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**Characterization of arbuscular mycorrhizal fungal
communities with respect to soil disturbance
in a volcanic ecosystem**

(土壌攪乱に着目した火山生態系におけるアーバスキュラー菌根菌
群集の特徴付け)

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General introduction

Slopes of active volcanoes are a unique ecosystem that is frequently disturbed by ash falling and erosion, and thus vegetation is generally poor, particularly near the crater (i.e. at higher elevations). Protection of volcanic slopes by vegetation is quite important for stabilization of the soil, particularly after a large eruption, by which a risk for catastrophic debris flows could be reduced. Members of primary vegetation on volcanic slopes, however, are limited within those that have disturbance-tolerant traits due to the frequent disturbance of the habitat. *Miscanthus sinensis* Anders. is a pioneer grass species that is a common member of primary vegetation in eastern Eurasia and South-East Asia (An *et al.*, 2008; Stewart *et al.*, 2009) and also common in volcanic slopes (Kondo *et al.*, 2016; Holle & Tsuyuzaki, 2018). Pioneer plants have developed various strategies to adapt to harsh environments, and one distinct strategy is the association with arbuscular mycorrhizal (AM) fungi.

AM fungi belong to the subphylum Glomeromycotina and form mutualistic associations with approximately 74% of land plants (Brundrett & Tedersoo, 2018). The primary function of mycorrhizal association is the enhanced uptake of immobile soil nutrients, in particular, phosphorus (P), through extensive hyphal networks constructed in soil (Smith & Read, 2008). Basically, AM fungi are susceptible to soil disturbance. Destruction of hyphal networks by mechanical disturbance and faunal grazing leads to loss of extraradical biomass. However, the fact that AM fungi are abundant in arable soil that are frequently disturbed by tillage/ploughing (e.g., Jansa *et al.*, 2002) implies that they have distinct traits; e.g., rapid colonization capability, rapid production of low-

cost hyphal biomass, and short-reproductive cycles via producing abundant spores (Hodgson *et al.*, 1999; Cornelissen *et al.*, 2001; Chagnon *et al.*, 2013).

However, information about AM fungi that inhabit volcanic ecosystems, which is essential for successful application of AM fungi for restoration of the ecosystem, is limited. The objective of this study is to characterize AM fungal communities associated with pioneer grass *M. sinensis* in a volcanic ecosystem with respect to disturbance tolerance; in Chapter 1 distribution of disturbance-tolerant AM fungi along a volcanic slope was investigated, and in chapter 2 the impact of periodic disturbance on the fungal community was evaluated in a one-year-pot experiment.

CHAPTER 1

Distribution of disturbance-tolerant AM fungi along a volcanic slope

1.1 Introduction

It has been demonstrated that mechanical destruction of hyphal networks by soil disturbance has a significant impact on AM fungal communities (Hart & Reader, 2004; Jansa *et al.*, 2002; Lekberg *et al.*, 2012; Schoor *et al.*, 2011), suggesting that disturbance could act as an ecological filter for selecting the fungi that are tolerant hyphal destruction. In volcanic ecosystems severity of disturbance is likely to increase with increasing elevation towards the crater (Del Moral & Grishin, 1999). In this context, AM fungi that are more tolerant to disturbance would preferentially inhabit at higher elevations, whereas those that are sensitive to disturbance would be more abundant at lower elevations where disturbance would be less severe.

In the present study, distribution of disturbance-tolerant AM fungi along a volcanic slope at Mt. Tarumae was investigated, in which here I hypothesized that the fungi that are tolerant to soil disturbance are selected at higher elevations (i.e. nearer to the crater). To test this hypothesis, I employed the soil trap culture approach and applied artificial disturbance to soil samples collected from rhizosphere of the pioneer grass *M. sinensis* along an elevation gradient in the volcanic slope.

1.2 Materials and methods

Sampling site

Mt. Tarumae volcano is located in the southeastern rim of Shikotsu caldera (42°41'26"N/141°22'36"E) in Shikotsu-Toya National Park, Hokkaido, Japan (Fig. 1.1a). The volcano occurred approximately 9,000 years ago, and the largest explosive eruptions that affected Northern Hemisphere temperature were recorded in 1667 and 1739 (Briffa *et al.*, 1998). The latest eruption with ash falls and mudflows occurred during 1978 – 1981, and in 2001 and 2003 temperature of the crater reached over 600°C, during which sand and mud were emitted from the crater (Furukawa and Nakagawa, 2010). The height is 1,041 m a.s.l., and there is a lava dome (120 m in height) in the crater. The upper limit of forest is around 630 m a.s.l., and above the limit, *M. sinensis* is distributed in three types of habitat, grassland, gullies, and slopes, on the north-facing slope. The grassland is an open flat area (inclination angles are less than 10°) located at 630 – 660 m a.s.l., in which *M. sinensis* and the shrub *Ledum palustre* subsp. *diversipilosum* var. *diversipilosum* largely dominate (Fig. 1.1b). In addition to the two dominant species, large colonies of *Spiraea betulifolia* var. *betulifolia* and *Weigela middendorffiana* are patchily distributed. *M. sinensis* grown in this habitat generally develop a large clump of multiple stems (50–150 cm in diam). Above the grassland (up to the vegetation limit at 850 m a.s.l), several gullies (0.3 – 2 m in depth, inclination angles are up to 15°) are developed along the slope of which the inclination angles are 20 – 40°. It seems likely that water availability in the gullies is higher than in the grassland and on the slope, particularly during spring when snow

melts. The gullies and slopes (Fig. 1.1c,d) are not fully covered with vegetation, but plant diversity is much higher than in the grassland; *Alnus maximowiczii*, *Sorbus sambucifolia*, *Fallopia sachalinensis*, *Pennellianthus frutescens*, and *Salix reinii* are patchily distributed in addition to *M. sinensis*, *L. palustre* subsp. *diversipilosum* var. *diversipilosum*, and *S. betulifolia* var. *betulifolia* (Fig. S1.1). *M. sinensis* grown in the gullies and on the slopes generally forms smaller clumps than those in the grassland (Fig. 1.1e).

Soil sampling

Ten individuals of *M. sinensis* were selected from each of the three habitats, grassland, gullies, and slopes (30 plants in total), along the north-facing slope towards the crater (Fig. 1.1f). After measuring colony diameter of *M. sinensis*, two rhizosphere soil samples (i.e. paired-soil samples), including root fragments, were collected from each individual with a stainless steel core sampler (5× 5 cm, 100 mL in vol) in June 2015 (14 samples) and August 2016 (16 samples) as AM fungal inocula for soil trap culture (Table S1.1). In addition to these paired-soil samples, about 100 g of root-zone soil was collected from each individual for chemical analysis. Twenty kilograms of bulk soil was also collected at which no vegetation was present (800 m a.s.l.) as a base medium for trap culture. Chemical properties of the soil samples were analyzed at Tokachi Federation of Agricultural Cooperatives, Obihiro, Japan (Table S1.1).

Disturbance treatment in soil trap culture

One of the paired-soil-core samples was excised from the core, sieved on a 2-mm stainless steel mesh to destruct hyphal networks of the fungi and designated as a disturbed inoculum, whereas the other sample was carefully taken from the core sampler and used without sieving as an intact inoculum. Either of the intact or disturbed inoculum (*ca.* 100 mL) was transferred to 350-mL plastic pots that were filled with 200 mL of the base medium (i.e. autoclaved bulk soil). Seeds of *M. sinensis* (Kaisessa, Otofuke, Japan) were sown onto the soil surface, covered with a thin layer of autoclaved river sand, grown only with tap water in a temperature/light/humidity-controlled greenhouse (day/night temperature 26/20°C; 14-h day length; 60% relative humidity), and thinned to 10 plants per pot two weeks after sowing. Two months later, the roots were harvested, washed with tap water, cut into 1-cm segments, randomized in water, blotted on a paper towel, frozen at -80°C more than a day, freeze-dried for 2 days, and stored at -30°C for DNA extraction.

