fig. 1b). Four individuals of *M. sinensis* and *L. mollis* in landward and seaward,
129 respectively, were selected in each sampling plot (5×5 m), and root-zone soil samples, including root
130 fragments, were collected from the four on June 17, 2020, as follows. For *M. sinensis*, a stainless-steel
131 core sampler [5×5 cm (diam/height), 100 mL in vol] was used to collect after removing the litter
132 layer. The core sampler could not be applied to *L. mollis* sampling because the root-zone soil (sand)
133 was too fragile to collect with the sampler due to the low density of the roots, but instead, root-soil
134 samples were collected by using a small shovel from an area of 10×10 cm at a depth of 10 cm (*ca.*
135 $100 - 200$ g plant⁻¹). Twenty kilograms of sand was also collected from the seaward slope where no
136 vegetation was present and autoclaved for 2 h as a "base medium" for soil trap cultures.

137

138 Trap cultures

139

140 The root-soil samples collected by the core samplers (landward slopes) and those collected by the
141 shovel (seaward slopes) were transferred to cylinder-shaped 37- μ m nylon mesh bags [5×5 cm
142 (diam/height)] (non-destructively for the landward samples) in 350-mL plastic pots in which the base
143 medium was filled around the mesh bag (four-replicated samples per plot and five plots in each of the
144 two slopes in a total of 40 pots). *M. sinensis* was used as a host plant for the samples from both slopes
145 based on the following two reasons; i) *L. mollis* seeds were unavailable, and ii) no significant
146 differences in the composition and diversity were observed between the communities of *L. mollis* field
147 roots and trap cultures with *M. sinensis* in the previous study (Kawahara and Ezawa 2013). *M. sinensis*
148 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 μ M 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. 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.

169

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-

174 ring sealed cap (Yasui Kikai, Osaka), and stored at -80°C for molecular analysis.

175

176 Molecular identification and life-history strategy assignment

177

178 The frozen roots in the 3-mL tube were ground with a metal cone in the presence of liquid nitrogen at

179 2,500 rpm for 2×5 s using Multi-Beads Shocker (Yasui Kikai, Osaka), and DNA was extracted and

180 purified from approx. 100 mg of ground sample by Maxwell RSC Instrument (Promega, Madison, WI)

181 using Maxwell RSC PureFood GMO and Authentication Kit (Promega) according to the

182 manufacturer's instructions, stored at -30°C, and used as template for PCR amplification. The divergent

183 domain 2 of the large-subunit ribosomal RNA gene (LSU rDNA) was amplified in a 25- μ L reaction

184 mixture of Expand High-Fidelity PCR System (Roche Diagnostics, Tokyo), 0.5 nmol μ L⁻¹ each of

185 FLd3 (forward) and FLR2 (reverse) primers that were linked to TruSeq-type forward- and reverse-

186 adapter sequences (Illumina, Tokyo), respectively, at the 5'-end (Niwa et al. 2018), and 0.2 - 2 μ L

187 template DNA using C1000 Touch™ Thermal Cycler (BIO-RAD, Tokyo) with the following

188 program: initial denaturation at 94°C for 2 min, followed by 30 cycles of denaturation at 94°C for 15 s,

189 annealing at 48°C for 40 s, polymerization at 72°C for 1 min, and final elongation at 72°C for 10 min.

190 PCR products were obtained from all samples from the landward slopes, except for one of the RD

191 samples, and from five SP, five RD, and 10 SL samples from the seaward slopes and sequenced on the

192 Illumina MiSeq platform (2×300 bp), and high-quality paired end reads (read 1 and read 2) of which

193 Phred quality score ≥ 20 were merged with a minimum overlap length of 10 nt using FLASH

194 (<http://ccb.jhu.edu/software/FLASH/>) at Bioengineering Lab (Sagamihara, Kanagawa, Japan). The

195 merged reads were subjected to blastn searches against the fungal LSU rDNA database consisted of

196 82,606 operational taxonomic units (OTUs) of fungi, including 524 and 82,082 OTUs of

197 glomeromycotinian (AM) and non-glomeromycotinian fungi, respectively, constructed by Niwa et al.

198 (2018) (<http://amfungi.kazusa.or.jp/>) and assigned to the OTUs at $\geq 95\%$ similarities over 330-bp
199 alignment with an E-value cut-off of $1e-30$. The maximum likelihood tree of the 524 AM fungal OTUs
200 with the 174 reference species of AM fungi (Delavaux et al. 2022) is available at
201 <http://amfungi.kazusa.or.jp/>. The two sets of the read count data from the same sampling plots (i.e., two
202 technical replication) were summed and treated as "one sample", whereas in the samples from which no
203 PCR product was obtained zero-data were assigned to all OTUs. Then the read count data were
204 normalized to 10^6 reads sample⁻¹ and transformed to logarithmic values ($\log_2$) or binary data
205 (presence/absence) after removing the read counts of non-glomeromycotinian fungi. At this stage,
206 samples in which all count data were zero were removed in subsequent analyses.

207
208 The OTUs that occurred uniquely in the SP and RD trap cultures, irrespective of the occurrence in the
209 SL trap cultures, were assigned to SP and RD fungi, respectively, and those that occurred in both SP
210 and RD trap cultures were defined as SP/RD fungi. Only the OTUs that occurred uniquely in the SL
211 trap cultures were assigned to SL fungi. Approximate phylogenetic positions of the OTUs detected in
212 this study were indicated by the maximum likelihood tree as follows. The OTU sequences were aligned
213 with the LSU rDNA sequences of the ascomycotan fungus *Exophiala spinifera* (MH876260) and
214 mucoromycotan fungus *Mortierella elongata* (MH047197) as outgroup with MAFFT ver. 7 (Katoh et al
215 2019), and the tree was inferred with raxmlGUI ver. 2.0.10 (Silvestro and Michalak 2012) in which the
216 GTR+I+G4 model was selected. Closest know species or genera of the OTUs were assumed in
217 reference to the phylogenetic tree of the 524 AM fungal OTUs with the 174 reference species at
218 <http://amfungi.kazusa.or.jp/>.