Molecular identification

Ten – 20 mg of the freeze-dried root sample was transferred to a 2.5-mL tube with an O-ring sealed cap (Yasui Kikai, Osaka, Japan) and ground using Multi-Beads Shocker (Yasui Kikai, Osaka, Japan) with a metal cone at 2,500 rpm for 2×60 s, and DNA was extracted with DNeasy Plant Mini Kit (Qiagen, Tokyo, Japan) according to the manufacturer's instructions. The divergent domain 2 of large-subunit ribosomal RNA

gene (LSU rDNA) was amplified in a 25- μ L reaction mixture of Expand High-Fidelity PLUS PCR System (Roche Diagnostics, Tokyo, Japan), 0.5 nmol μ L⁻¹ each of the fungi-specific primers, FLd3 (forward) and FLR2 (reverse) to which TruSeq-type forward- and reverse-adapter sequences (illumina, Tokyo, Japan), respectively, were linked at the 5'-end (Niwa *et al.*, 2018), and 1 μ L template DNA using C1000 TouchTM Thermal Cycler (BIO-RAD, Tokyo, Japan) 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, and polymerization at 72°C for 1 min, and final elongation at 72°C for 10 min. The PCR products were purified with Agencourt AMPure XP SPRI magnetic beads (Beckman Coulter, Tokyo, Japan) and sequenced on the illumina MiSeq platform (2 × 300 bp) at Bioengineering Lab (Atsugi, Kanagawa, Japan). The nucleotides with a quality value (QV) < 20 in the 3' end and the adapter-index sequence in the 5' end were trimmed, and those shorter than 40 bp were excluded. After the quality filtering, the paired-end reads (read 1 and read 2) were merged with a minimum overlap length of 10 nt using FLASH (<http://ccb.jhu.edu/software/FLASH/>). The merged sequence reads were subjected to BLASTn searches against the fungal LSU rDNA database consisted of 8,2494 operational taxonomic units (OTUs) of fungi, including 412 OTUs of AM fungi, and assigned to the OTUs at a criterion of $\geq 95\%$ similarities over 330 bp with an E value $\leq 10^{-100}$, which was executed in the open web interface “Arbuscular mycorrhizal fungi classification pipeline” (<http://amfungi.kazusa.or.jp>) (Niwa *et al.*, 2018).

Statistical analysis

Total sequence reads assigned to AM fungal OTUs were standardized to 10^4 reads per sample by resampling for community analysis. All statistical analyses were performed on R 3.2.3 platform (R Core Team, 2013). Shannon-Wiener indices, Bray-Curtis dissimilarity indices, Mantel test (9,999 permutations), and permutational multivariate analysis of variance (PERMANOVA) (9,999 permutations) were performed with vegan package (Oksanen *et al.*, 2013). In Mantel test Bray-Curtis dissimilarity index was employed as a measure of community distance.

Community nestedness was evaluated using nested overlap and decreasing fill (NODF) as an index (Almeida-Neto *et al.*, 2008), in which the hypothesis whether species (OTU)-poor communities are subsets of species (OTU)-rich communities was tested. For this analysis, OTU composition data of the intact and disturbed communities from the paired-soil samples were combined and transformed to presence-absence data, and then a maximally stacked matrix in which orders of the columns (soil samples) and rows (OTUs) were sorted by OTU richness and the occurrence of OTU (number of soil sample in which the OTU was detected), respectively, was constructed. A two-sided test was performed using the function *oecosimu* in the *vegan* package, in which the significance of NODF among columns ($NODF_{\text{column}}$) was tested against 1,000 randomly generated matrices (Monte Carlo procedure) under the column-totals fixed null model that assumes the number of species in each sample remains constant. The index ranges from 0 (non-nested) to 100 (fully nested) and was deemed significant if the value is higher than the expected value obtained from the randomly generated matrices. Z-scores

of $NODF_{\text{column}}$ were calculated as $(NODF_{\text{column}} - NODF_{\text{column-exp}}) / SD_{\text{exp}}$, where SD_{exp} is standard deviation of the expected values. A significant positive Z-score indicates that there is a nested pattern in the matrix, while a significant negative Z-score indicates anti-nestedness.

Individual OTUs were characterized with respect to soil disturbance based on gain and loss of the sequence reads by the disturbance treatment in comparison with those in the intact treatment. All read number data were first transformed to logarithmic values ($\log_{10}$) according to Anderson *et al.* (Anderson *et al.*, 2006), and 'responsiveness' and 'robustness' of OTU_j were calculated by the following equations:

$$a) \text{ Responsiveness of } OTU_j = \frac{D_j - I_j}{D_j + I_j}$$

$$b) \text{ Robustness of } OTU_j = 1 - \left| \frac{D_j - I_j}{D_j + I_j} \right|$$

where D_j and I_j are total read numbers of OTU_j detected in the disturbed and intact treatments, respectively, across all samples. The former index (a) of the OTUs that gained and lost sequence reads by disturbance shows positive and negative scores, respectively, whereas the latter index (b) of the OTUs that showed large and small differences in sequence-read abundance between the disturbance and intact treatments indicates low and high scores, respectively, between 0 and 1.

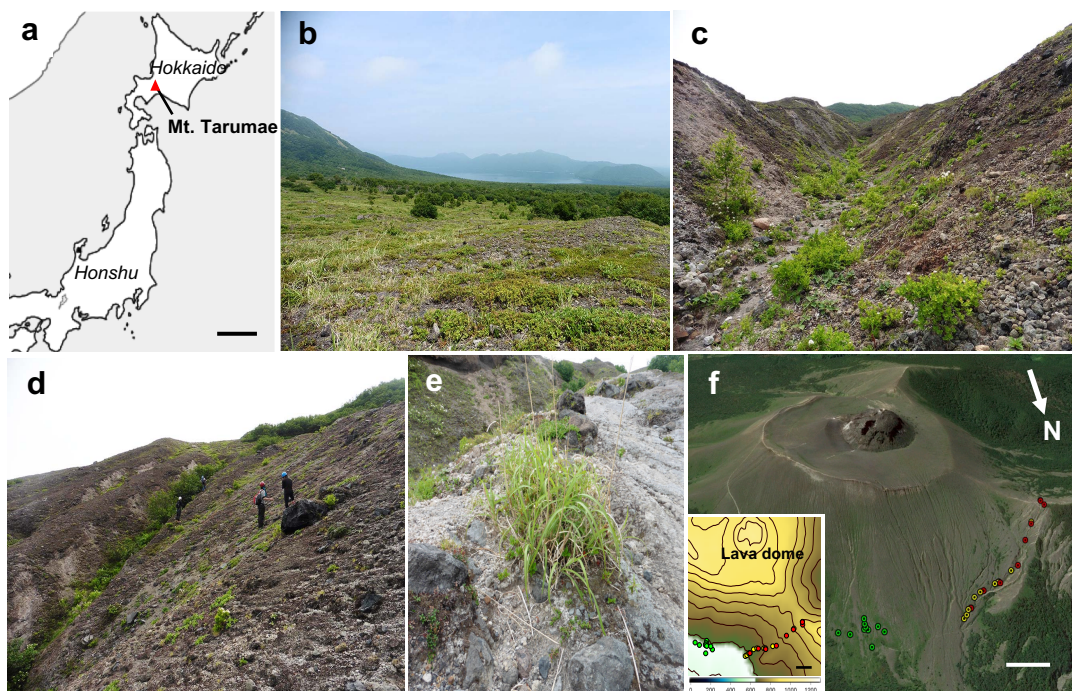


Fig. 1.1 a Location of Mt. Tarumae volcano in Japan. Scale, 200 km. Three habitat types in the mountain: b, grassland; c, gully; d, slope. e, *Miscanthus sinensis* grown on a slope. f Sampling points; green circles, grassland; yellow circles, gully; red circles, slope. Scales, 200 m. 3D- and 2D-maps were generated by Google Earth (<https://www.google.co.jp/intl/ja/earth/>) and GMT 5.4.5 (<http://gmt.soest.hawaii.edu/>), respectively, using the GPS data of sampling points (Supporting Information Table S1). The colors in the 2D map represent elevations.