219
220 To assess the possible biases in the community compositions revealed by the trap cultures, the
221 sequence data obtained by Kawahara and Ezawa (2013) were subjected to blastn searches and re-
222 assigned to the recent AM fungal OTUs with the same criteria applied to the present sequence reads.

223

224 Statistical analysis

225

226 *F*-test for equality of variance and Welch's *t*-test were conducted on the R 4.2.1 platform (R Core Team
227 2020), in which Bonferroni correction was applied for multiple comparisons. Rarefaction analysis, non-
228 metric multidimensional scaling (NMDS) using Bray-Curtis dissimilarity index as a distance metric, β -
229 diversity analysis (Jaccard index), and permutational analysis of variance (PERMANOVA) were
230 performed with the vegan package (Oksanen et al. 2020) on the R platform. β -diversity analysis was
231 applied only to the OTUs that were detected from three or more samples. Scatter plots were drawn with
232 the dabeatr package on the R platform (Ho et al. 2019). Venn diagrams were drawn with Google Charts
233 (<https://developers.google.com/chart>). To assess preferences for the SP, RD, and SL strategies at the
234 family and genus levels, as well as preferences for the habitats, the numbers of samples in which the
235 individual OTUs were detected in each trap culture were summed within the same families/genera,
236 normalized, and subjected to principal component analysis using the vegan package.

237

238 Results

239

240 Trap culture overview

241

242 The amplicon sequences generated over 50,000 paired reads for each sample, which were merged and
243 subjected to blastn searches to assign to the fungal OTUs. Rarefaction analysis indicated that AM
244 fungal OTU-accumulation curves were leveling off, suggesting that the present sampling strategy (5
245 plots $\times$ 2 technical replication = 10 samples per habitat) provided reasonable coverages of AM fungal
246 diversity in the two slope habitats (Supplementary material Fig. S1). Combining of the two datasets of

247 the technical replication generated 15 community data from the landward samples (5 each from the
 248 three trap cultures) and 12 community data from the seaward samples (3, 4, and 5 data from the SP,
 249 RD, and SL trap cultures, respectively). These data consisted of 33,000 – 109,000 of fungal OTU-
 250 assigned reads (ca. 76,000 reads per sample on average) in which 23 – 100% (85% on average) were of
 251 AM fungi (Supplemental material Table S2).
 252
 253 Overall, 83 AM fungal OTUs were detected in total; 82 and 18 AM fungal OTUs occurred in the
 254 landward and seaward samples, respectively, in which 17 OTUs were common between the two slope
 255 habitats. The NMDS sample plot suggested that difference in the community compositions between the
 256 two habitats were obvious, but that among the trap cultures were ambiguous (Fig. 3a). Two-way
 257 PERMANOVA, however, indicated that both the habitat factor ($R^2 = 0.387/ P < 0.001$) and the trap
 258 culture factor ($R^2 = 0.100/ P < 0.027$) were significant. Subsequent one-way PERMANOVA within
 259 each habitat, as expected, showed that the trap culture factor was significant only in the landward
 260 samples ($R^2 = 0.242/ P = 0.044$) and not in the seaward samples ($R^2 = 0.279/ P = 0.094$). OTU richness
 261 in the SL trap cultures was significantly higher than those in the SP and RD trap cultures in the
 262 landward samples, but no significant differences were observed among the seaward trap cultures (Fig.
 263 3b). Total OTU richness was also highest in the SL trap cultures (72 OTUs), followed by the SP trap
 264 cultures (51 OTUs), and lowest in the RD trap cultures (28 OTUs), in which 84 and 89% of the OTUs
 265 in the SP and RD trap cultures, respectively, were also detected in the SL trap cultures; that is, they
 266 remained colonized six months after disturbance (Fig. 3c). These results imply that the extension of
 267 culture period to six months assisted the appearance of new OTUs, even in the presence of the rapid
 268 regenerators.
 269

270 Characterization of life-history strategies for disturbance tolerance

271

272 Based on the occurrence patterns of the OTUs in the trap cultures, 23, 28, 5, and 27 OTUs were
273 assigned to SP/RD, SP, RD, and SL strategists (Supplemental material Table S3). Subsequently, their
274 phylogenetic positions at the genus level were inferred in reference to the maximum likelihood tree
275 (<http://amfungi.kazusa.or.jp/>) as follows: 20 SP/RD, 12 SP, 4 RD, and 14 SL fungi in the 8 genera of
276 the Glomeraceae; 2 SP/RD, 11 SP, and 1 SL fungi in the 5 genera of the Gigasporaceae; 2 SP and 1 SL
277 fungi in the genus *Acaulospora* (Acaulosporaceae); 2 SL fungi in the genus *Archaeospora*
278 (Archaeosporaceae); 2 SP, 1 RD, and 4 SL fungi in the genus *Entrophospora* (Entrophosporaceae); 3
279 SL fungi in the genus *Diversispora* (Diversisporaceae); 1 SP/RD, 1 SP, and 2 SL fungi in the genus
280 *Paraglomus* (Paraglomeraceae) (Fig. 4 and Supplemental material Table S3).

281

282 In this ecosystem SP/RD strategists were most prevalent (Fig. 5a), and among them, the five OTUs
283 216_Par, 007_Rhz, 049_Rhz, 122_UnG, and 064_UnG that are close relatives of *Paraglomus*
284 *brasilianum*, *R. irregularis*, *Halonatospora panshihalos*, *Dominikia achra*, and *Nanoglomus* sp.,
285 respectively, occurred most frequently across the two slope habitats (Fig. 4). The occurrences of SP,
286 RD, and SL strategists were less than that of the SP/RD strategists and not significantly different
287 among them (Fig. 5a). The family level analysis on the preferences for the strategies, which was
288 calculated based on the prevalence of each family among the samples, indicated that the Glomeraceae
289 and Paraglomeraceae OTUs showed higher preferences for the RD strategy to the SP strategy, whereas
290 the Gigasporaceae OTUs showed a distinct preference for the SP strategy (Fig. 5b), illustrating clear
291 differentiation of the life-history strategies at the family level. The Entrophosporaceae
292 (*Entrophospora*), Acaulosporaceae (*Acaulospora*), Archaeosporaceae (*Archaeospora*), and
293 Diversisporaceae (*Diversispora*) OTUs preferred the SL strategy, although some OTUs in the former
294 two family (genera) showed the SP strategy. The genus level analysis was conducted for more detailed

295 characterization of the preferences not only for strategy but also for the habitats, mainly focusing on the
296 Glomeraceae OTUs that employ diverse strategies and showed distinct distribution patterns (i.e.,
297 habitat preferences). The *Glomus* and *Microdominikia* OTUs preferred the SP strategy and mainly
298 occurred in the landward habitat (Fig. 5c). Whereas the *Halonatospora*, *Dominikia*, *Nanoglomus*, and
299 *Rhizophagus* OTUs in addition to the *Paraglomus* OTU, which include the dominant five OTUs,
300 preferred the RD strategy and showed broad distribution across the habitats. It is noteworthy that, in
301 this PCA plot, the two factors RD strategy and occurrences in the seaward habitat are correlated (i.e.,
302 "RD" and "Sea" arrows point the same direction in the plot), implying that those that occurred in the
303 seaward habitat preferred the RD strategy. The *Gigaspora*, *Scutellospora*, and *Racocetra* OTUs in the
304 Gigasporaceae showed strong preferences for the SP strategy and mainly occurred in the landward
305 habitat.

306
307 OTU richness of the SP/RD strategists in the landward samples was not significantly different from
308 that of the SP strategists in the same habitat, but was higher than that of the other strategists in both
309 habitats (Fig. 6a). Richness of the SP and SL strategists in the landward samples and that of the SP/RD
310 strategists in the seaward samples were not different from each other, but higher than that of the RD
311 strategists in the landward and the SP strategists in the seaward samples. RD and SL strategists were
312 absent in the seaward samples. β -diversity was highest among the SL fungal communities, followed by
313 the SP and RD communities in the landward and the SP communities in the seaward samples, and
314 lowest in the SP/RD strategists in the samples from both habitats (Fig. 6b).

315
316 Potential biases of the community compositions caused by the destructive collection of the seaward
317 samples, as well as by the trap culture approach itself, were assessed by re-analyzing the previous data
318 (Kawahara and Ezawa 2013) together with the present data, addressing the following possibilities that

319 i) the destructive collection of the root-soil samples damaged SL fungi thus inhibited their occurrence,
 320 that ii) the employment of *M. sinensis* that is not a native host in the habitat as a trap culture host
 321 inhibited the colonization of those that have strong preferences to *L. mollis*, and that iii) the fungi that
 322 can occur in trap culture are inherently disturbance tolerant. To approach these possibilities, we
 323 searched the OTUs that occurred uniquely in the field roots, but were absent in the present trap
 324 cultures, which are likely disturbance-sensitive (SL) fungi. The re-assignment of the previous sequence
 325 data to the present OTUs identified 278_Rhz, 019_Rhz, 040_Rhz, 188_Aca, and 378_Unc, close
 326 relatives of *Epigeocarpum japonicum*, *R. fasciculatus*, and *Sclerocystis sinuosa* in the Glomeraceae,
 327 *Ac. scrobiculata* (Acaulosporaceae), and *Ambispora* sp. (Ambisporaceae), respectively, as potential SL
 328 fungi missed in this study. 019_Rhz was common between the two slopes, and 278_Rhz was specific to
 329 the landward habitat. The three OTUs, 040_Rhz, 188_Aca, and 378_Unc, were specific to the seaward
 330 habitat (i.e., specific to *L. mollis*) (Supplementary material Table S4 and Fig. S2). On the other hand, it
 331 was also found that five of the 27 SL fungi in this study, 008_Rhz, 119_UnG, 206_Div, 318_Div, and
 332 229_Par that are close relatives of *R. irregularis*, *Do. achra*, *Di. densissima*, *Di. sabulosa*, and *P.*
 333 *occultum*, respectively, occurred in the previous trap cultures and thus are likely rapid regenerators.
 334 These observations support the possibilities that i) there are potential SL fungi missed in this study, ii)
 335 in which some could be *L. mollis*-specific, and that iii) some of the SL fungi are potentially rapid
 336 regenerators.

338 Discussion

340 The present study provides experimental support for the previous study (Kawahara and Ezawa 2013)
 341 through characterizing the AM fungal life-history strategies for regeneration after disturbance. We
 342 demonstrated that soil-borne propagules and colonized roots play a major role in the rapid regeneration

343 of AM fungi inhabiting a frequently disturbed ecosystem, supporting the first hypothesis. Intriguingly,
344 many SP strategist fungi were also capable of regenerating from the colonized roots, that is, they are
345 SP/RD strategists. The extension of growth period for the trap cultures assisted the appearance of new
346 OTUs that required longer time to regenerate after disturbance; they are designated as disturbance-
347 sensitive SL fungi, supporting the second hypothesis. The majority of the rapid regenerators belong to
348 the families Glomeraceae and Paraglomeraceae, rhizophilic/ruderal fungi, but unexpectedly, a number
349 of SP fungi were also found in the Gigasporaceae that has been characterized as the family of
350 edaphophilic/competitor fungi. Similarly, SL fungi were distributed not only in the Gigasporaceae and
351 Diversisporaceae but also across all the families occurred in this study. Accordingly, the third
352 hypothesis was only partially supported.