1.3 Results

Assignment to operational taxonomic units of arbuscular mycorrhizal fungi

PCR products were successfully obtained from all samples, and sequencing of these products generated 70,000 – 170,000 paired-end reads that were merged into 48,000 – 130,000 sequences. Among them, 41 and 8% in average were assigned to the OTUs of AM fungi (i.e. glomeromycotinan fungi) (Tables S1.2 – S1.3) and those of non-glomeromycotinan fungi (data not shown), respectively. The rest of the reads could not be assigned to none of the OTUs in the database. Increases in AM fungal diversity by subsequent BLASTn searches of these unassigned sequences against the GenBank database were only marginally (data not shown) and were not reflected in the results. Rarefaction curves of AM fungal OTU richness were constructed based on both the numbers of sample and sequence read to assess the sampling effort status (Fig. S1.2). Both curves showed leveling off, suggesting that the number of sampling provided reasonable coverage of AM fungal diversity in the site.

Environmental factors that drive the communities

Environmental factors employed for community analysis are listed in Supplementary Information Table S1.4. Pairwise correlation analysis between these factors indicated that elevation was correlated with none of the plant and soil factors, whereas colony diameter of *M. sinensis* was positively correlated with NH₃-nitrogen. Exchangeable bases were generally correlated positively with each other and negatively with pH.

Among the three habitats, the colony diameter is larger in the grassland and gully habitats than in the slope habitat (Table S1.5). pH was lowest in the slopes and highest in the gullies, while soil phosphate, exchangeable bases, and NH₃-nitrogen were generally higher in the grassland and slopes than in the gullies.

Prior to the main analyses, the OTU composition data of the intact and disturbed communities obtained from the paired sample were combined, which resulted in 14 and 16 community data collected in 2015 and 2016, respectively, and the influence of sample collection year on the composition was assessed by PERMANOVA; no significant difference in the composition was observed between the years (Pseudo- $F=2.309$, $P = 0.061$), indicating that annual variation between the two sets was minimum. Accordingly, the data collected in the two years were combined in subsequent analyses.

Correlation analysis between AM fungal diversity and environmental factors indicated that OTU richness was decreased with increasing elevation and increased with increasing colony diameter of *M. sinensis* (Table 1.1). The diversity indices were also decreased with increasing elevation and increased with increasing soil pH. OTU richness was not significantly different among the three habitats, but the diversity indices were lower in the slope habitat than in the grassland and gully habitats (Fig. 1.2). Mantel test revealed that elevation ($r = 0.243$, $P = 0.007$) and soil phosphate ($r = 0.116$, $P = 0.042$) were the significant environmental factors that drive the communities (Supplementary Information Table S1.6). PERMANOVA indicated that the community compositions were significantly differentiated among the habitats (Pseudo- $F = 2.437$, $P = 0.01$). The fact that these factors drive not only the community compositions but also the diversity implies that changes in the composition were concurrent with changes in the diversity, which led to the idea that there is either nestedness or anti-nestedness

(e.g., species turnover) structure among the communities. To test this idea, I constructed a matrix (Supplementary Information Table S1.7) and performed nestedness analysis; significant nested structure was observed among the columns ($\text{NODF}_{\text{column}} = 73.69$, $Z = 11.70$, $P < 0.001$), as well as in the whole matrix ($\text{NODF}_{\text{total}} = 58.61$, $z = 8.72$, $P < 0.001$) (Fig. 1.3). This implies that species (OTU)-poor communities (i.e. those at higher elevations and in the slopes) were subsets of species (OTU)-rich communities (i.e. those at lower elevations and in the grassland and gullies). The matrix further suggested that the OTUs that occurred both in the intact and disturbed treatments, that is, disturbance-tolerant (or unresponsive) OTUs, are widely distributed across the ecosystem, which was tested subsequently.

Impact of soil disturbance

Bray-Curtis dissimilarity indices between the intact and disturbed communities from the paired samples were first calculated, and correlation analysis between the indices and the environmental/habitat factors was performed to assess the impact of disturbance on the compositions; elevation was the only factor that showed a significant negative correlation with the indices, that is, the higher the elevation, the smaller the impact (Table 1.2). The index was lower in the slopes than in the grassland and gullies (Fig. 1.4).

Lastly, individual OTUs were characterized in terms of responsiveness and robustness to soil disturbance with respect to their occurrence. The OTUs that occurred in fewer samples were more responsive to disturbance; they showed either a higher or lower responsiveness than those occurred more frequently (Fig. 1.5a). As expected, the

OTUs that occurred in more samples showed higher robustness, in which a logarithmic correlation between robustness and occurrence was observed ($r^2 = 0.671$, $P < 0.001$) (Fig. 1.5b), supporting the idea that AM fungal OTUs that are less responsive to (i.e. more robust against) soil disturbance are more widely distributed in the ecosystem.

Table 1.1. Correlations of arbuscular mycorrhizal fungal diversity in the rhizosphere of *Miscanthus sinensis* with environmental factors in Mt. Tarumae volcano.

Factor	OTU richness		Shannon-Wiener index	
	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>
Elevation	-0.457	0.010	-0.584	< 0.001
Colony diameter	0.384	0.035	0.320	0.084
pH	0.091	0.629	0.370	0.040
Truog-P	0.160	0.398	0.127	0.501
Ca	0.045	0.812	0.194	0.303
Mg	0.115	0.543	0.118	0.532
K	0.010	0.956	0.004	0.980
CEC	0.134	0.475	0.015	0.936
NH ₃	0.179	0.341	0.113	0.549
NO ₃	0.051	0.784	0.267	0.153
Total N	0.135	0.475	0.801	0.673

Table 1.2. Correlations of Bray-Curtis dissimilarity indices between the intact and disturbed communities of arbuscular mycorrhizal fungi with environmental factors in Mt. Tarumae volcano.

Factor	<i>r</i>	<i>P</i>
Elevation	-0.421	0.021
Colony diameter	0.277	0.137
pH	-0.058	0.758
Truog-P	0.079	0.677
Ca	-0.022	0.905
Mg	0.111	0.557
CEC	0.213	0.256
K	0.255	0.173
NH ₃	0.289	0.121
NO ₃	0.219	0.244
Total N	0.264	0.157

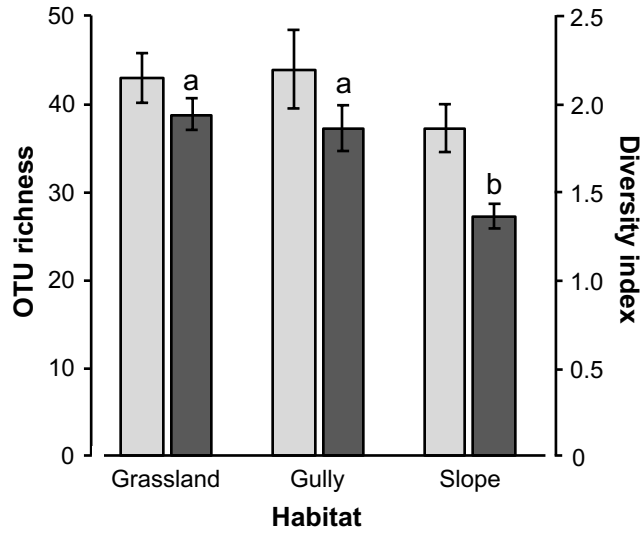


Fig. 1.2 OTU richness (light grey) and Shannon-Wiener diversity index (dark grey) of arbuscular mycorrhizal fungal communities in the rhizosphere of *Miscanthus sinensis* grown in grassland, gullies, and slopes in Mt. Tarumae volcano. The community composition data of the intact and disturbance treatments from the paired samples were combined before calculation. The values represent the mean $\pm$ SE ($n = 10$). Different letters indicate significant difference (Tukey HSD test, $P < 0.05$).