353

354 The dual strategy (SP and RD) for rapid regeneration would be the nature of the rhizophilic fungi. The
355 production of abundant spore (i.e., soil-propagule) is a prevalent trait in the Glomeraceae (Chagnon et
356 al. 2013; Klironomos and Hart 2002) and probably in the Paraglomeraceae. In fact, 34 out of the 51 SP
357 fungi belong to these families. Whereas the RD strategy was also prevalent in the two families; 25 of
358 the 28 RD fungi belong to the families. Furthermore, the five dominant OTUs in the genera
359 *Halonatospora*, *Dominikia*, *Nanoglomus*, *Rhizophagus*, and *Paraglomus* in these families showed
360 broad distributions across the habitats and higher preferences to the RD strategy (i.e., higher
361 occurrences in the RD treatment), suggesting that the RD strategy is more important than the SP
362 strategy for the survival in severely/frequently disturbed environments.

363

364 For rapid regeneration after disturbance, various strategies known as the ruderal traits (Chagnon et al.
365 2013) may function together. SP fungi may have short life cycles (Declerck et al. 2001) thus could
366 produce spore more rapidly than those that have longer life cycles. The efficient mechanisms for
367 bridging/healing of fragmented extraradical hyphae (de la Providencia et al. 2007) would sustain not

368 only survival of detached extraradical hyphae but also their inoculum potential in the soil. Rapid hyphal
369 growth (Avio et al. 2006; Hart and Reader 2005) is a quite important trait for SP and RD fungi for
370 rapid colonization and reconstruction of hyphal networks. It was unexpected, however, that most of the
371 rapid regenerators (SP/RD, SP, and RD fungi) remained colonized in the SL trap cultures (i.e., six
372 months after disturbance), which implies that their life cycles are not as short as we considered and that
373 they are not necessarily less competitive against competitor fungi. Further study is necessary to
374 elucidate the involvement of these "typical ruderal traits", which were not considered in this study, in
375 the disturbance tolerance of the fungi.

376
377 We hypothesized that the members in the Gigasporaceae would be SL fungi because they colonize
378 roots mainly via spores, rarely via the colonized roots, and produce spores at later stages of their life
379 cycles (Biermann and Linderman 1983; Jasper et al. 1993; Klironomos and Hart 2002; Oehl et al.
380 2009), which are typical of the competitor traits (Chagnon et al. 2013). In line with these studies, the
381 Gigasporaceae showed a strong preference to the SP strategy, confirming that their intraradical hyphae
382 may not be major inoculum source in the soil. Whereas the occurrence of the Gigasporaceae OTUs in
383 the SP trap cultures implies that they are capable of regenerating rapidly via extraradical hyphae
384 detached from the roots (de la Providencia et al. 2007; Jasper et al. 1993) and/or via mature spores
385 produced within two months. Their preferences for the landward habitat, however, suggest that their
386 propagules in the soil are susceptible to severe/frequent disturbance. For further characterization of the
387 life-history strategies of Gigasporaceae fungi, the inoculum potential of extraradical hyphae and timing
388 of their sporulation need to be evaluated by comparing those inhabiting disturbed and undisturbed
389 ecosystems.

390
391 β -diversity of the SL fungal community was higher than that of the communities of the SP/RD and SP

392 strategists in the same habitat, reflecting that most of the SL fungi were unique to each plot. This
393 patchy distribution of the SL fungi suggests that stochastic processes, rather than environmental factors
394 such as disturbance severity, may play an important role in structuring the community (e.g., Dumbrell
395 et al. 2010; Lekberg et al. 2012). This could be interpreted by the reproductive strategy of competitor
396 fungi; they generally invest more in vegetative growth than in reproductive growth (Chagnon et al.,
397 2013), constructing dense hyphal networks through which they explore new hosts, and thus their
398 dispersal ability may not be so high as the SP (ruderal) strategists that are capable of dispersing over
399 long distances. It is considered, due to these reasons, that the SL fungi showed stochastic (i.e.,
400 heterogeneous) distribution, compared with those that have high-dispersal ability.

401
402 The possibility that the fungi that occur in trap cultures are inherently disturbance tolerant is
403 considered. The SL fungi were distributed across diverse families, including the members of the ruderal
404 families Glomeraceae and Paraglomeraceae, suggesting that not all the SL fungi are competitor
405 strategists, but potentially ruderal strategists. They might not be able to establish within two months,
406 probably due to the rapid occupation of the niche by the dominant rapid regenerators that produced
407 abundant propagules in the soil. This observation suggests that our approach has limitations for
408 detecting SL fungi (i.e., disturbance-sensitive fungi), and more detailed characterization, e.g., spore
409 productivity and resilience of hyphal networks, is necessary in the future.

410
411 The absence of SL fungi in the seaward slopes is supported by the previous findings that the AM fungal
412 community in the slopes was robust against soil disturbance, that is, most fungi in the habitat were
413 disturbance tolerant (Kawahara and Ezawa 2013). However, biases in the community compositions
414 caused by the destructive collection of the samples, as well as by using the non-native host in the trap
415 cultures, cannot be excluded. Among the three OTUs that occurred uniquely in the seaward field roots
416 in the previous study, the two OTUs, 188_Aca (*Ac. scrobiculata*) and 378_Unc (*Ambispora* sp.) that

417 belong to the families of "ancestral" fungi (Weber et al. 2019), are more likely to be SL fungi than the
418 *Rhizophagus* OTU and potentially *L. mollis*-specific . Similar to the proposed mechanism underlying
419 the occurrence of potential ruderal fungi in the SL trap cultures, the presence of the massive amounts of
420 propagules of the dominant rapid regenerators, which had continuously been supplied from the other
421 side of the slopes to the habitat, was likely to overshadow SL fungi in the habitat. For more detailed
422 studies, the improvement of isolation (detection) method for disturbance-sensitive fungi is necessary,
423 although it may not be easy due to the coexistence of rapid regenerators.