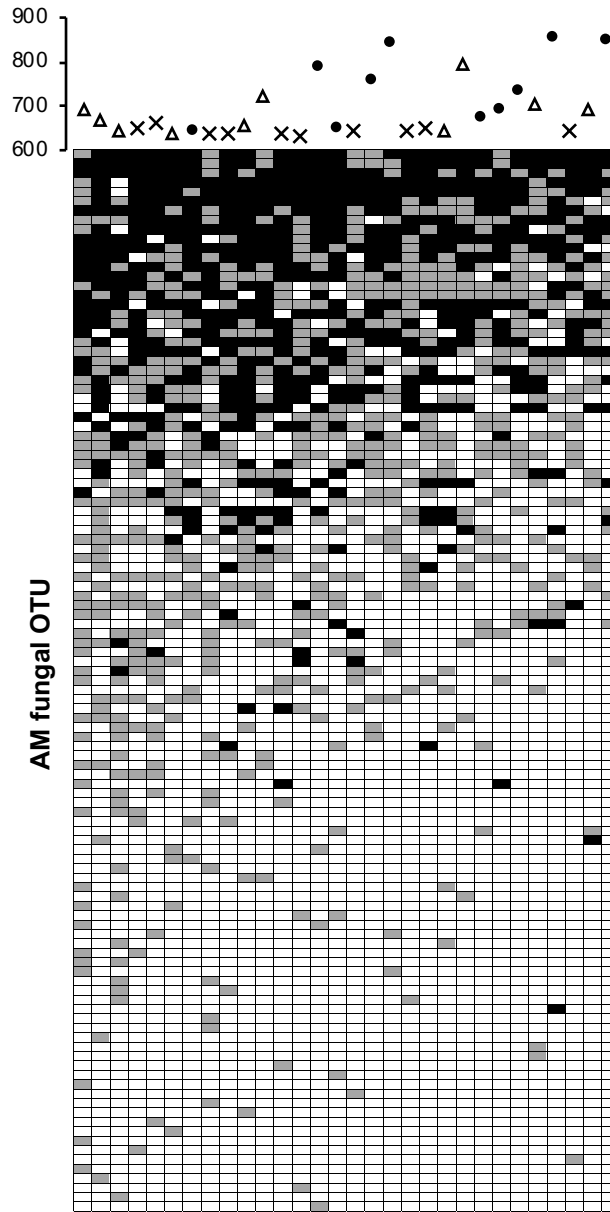


Fig. 1.3 Nestedness structure of arbuscular mycorrhizal fungal communities in the rhizosphere of *Micanthus sinensis* grown in Mt. Tarumae volcano. The community composition data of the intact and disturbance treatments from the paired samples were combined and converted to presence-absence data, and the columns (soil samples) and rows (AM fungal OTUs) were sorted by richness (number of OTU) and occurrence (number of soil sample), respectively. Grey and black cells indicate the OTUs that were detected either in the intact or disturbance treatment and those that were detected in both treatments, respectively.

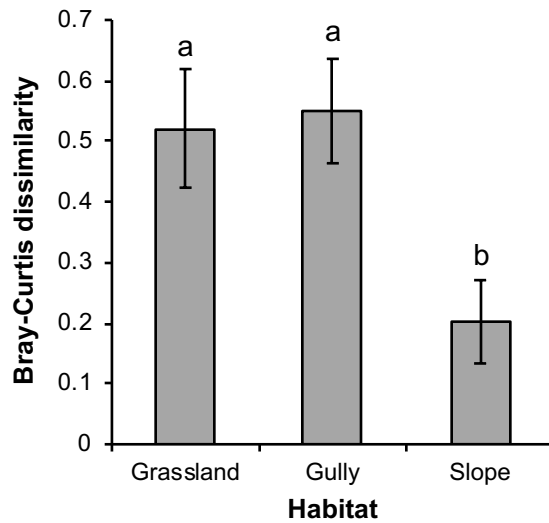


Fig. 1.4 Bray-Curtis dissimilarity indices between the intact and disturbed communities of arbuscular mycorrhizal fungi in the rhizosphere of *Miscanthus sinensis* grown in grassland, gullies, and slopes in Mt. Tarumae volcano. The index is employed as a measure of the impact of soil disturbance. The values represent the mean $\pm$ SE ($n = 10$). Different letters indicate significant difference (Tukey HSD test, $P < 0.05$).

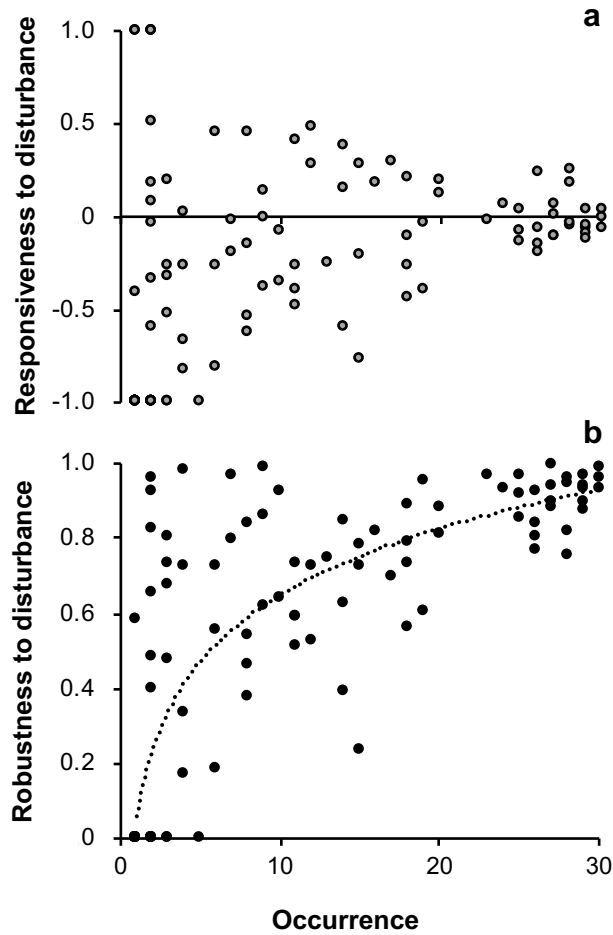


Fig. 1.5 Characterization of individual arbuscular mycorrhizal fungi in the rhizosphere of *Miscanthus sinensis* in Mt. Tarumae volcano with respect to responsiveness (a) and robustness (b) to soil disturbance. Responsiveness (RE) and robustness (RO) to disturbance represent the ratios of gained/lost sequence reads by the disturbance treatment to total read number; RE indicates positive and negative scores for the OTUs that gained and lost sequence reads, respectively, whereas RO indicates $[1 - |\text{RE}|]$. Occurrence is the number of paired samples in which the OTU occurred. RO showed a negative logarithmic correlation with occurrence: $r^2 = 0.671$, $P < 0.001$.

1.4 Discussion

The present study demonstrates, via applying artificial disturbance to trap culture, that soil disturbance is a major selection pressure for AM fungi associated with pioneer vegetation on the volcanic slope. The impact of disturbance on the community compositions was smaller in the communities in the slope at higher elevations, supporting the hypothesis that AM fungi that are tolerant to soil disturbance are selected near the crater. Furthermore, I also found that the communities in the slope at higher elevations (i.e. species-poor communities) are subsets of those in the grassland and gullies at lower elevations (i.e. species-rich communities); there is nested structure along a disturbance gradient in which the disturbance-tolerant fungi are distributed widely in the ecosystem, that is, they are generalists.

Nested structure in AM fungal community is also observed along soil-acidity gradients, in which acid-tolerant fungi are the generalists that are widely distributed across ecosystems (Kawahara *et al.*, 2016). It has been suggested, however, that fungi in extreme environments generally evolve towards specialists that tolerate or prefer the environments but are unable to compete in moderate environments (Gostinčar *et al.*, 2010). Therefore, one might expect, e.g., species turnover under strong selection pressure; in other words, disappearance of sensitive fungi and appearance of tolerant fungi. The nature of generalist/specialist fungi could be interpreted according to the basic principles of biological conservation; species with broad-niche width exhibit a high capacity to maintain fitness across harsh environments, while species with narrow-niche width experience with a higher risk of extinction under stressed conditions (Evans *et al.*, 2005; Boulangeat *et al.*, 2012). Given that the significance of AM fungal

association in the establishment of vegetation in harsh environments (Liu *et al.*, 2011; Gai *et al.*, 2012; Pan *et al.*, 2013) I propose that community nestedness in AM fungal community in volcanic ecosystems has significant implications for resilience of vegetation after disturbance, e.g., by eruption and erosion; a broad distribution of disturbance-tolerant fungi enables rapid mycorrhization of plants, which secures rapid regeneration of vegetation.

Elevation represents both physical and climatic factors and is an ecologically important driver for animal, plant, and microbial communities (Körner, 2007), including soil fungal communities (Pellissier *et al.*, 2014) and AM fungal communities (Li *et al.*, 2014; Gelm, 2017; Shi *et al.*, 2014). However, difference in elevation between the forest limit (630 m a.s.l.) and the vegetation limit (850 m a.s.l.) in Mt. Tarumae, where I collected the samples, is only 220 m. This suggests that the influence of climatic factors on the development of vegetation gradient, as well as on the differentiation of the habitats, would be minimum, and rather, physical factors such as soil disturbance and water availability may drive the vegetation distribution and the habitat differentiation in the ecosystem. Inclination angles could be an important factor for soil stability in volcanic ecosystems, e.g., greater angles have higher potential for surface runoff that is the main driver for soil disturbance (i.e. erosion) (El Kateb *et al.*, 2013). In Mt. Tarumae this physical (i.e. microgeographic) factor would be one primary driver for the differentiations of the habitats and thus fungal communities.

The impact of soil disturbance on AM fungal community has been demonstrated in various ecosystems, e.g., coastal sand dune (Kawahara & Ezawa, 2013), grassland (Lekberg *et al.*, 2012; García de León *et al.*, 2016), arable land (Boulangeat *et al.*, 2012; Yang *et al.*, 2016), and shrubland/forest (García de León *et al.*, 2018). Generally,

microorganisms are sensitive to disturbance and not readily recoverable (Allison & Martiny, 2008; Yang *et al.*, 2017). In AM fungi, those that require a longer period to occur are less competitive or become extinct after soil disturbance (Schnoor *et al.*, 2011), which were designated as disturbance-sensitive fungi in the present study. On the other hand, in a global study it was found that anthropogenic disturbance did not always decreased AM fungal diversity; for example, at sites where natural diversity was low, disturbance increased the diversity (García de León *et al.*, 2018). In a coastal dune ecosystem where zonal distribution of vegetation between the seaward (frequently disturbed) and landward (stable) slopes has developed, soil disturbance increased AM fungal richness in the landward soil, suggesting that potential richness in the soil is high due to the immigration of AM fungal propagule from the other side of the slope (Kawahara & Ezawa, 2013). In fact, a couple of OTUs occurred newly after disturbance in the present study, although they were rather rare OTUs that occurred in a limited number of samples (Fig. 1.55a). These observations suggest that not only fungal sensitivity/tolerance to disturbance but also potential richness/diversity should be taken into account to assess the impact of disturbance on AM fungal community. In this context, one interpretation for the larger shifts in the community compositions after disturbance in the grassland and gullies is that potential richness/diversity in the soils were higher due to the immigration of propagule from neighboring habitats.

Absolute and/or relative abundance of the propagule may also affect the responsiveness of the fungi to disturbance. Soil disturbance did not decrease mycorrhizal colonization in a pasture soil in which propagule density of the fungi was high, but halved colonization in the soils collected from forest and heathland in which the densities were low (Jasper *et al.*, 1991). The negative impact of soil disturbance on

mycorrhizal colonization could be reduced with increasing the propagule density of the fungi (McGonigle & Miller, 2000). These observations suggest that resilience (i.e. rate of recovery) of the fungi after disturbance depends on propagule density. It is likely, therefore that the robustness of generalist fungi to soil disturbance observed in the present study could be interpreted not only by their tolerance to mechanical disturbance but also by the abundance of propagule in the ecosystem. In addition, in the slope habitat vegetation is only patchily distributed; in other words, individual plants are isolated from each other (Fig. 1.1), which would limit the migration of propagule among the patches. Accordingly, in the slope habitat potential richness/diversity is likely to be low, and this might be one factor for the robustness of the communities to disturbance in the habitat.

CHAPTER 2

Periodic disturbance as selection pressure for AM fungi

2.1 Introduction

It has been demonstrated that the AM fungi play a significant role in the establishment of primary vegetation; e.g., *M. sinensis* and the legume pioneer *Lespedeza cyrtobotrya* are highly dependent on AM associations for their establishment in acidic soil (Maki *et al.*, 2008). AM fungi are sensitive to various disturbance, but the impact differs among species and is context-dependent (i.e. differs among disturbance types) (van der Heyde *et al.*, 2017). Soil disturbance reduced AM fungal diversity in grasslands (Schnoor *et al.*, 2011; Brundrett & Ashwath, 2013) and altered the community composition (Rosendahl & Stukenbrock, 2004; Jansa *et al.*, 2006). Whereas soil disturbance increased AM fungal diversity in a dry forest (Guadarrama *et al.*, 2014) and had a little impact on the community in a grassland in which disturbance-tolerant AM fungi dominate (Lekberg *et al.*, 2012). Our previous study demonstrated that in a volcanic slope the fungi that inhabit nearer to the crater are more tolerant to soil disturbance and generalists that are distributed across the ecosystem. These observations suggest that the community members are selected along a disturbance gradient on the slope, although there is little evidence that soil disturbance acts as selection pressure for the fungi in the ecosystem.

In this study, I designed a one-year-greenhouse experiment in which the impact of periodic soil disturbance on the fungal community was evaluated, addressing the

hypothesis that frequent disturbance differentiates AM fungi community in comparison with that grown without disturbance. To test this hypothesis, rhizosphere soils of *M. sinensis* grown in a volcanic slope were collected as an AM fungal inoculum and subjected to soil trap culture with *M. sinensis* seedlings.

2.2 Materials and methods

Sampling site

Mt. Tarumae volcano is an active volcano located in the southeastern rim of Shikotsu caldera (42°41'26"N/141°22'36"E) in Shikotsu-Toya National Park, Hokkaido, Japan. The height is 1,041 m a.s.l., and there is a lava dome (120 m in height) in the crater. The volcano occurred approximately 9,000 years ago, and the largest explosive eruptions that affected Northern Hemisphere temperature were recorded in 1667 and 1739 (Briffa *et al.*, 1998). The latest eruption with ash falls and mudflows occurred during 1978 – 1981, and in 2001 and 2003 temperature of the crater reached over 600°C, during which sand and mud were emitted from the crater (Furukawa & Nakagawa, 2010). Three different habitats, grassland, gullies, and slopes are differentiated from the boarder of forest limit (630 m a.s.l.) to the crater. The gullies and slopes are not fully covered with vegetation, while the open and flat grassland distributed between 630 – 660 m a.s.l. is covered with rich vegetation. In the grassland *M. sinensis* and the shrub *Ledum palustre* subsp. *diversipilosum* var. *diversipilosum* largely dominate, and *M. sinensis* grown in this habitat generally develop a large clump of multiple stems (50 – 150 cm in diam). In addition to the two dominant species, *Spiraea betulifolia* var. *betulifolia* and *Weigela middendorffiana* are also patchily distributed in the grassland.