424

425 The mesh-separated compartment culture system successfully differentiated AM fungal strategies
426 responsible for regeneration after disturbance. To characterize soil-borne propagules of the fungi, the
427 destructive method (i.e., wet sieving) has generally been employed in previous studies (e.g.,
428 Klironomos and Hart 2002; Varela-Cervero et al. 2016). It is likely, however, that the isolation of spore
429 and hyphae by the destructive manners would lead to underestimation of inoculum potential via
430 damaging/losing the propagules. The compartment culture system had originally been established three
431 decades ago to assess the impact of destruction of extraradical hyphae on their inoculum potential
432 (Jasper et al. 1989) and has recently been applied to physiological studies on fungal nutrient uptake
433 (e.g., Hodge & Fitter, 2010; Kikuchi et al., 2016). The present study demonstrated that the application
434 of this technique enables us to assess the potential of extraradical mycelia in a non-destructive manner,
435 in which extraradical mycelia were separated from the mycorrhizal roots simply by removing the mesh
436 bag, minimizing the damages of the propagules. This culture system, however, cannot separate spores
437 and extraradical hyphae that may differ in inoculum potential. Given that spores are likely to survive a
438 longer period than detached hyphae, further experiments that take into account a time factor are
439 necessary to assess the significance of these two types of inocula in regeneration after disturbance.

440

441 **Conclusion**

442

443 We employed Ishikari sand dunes as a model ecosystem and experimentally characterized the life-
444 history strategies of disturbance-tolerant/-sensitive AM fungi by taking into account the time factor
445 necessary for regeneration after disturbance. We demonstrated the significance of both soil-borne
446 propagules and colonized roots in the rapid regeneration of the fungi and the differences in their life-
447 history strategies at the higher taxonomic levels. The Glomeraceae and Paraglomeraceae fungi
448 dominated in the more severely disturbed habitat seaward slopes and showed preferences for the RD
449 strategy, whereas the Gigasporaceae fungi occurred mostly in the less severely disturbed habitat
450 landward slopes, even though they are SP strategists, suggesting the significance of the RD strategy in
451 severely and frequently disturbed environments. On the other hand, the detection and characterization
452 of the disturbance-sensitive fungi (i.e., SL fungi) was not easy because of the co-occurrence of rapid
453 regenerators, as well as potential biases caused by trap culture. Further improvement of research
454 approaches for characterizing SL fungi is required. Taken together, our findings provide a new insight
455 into the life-history strategies of AM fungi in the ecosystems where soil disturbance occurs frequently
456 and will contribute to the practical application of AM fungi to restoration of post-disturbed ecosystems.

457

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459

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463

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603

604 **Statements and Declarations**

605

606 **Competing interests**

607

608 The authors have no relevant financial or non-financial interests to disclose.

609

610 **Author contributions**

611

612 All authors contributed to the study conception, design, and writing the manuscript.

613

614 Data availability

615

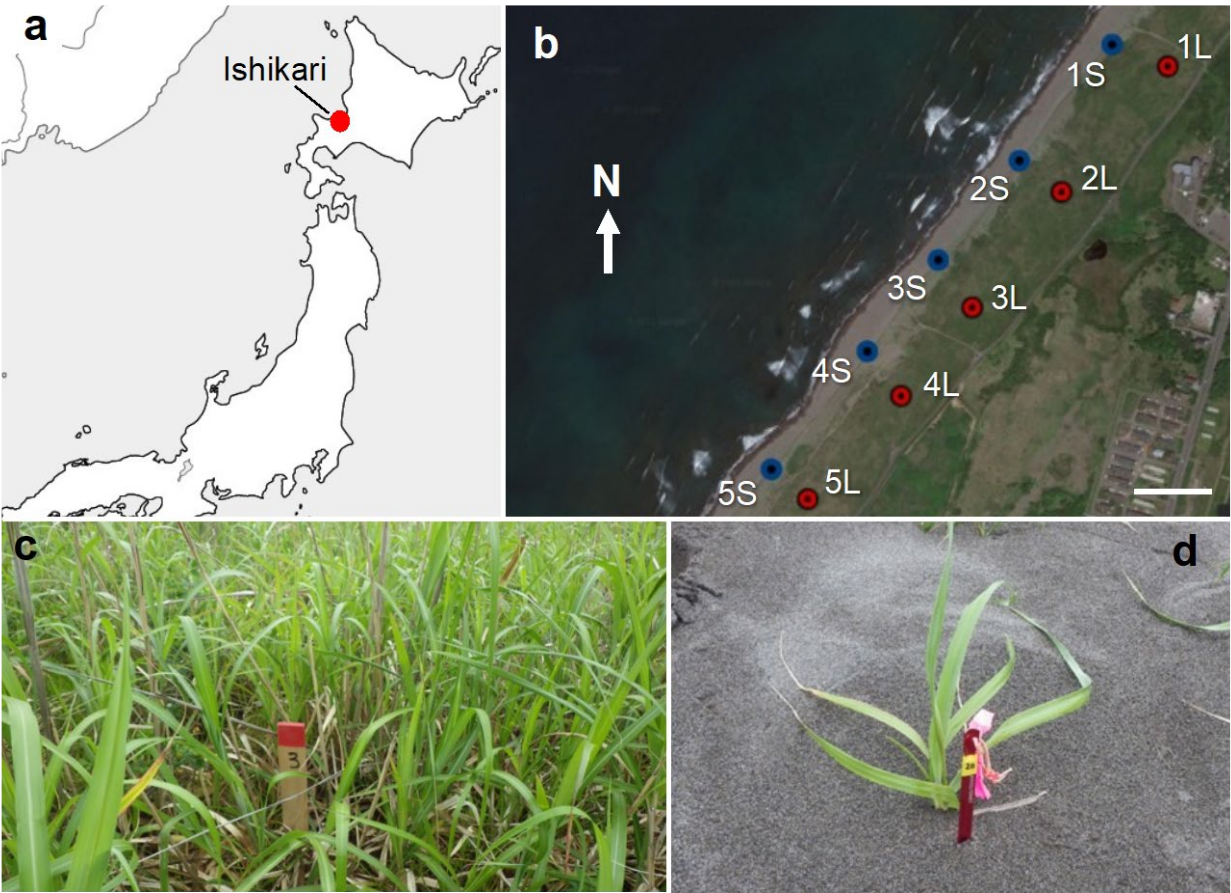
616 The sequence reads have been deposited in the Sequence Read Archive of National Center for

617 Biotechnology Information under the accession number PRJNA949820.

618 **Figures**

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622

623 **Fig. 1** a) Location of Ishikari coastal dune in Hokkaido, Japan. Scale, 250 km. b) Sampling plots and
624 ID in the seaward (1S – 5S) and landward (1L – 5L) slopes in the dunes. The image was generated by
625 Google Earth (<https://www.google.co.jp/intl/ja/earth/>) using the GPS data of the plots (Supplemental
626 material Table S1). Scale, 100 m. c) *Miscanthus sinensis* grown in a landward slope. d) *Leymus mollis*
627 grown in a seaward slope.

628

643 **Fig. 3** Analysis of trap culture communities. Root-
 644 soil samples were collected from the landward and
 645 seaward slopes in Ishikari dunes, and *M. sinensis*
 646 seedlings were grown aa) Nonmetric multi-
 647 dimensional scaling of AM fungal communities in
 648 the trap cultures of soil-propagule mediated
 649 regenerator (circles), root-direct regenerator
 650 (triangles), and slow regenerator (squares) raised
 651 from the landward and seaward samples. Bray-Curtis
 652 dissimilarity index was applied as a distance metric.
 653 Dimensions, 2; Stress = 0.141. b) AM fungal OTU
 654 richness in the trap cultures of soil-propagule
 655 mediated regenerator (SP), root-direct regenerator
 656 (RD), and slow regenerator (SL) raised from the
 657 landward and seaward samples. Welch's t-test: *, $P <$
 658 0.05; ***, $P < 0.001$. c) Venn diagram of AM fungal
 659 OTUs occurred in the trap cultures. Abbreviations
 660 are the same as above. The OTU numbers in each
 661 area are indicated. The common OTUs that occurred
 662 between the SL cultures and either of the SP or RD
 663 cultures are highlighted with a bold black line.