Since the previous study revealed that AM fungal diversity was highest in the grassland habitat, AM fungal inocula for this study were collected from this habitat. Three rhizosphere soil samples, including root fragments, were collected from each of four *M. sinensis* plants grown in the grassland using a stainless steel core sampler (5 × 5 cm, 100 mL in vol) in August 2016 (Supplementary Table S1). In addition, 10 kg of

bulk soil was also collected at which no vegetation was present (800 m a.s.l.) as a base medium for trap culture.

Disturbance treatment in soil trap culture

One of the three soil-core samples collected from one individual plant was sieved on a 2-mm mesh to destruct hyphal networks of the fungi and designated as a disturbed inoculum, whereas the second one was carefully taken from the core sampler and used without sieving as an intact inoculum ($n = 4$). Either of the intact or disturbed inoculum (*ca.* 100 mL) was transferred to 350-mL plastic pots that were filled with 200 mL of the base medium (i.e. autoclaved bulk soil). Seeds of *M. sinensis* (Kaisessa, Otofuke, Japan) were sown onto the soil surface, covered with a thin layer of autoclaved river sand, grown only with tap water in a temperature/light/humidity-controlled greenhouse (day/night temperature 26/20°C; 14-h day length; 60% relative humidity), and thinned to 10 plants per pot two weeks after sowing. The soils in the disturbance treatment were sieved on the 2-mm mesh to destruct hyphal networks one month after sowing and put back to the pot with the seedlings. The plants were grown for 12 months in total, during which this disturbance treatment was repeated every month. A small part of the roots was collected every 2 months using a small stainless steel core sampler (1.5×10 cm), washed with tap water, blotted on a paper towel, frozen at -80°C more than a day, freeze-dried for 2 days, and stored at -30°C for DNA extraction (6 time points). The small whole created by the sampling was filled with autoclaved river sand. Root fragments in the third soil-core sample were collected, washed, frozen, freeze-dried, and stored for DNA extraction to analyze compositions of initial (zero time) community. In

summary, 52 samples comprised of the zero-time samples and those collected at the 6 time points of each of the two treatments ($n = 4$) were subjected to molecular analysis.

Molecular identification

The freeze-dried root sample was transferred to a 2.5-mL tube with an O-ring sealed cap (Yasui Kikai, Osaka, Japan) and ground using Multi-Beads Shocker (Yasui Kikai, Osaka, Japan) with a metal cone at 2,500 rpm for 2×60 s, and DNA was extracted with DNeasy Plant Mini Kit (Qiagen, Tokyo, Japan) according to the manufacturer's instructions. The divergent domain 2 of large-subunit ribosomal RNA gene (LSU rDNA) was amplified with $0.5 \text{ nmol } \mu\text{L}^{-1}$ each of the fungi-specific primers, FLd3 (forward) and FLR2 (reverse) to which TruSeq-type forward- and reverse-adaptor sequences (illumina, Tokyo, Japan), respectively, were linked at the 5'-end (Niwa *et al.*, 2018), and $1 \text{ } \mu\text{L}$ template DNA. In a total amount of $25\text{-}\mu\text{L}$ reaction mixture of Expand High-Fidelity PLUS PCR System (Roche Diagnostics, Tokyo, Japan) was amplified using C1000 Touch™ Thermal Cycler (BIO-RAD, Tokyo, Japan) 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, and polymerization at 72°C for 1 min, and final elongation at 72°C for 10 min. The PCR products were purified with Agencourt AMPure XP SPRI magnetic beads (Beckman Coulter, Tokyo, Japan) and sequenced on the illumina MiSeq platform (2×300 bp) at Bioengineering Lab (Atsugi, Kanagawa, Japan). The nucleotides with a quality value (QV) < 20 in the 3' end and the adapter-index sequence in the 5' end were trimmed, and those shorter than 40 bp were excluded. After the quality filtering, the paired-end reads (read 1 and read 2) were merged with a

minimum overlap length of 10 nt using FLASH (<http://ccb.jhu.edu/software/FLASH/>). The merged sequence reads were subjected to BLASTn searches against the fungal LSU rDNA database consisted of 82,494 operational taxonomic units (OTUs) of fungi, including 412 OTUs of AM fungi, and assigned to the OTUs at a criterion of $\geq 95\%$ similarities over 330 bp with an E value $\leq 10^{-100}$, which was executed in the open web interface “Arbuscular mycorrhizal fungi classification pipeline” (<http://amfungi.kazusa.or.jp>) (Niwa *et al.*, 2018).

Statistical analysis

All statistical analyses were performed with R 3.2.3 (R Core Team, 2013). Total sequence reads assigned to AM fungal OTUs were standardized to 15,000 reads per sample by resampling for community analysis. Bray-Curtis dissimilarity indices (read-abundance data based) were calculated to construct distance matrices of the AM fungal community using the `vegdist` command in `vegan` (Oksanen *et al.*, 2013) and used for community dissimilarity analysis. Permutational multivariate analysis of variance (PERMANOVA) (9,999 permutations) was carried out to assess the impact of the time period and disturbance treatment in which Bray-Curtis index was used using the `Adonis` command in `vegan` package.

2.3 Results

Assignment to operational taxonomic units

PCR products were successfully obtained from all samples, and high-quality paired-end reads were merged into 15,000 – 45,000 sequences per sample. Among them, 75 and 15% in average were assigned to the OTUs of AM fungi (i.e. glomeromycotinan fungi) and those of non-glomeromycotinan fungi (data not shown), respectively, and the rest of the reads could not be assigned to none of the OTUs in the database. BLASTn searches of the unassigned reads against the GenBank database increased AM fungal diversity only slightly (data not shown), and thus the results were not included in subsequent analysis. In total, 105 AM fungal OTUs were detected, in which 57, 13, 11, 6, 6, 4, 3, 2, 1, 1, 1 OTUs were assigned to the genera/taxa *Rhizophagus* (Rhz), *Scutellospora* (Scu), uncultured *Glomus* (UnG), *Funneliformis* (Fun), *Acaulospora* (Aca), *Claroideoglomus* (Cla), *Paraglomus* (Par), *Ambispora* (Amb), *Glomus* (Glo), *Gigaspora* (Gig), and *Archaeospora* (Arc), respectively (Supplementary Table S2.2 and S2.3). Rarefaction curves of AM fungal OTU richness were constructed based on both the numbers of sample and sequence read to assess the sampling effort status (Fig. S2.1). Both curves showed leveling off, suggesting that our sampling provided reasonable coverage of AM fungal diversity in the site.

It has been demonstrated that different rDNA sequences coexist in a single nucleus of Glomeromycotinan species (Lin *et al.*, 2014), raising possibility that sequence reads that were assigned to different OTUs are not necessarily originated from different

individuals. Accordingly, we conducted pairwise comparison of the read abundances between OTUs within the individual genera/taxa over 52 samples for searching the OTUs that potentially belong to the same individuals. We found four groups in which the read abundances of two to three OTUs were highly correlated ($r > 0.9$, $P < 0.001$): Rhz055 and 059; Rhz041 and 057; Aca195, 361, and 327; UnG067, 068, and 070 (Table 2.1). Phylogenetic distance of these OTUs within the individual groups was also visually assessed on the phylogenetic tree constructed with all OTUs in the database (Niwa *et al.*, 2018), confirming that the OTUs of which the read abundance were correlated were also phylogenetically closely related. I therefore combined these OTUs, which reduced the total number of OTU from 105 to 99, and treated as single taxonomic units in subsequent analysis.

Community succession during the culture period

AM fungal OTU richness was decreased during the culture period both in the disturbance and intact treatments (Fig. 2.1). Community differentiation during the period was expressed by Bray-Curtis dissimilarity indices between the communities at time zero and those at each time point. In both treatments the community compositions were highly differentiated in the first four months, converged to those observed at time zero at the sixth and eighth months, and then redifferentiated thereafter (Fig. 2.2), implying that community differentiation and conversion repeated periodically within a year.