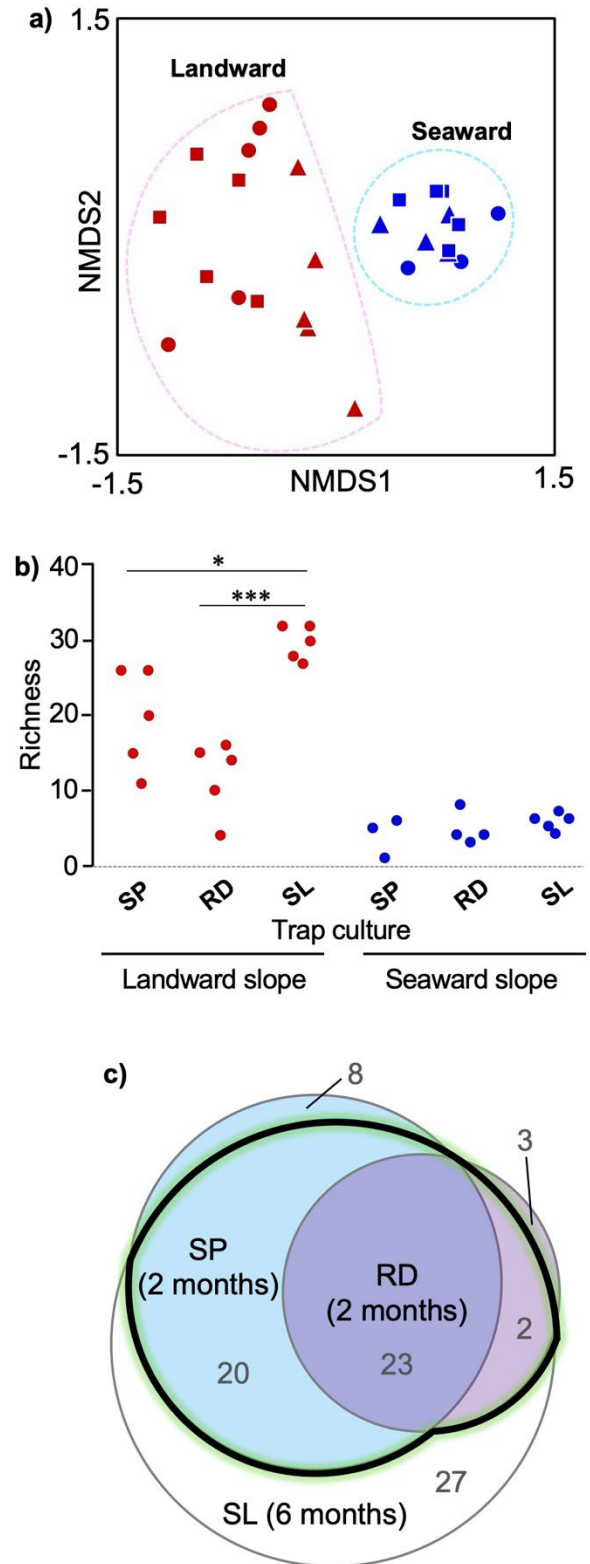
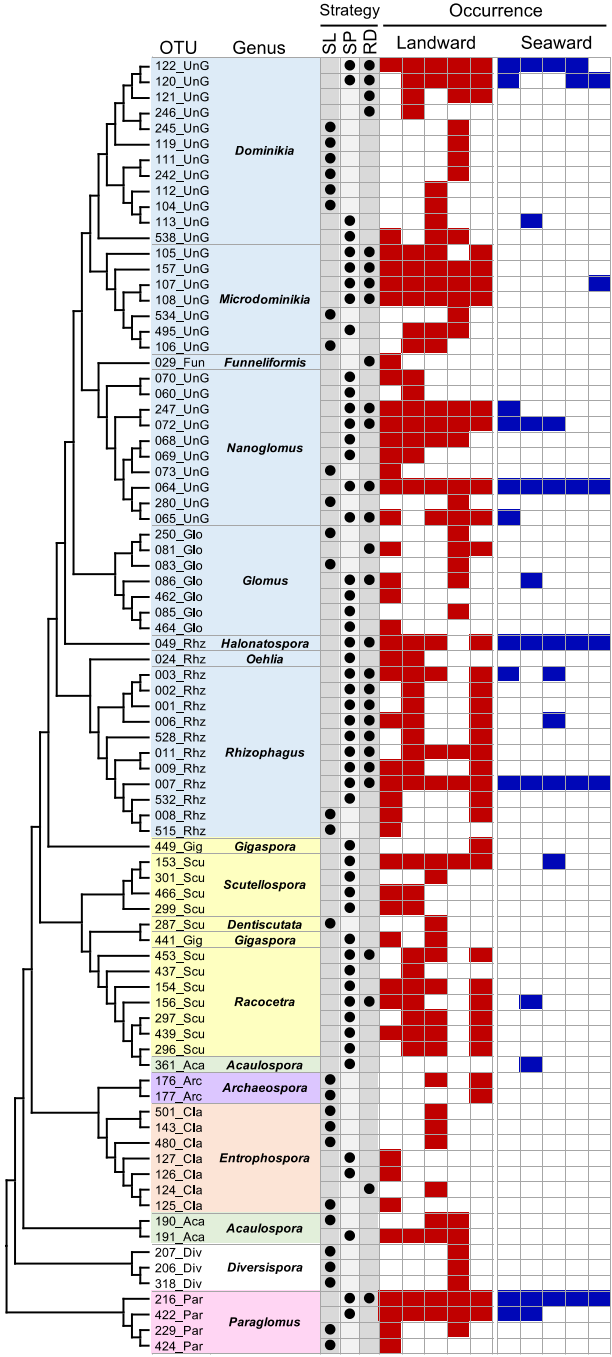
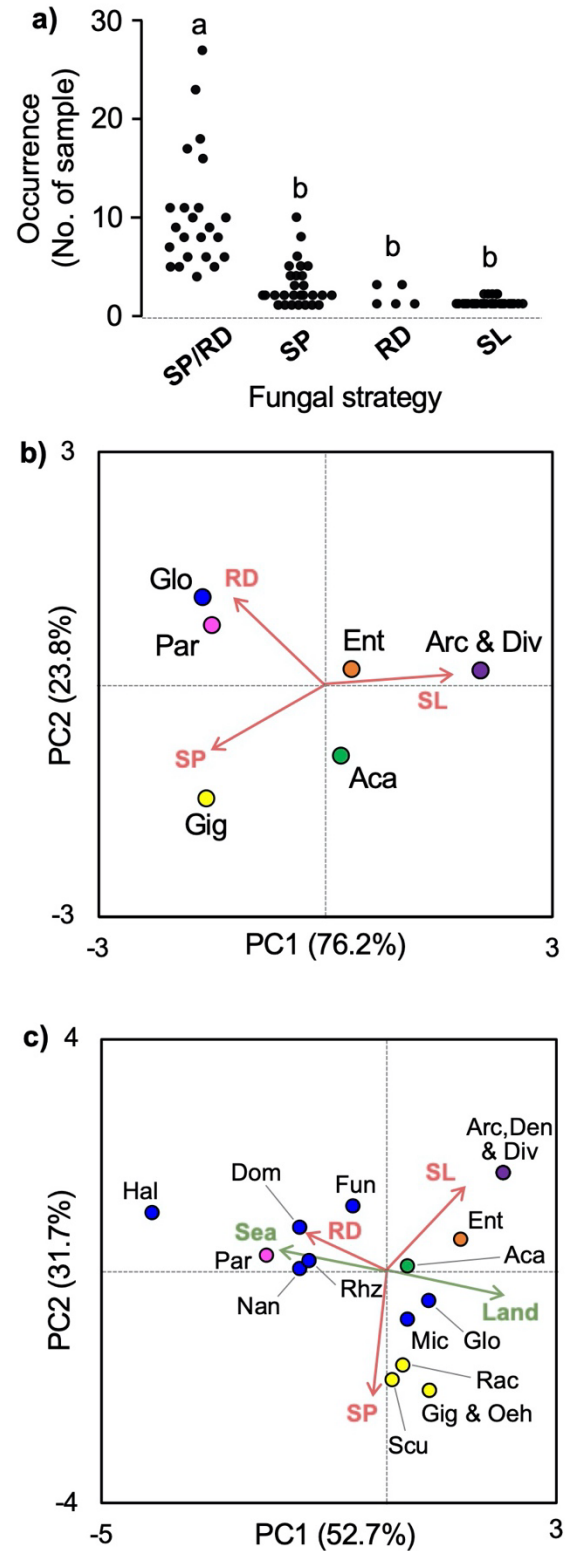
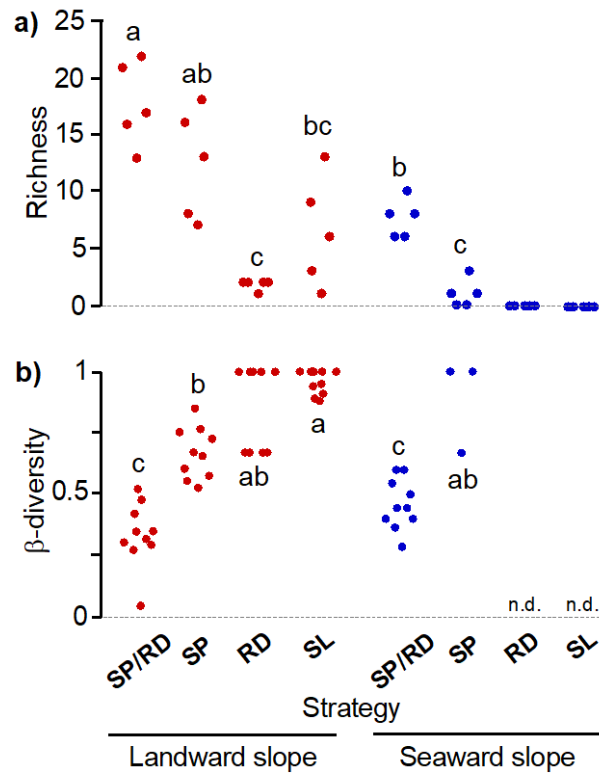


Fig. 4 Taxonomic positions and assigned strategy of all AM fungal OTUs detected in this study and their occurrences in the five plots in the landward and seaward slopes. The disturbance-tolerance strategies, soil-propagule mediated regenerator (SP), root-direct regenerator (RD), and slow regenerator (SL), were assigned to each OTUs based on their occurrence patters in the trap culture experiment (Supplementary material table S3). Maximum likelihood tree is 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*.



685 **Fig. 5 a)** Prevalence of AM fungal strategies for
 686 disturbance tolerance in Ishikari dune ecosystem.
 687 Occurrence (numbers of samples) of the OTUs that
 688 have either of the soil-propagule mediated
 689 regenerator/root-direct regenerator (SP/RD), SP, RD, or
 690 slow regenerator (SL) strategies occurred (each dot
 691 represents each OTU). Different letters indicate
 692 significant difference (Welch's *t*-test, $P < 0.05$). b and
 693 c) Principal component analysis on the preferences for
 694 the SP, RD, and SL strategies at the family (b) and
 695 genus (c) levels. Occurrence (numbers of sample) of
 696 the OTU that employ either of the strategies were
 697 summed within the same families (a) or genera (b),
 698 normalized, and subjected to the analysis. In the genus
 699 level analysis, their occurrence data in the landward
 700 (Land) and seaward (Sea) samples were also included.
 701 Abbreviations of the families/ genera are: Aca,
 702 Acaulosporaceae/ *Acaulospora*; Arc,
 703 Archaeosporaceae/ *Archaeospora*; Den, *Dentiscutata*;
 704 Div, *Diversisporaceae/ Diversispora*; Dom, *Dominikia*;
 705 Ent, *Entrophosporaceae/ Entrophospora*; Fun,
 706 *Funneliformis*; Gig, *Gigasporaceae/ Gigaspora*; Glo,
 707 *Glomeraceae/ Glomus*; Hal, *Halonatospora*; Mic, *Microdominika*; Nan, *Nanoglomus*; Oeh, *Oehlia*; Par,
 708 *Paraglomeraceae/ Paraglomus*; Rac, *Racocetra*; Rhz, *Rhizophagus*; Scu, *Scutellospora*.





709

710

711 **Fig. 6** Richness (a) and β -diversity (b) of AM fungal OTUs to which either of the disturbance-tolerance
 712 strategies, soil propagule-mediated/root-direct regenerator (SP/RD), SP, RD, or slow regenerators (SL)
 713 was assigned. Richness represents the numbers of OTU. β -diversity represents Jaccard dissimilarity
 714 indices calculated by pairwise comparison between all combinations of the samples from different plots
 715 and was calculated only for the strategy categories in which the OTU occurred three or more plots
 716 (n.d., not determined). Welch's t -test, followed by Bonferroni corrections, were applied. Different
 717 letters indicate significant difference at $P < 0.05$.

718 **Supplemental material**

719

720 1. Supplemental material 1.pdf

721 Fig. S1. Rarefaction analysis on AM fungal OTU richness in the trap cultures.

722 Fig. S2 Venn diagram of AM fungal OTUs occurred in this study (light yellow) and Kawahara and
723 Ezawa (2013).

724 Table S1. GPS data for the sampling plots.

725

726 2. Supplemental material 2.xlsx

727 Table S2. Raw read count of blastn searches against the LSU rDNA database consisted of 82,606
728 fungal operational taxonomic units (OTUs).

729 Table S3. Assignment of life-history strategy and log-transformed ($\log_2$) read count data of the AM
730 fungal OTUs.

731 Table S4. Summary of blast searches for the phylotypes in Kawahara and Ezawa (2013) against the 524
732 AM fungal operational taxonomic units (OTUs).

733 Table S5. AM fungal operational taxonomic units (OTUs) occurred in the SP, RD, and SL trap cultures
734 in this study and in the field and trap culture roots in Kawahara and Ezawa (2013).

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