Impact of periodic disturbance

To assess the impact of periodic disturbance along the culture period, two-way PERMANOVA was performed. Culture period had a significant impact on the community compositions, but periodic disturbance did not (Table 2.2). Interactions between the two factors, however, were significant, implying that the impact of periodic disturbance did appear in some time points, but not always. Accordingly, the impact of disturbance was assessed at each time point; Bray-Curtis dissimilarity indices were calculated between the intact and disturbed communities originated from the paired samples. The compositions were highly differentiated after two months between the treatments, converged in the fourth month, and then redifferentiated after the eighth month (Fig. 2.3). Dynamics of the three taxonomic units Aca195/327/361, Rhz055/059, and Rhz002 that showed distinct patterns during the culture period were analyzed in detail. Aca195/327/361 that belongs to the genus *Acaulospora* that were the most abundant genus throughout the culture period increased in the first two months and maintained its abundance by the end of culture period in both treatments (Fig. 2.4a). The abundance of Rhz055/059 decreased consistently and almost disappeared after six months in both treatments, but in the intact treatment the abundance was recovered to the initial level in the twelfth month (Fig. 2.4b). Rhz002 also decreased by the sixth month, increased a little in the eighth month, and then decreased again in both treatments (Fig. 2.4c).

Table 2.1. Pairwise comparison of the read abundance between OTUs.

Factor	<i>r</i>	<i>P</i>
Rhz055/059	0.921	<0.001
Rhz041/057	0.979	<0.001
Aca195/327	0.959	<0.001
Aca195/361	0.901	<0.001
Aca327/361	0.948	<0.001
UnG067/68	0.921	<0.001
UnG067/070	0.988	<0.001
UnG068/070	0.935	<0.001

Table 2.1. Assessment of the impact of periodic disturbance and culture period on AM fungal community compositions by PERMANOVA^a using Bray-Curtis index as a measure of dissimilarity.

Factor	Pseudo- <i>F</i>	R ²	<i>P</i>
Periodic disturbance (D)	1.147	0.006	0.308
Culture period (P)	22.288	0.705	<0.001
D*P	2.156	0.068	0.020

^a 9999 permutations

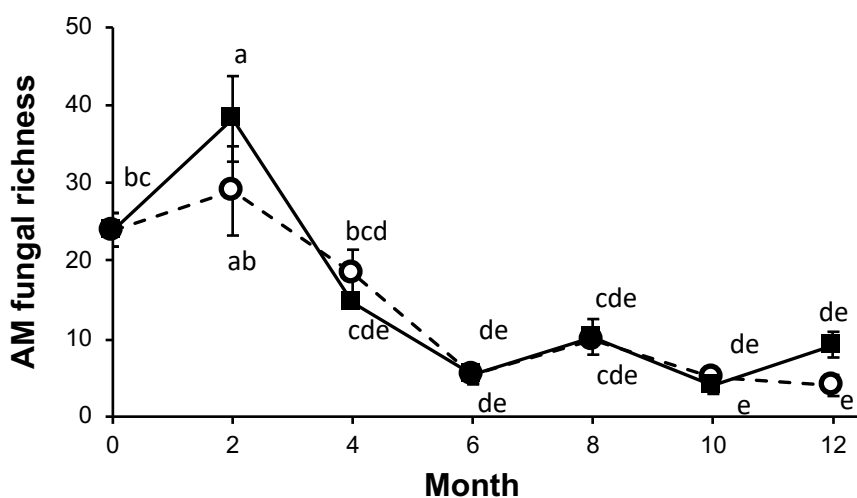


Fig. 2.1 Time course changes in AM fungal richness in the intact (solid line) and disturbed (dotted line) communities. The values represent the mean $\pm$ SE ($n = 4$). The different letters indicate significant differences among the culture periods (Tukey HSD test, $p < 0.05$).

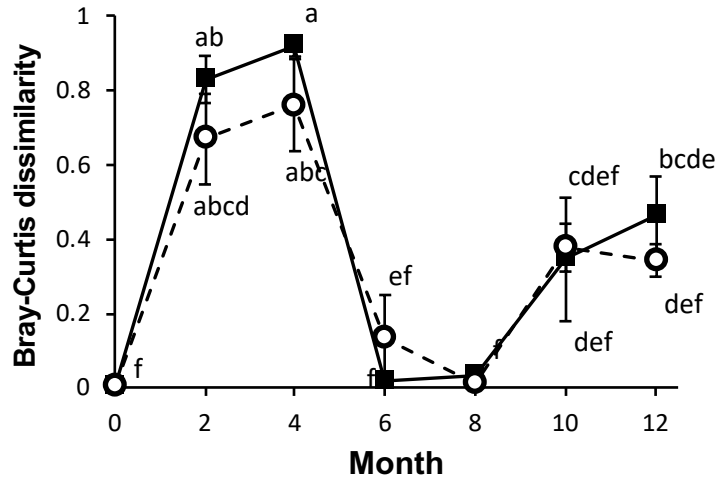


Fig. 2.2 Community differentiation during the culture period within the intact treatment (solid line) and within the disturbance treatment (dotted line). Bray-Curtis dissimilarity indices between the communities at time zero and those at each time point. The values represent the mean $\pm$ SE ($n = 4$). The different letters indicate significant differences among the culture periods (Tukey HSD test, $p < 0.05$).

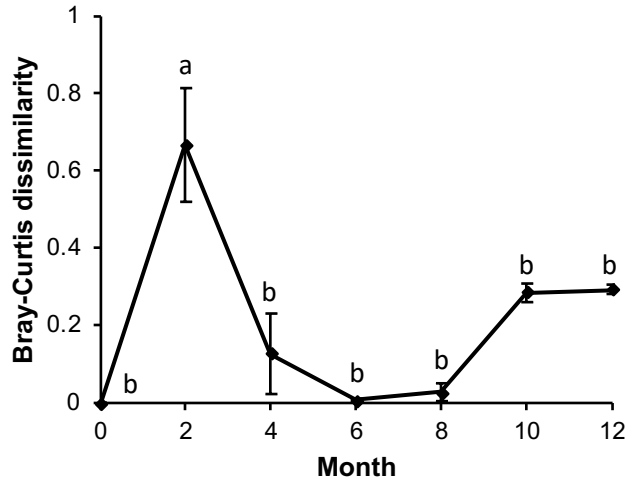


Fig. 2.3 Time course assessment of the impact of disturbance on the community compositions. Bray-Curtis dissimilarity indices between the intact and disturbed communities were calculated at each time. The values represent the mean $\pm$ SE ($n = 4$). The different letters indicate significant differences among the culture periods (Tukey HSD test, $p < 0.05$).

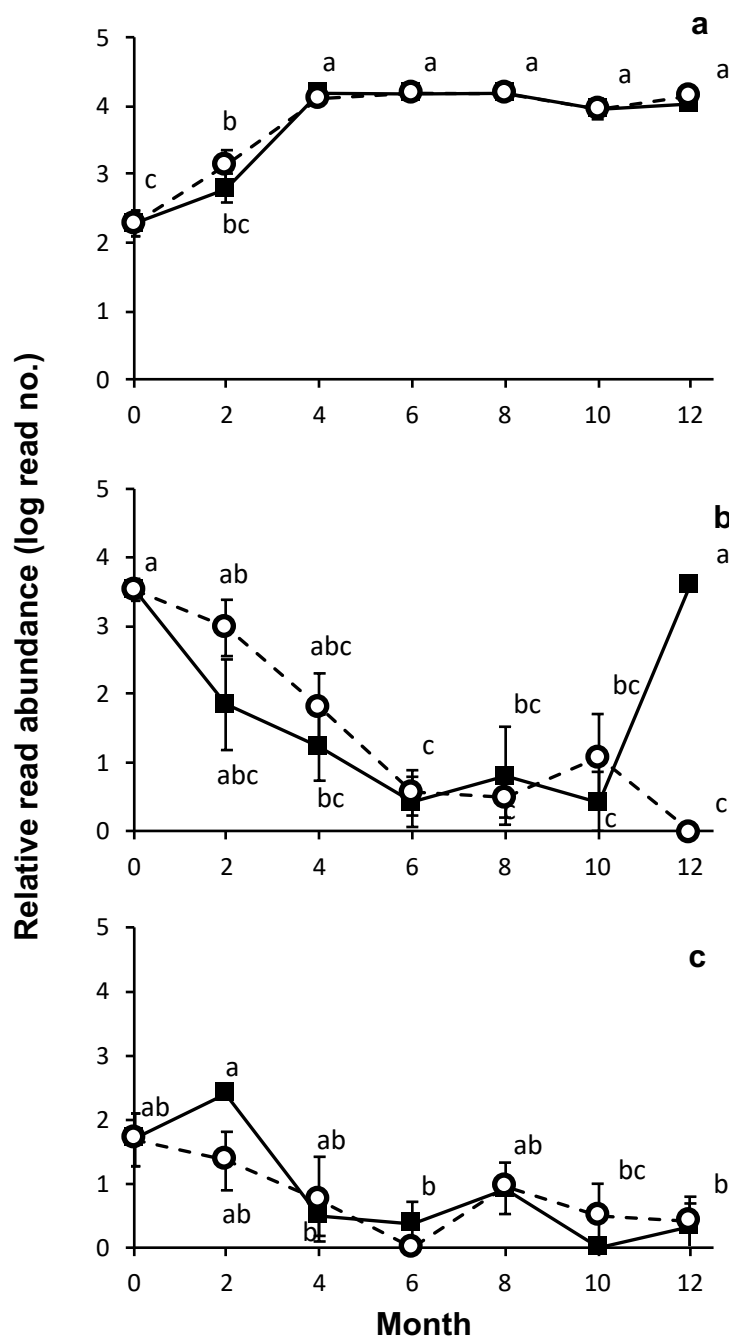


Fig. 2.4 Dynamics of the three major taxonomic units, (a) Aca195/327/361, (b) Rhz055/059, and (c) Rhz002 that showed distinct patterns during the culture period between the intact (solid line) and disturbed (dotted line) communities. The values represent the mean $\pm$ SE ($n = 4$). The different letters indicate significant differences among the culture periods (Tukey HSD test, $p < 0.05$).

2.4 Discussion

In the present study, consistent decreases in AM fungal richness was observed during the culture period in both treatments, suggesting that not only soil disturbance but also soil stability act as selection pressure for the fungi. We further observed that community divergence and convergence occurred periodically both within and between the treatments, reflecting the dynamic feature of AM fungal community.

Decreases in AM fungal richness by soil disturbance have been observed in various ecosystems (Brundrett & Ashwath, 2013; Schnoor *et al.*, 2011). AM fungi that are sensitive to soil disturbance may require a longer period to colonize and thus are less competitive than those that can produce abundant spore and thus regenerate rapidly (Chagnon *et al.*, 2013; Schnoor *et al.*, 2011). Consistent decreases in richness during the culture period, however, were also observed in the intact treatment in the present study, indicating that not only soil disturbance but also soil stability acts as selection pressure for the fungi. According to the C-S-R (competitor, stress tolerator, and ruderal) framework for the life-history strategy of AM fungi (Chagnon *et al.*, 2013), it is likely that the fungi that dominate under stable conditions are the competitors that produce fewer spores and proliferate mainly via hyphal networks in the soil. However, the dominance of *Acaulospora* sp. (Aca195/327/361) both in the disturbance and intact treatments suggests that the traits of the fungi selected under stable conditions are not necessarily those of the typical competitors. In other words, the ruderal-type fungi that produce abundant spores and colonize rapidly could also be competitive both under the disturbed and stable conditions. Periodic divergence and convergence of community composition were observed, that is, succession occurred within a year irrespective of the

present or absence of disturbance treatments. Succession in AM fungal community has been observed in various ecosystems (Caruso *et al.*, 2012), e.g., agricultural ecosystems (Bainard *et al.*, 2014; Chen *et al.*, 2016; Gao *et al.*, 2018; Higo *et al.*, 2015), a forest ecosystem (Voříšková *et al.*, 2014), and energy crop plantation (Barnes *et al.*, 2016). Driving force of succession is likely to be the difference in the life-history strategy of the fungi. For example, in early stages rapid colonizers dominate but disappear in the later stages due to their shorter life spans, after which slow colonizers appear. According to root growth, however, new niches may consistently be generated, which other strategists could also colonize.

Community differentiation by disturbance (i.e. the impact of disturbance) was observed in the early (2nd month) and later (10th - 12th month) stages. Interestingly, these differentiations were concurrent with community divergence, suggesting that selection by disturbance occurs in certain stages of succession. Community convergence between the intact and disturbance treatments, however, was observed from 4th to 8th month during which *Acaulospora* sp. (Aca195/327/361) dominated in both treatments. These results suggest that the species is not only tolerant to disturbance but also competitive even under the stable conditions. The impact of disturbance observed in the later stages were rather smaller than that observed in the early stage. This might be due to the lower OTU richness that would restrict large shifts in the compositions. Several taxonomic groups showed distinct responses to soil disturbance. One of *Rhizophagus* sp. (Rh055/059) gradually declined and disappeared in the disturbance treatment, but reappeared in the intact treatment, whereas the abundance of *Acaulospora* sp. (Aca195/327/361) increased by the 4th month and maintained by the end of experiment in both treatments. Differential responses to soil disturbance among

AM fungal species has been reported frequently, and those that belong to the family Acaulosporaceae are likely to be more tolerant to mechanical disturbance (van der Heyde *et al.*, 2017); loss of biomass by disturbance may be smaller in the species, probably due to their smaller biomass of extraradical mycelium, compares to that of the other fungi (Hart & Reader, 2002). Interestingly, increases in *Acaulospora* spp. at higher elevations in an alpine ecosystem (Li *et al.*, 2018), and several novel species in the genus *Acaulospora* were found exclusively in extremely high elevations (Palenzuela *et al.*, 2013; Oehl *et al.*, 2014; Li *et al.*, 2015). Although the present study demonstrated that one distinct trait of *Acaulospora* spp. is disturbance tolerance, the high adaptability of *Acaulospora* spp. to montane/alpine ecosystems needs to be studied in the future.

General discussion

In the present study, AM fungal community in a volcanic ecosystem was characterized with respect to soil disturbance. There is nested structure along the elevation gradient, in which the generalist fungi that are robust against disturbance were enriched near the crater. Traits involved in disturbance tolerance are of interest. They may produce abundant spore, which enables rapid recolonization in the roots, and/or be capable of curing hyphal networks rapidly after disturbance; these traits are of the "ruderal" fungi (Chagnon *et al.*, 2013). The fact that the disturbance-tolerant fungi are generalists raises a new question how they maintain competitiveness under non-stressed conditions in which cost for the tolerant traits would have a negative impact on their competitiveness. One possibility is that generalist fungi could minimize the costs for tolerant traits in moderate environments (Kawahara *et al.*, 2016), e.g., through rapid changes in the frequency of nucleotypes (Angelard *et al.*, 2014). Isolation and characterization of the disturbance-tolerant/sensitive fungi are necessary to examine this idea and, further, to understand the role of these fungi in the establishment and resilience of vegetation in either naturally or anthropogenically disturbed environments.

In Chapter 2, I demonstrated that soil disturbance acts as selection pressure for the fungi, which strengthens the observations that AM fungi that are more tolerant to disturbance inhabit at higher elevations (i.e. nearer to the crater) in Chapter 1. The dominance of *Acaulospora* sp. (Aca195/327/361) was also observed in Chapter 1, confirming that this species well adapts to the volcanic ecosystem. Isolation and further characterization of the fungus are a key to understand the role of the fungus in the establishment and resilience after disturbance.

Summary

- Slopes of active volcanoes are a harsh habitat for plants; soil disturbance due to ash falling and erosion occurs frequently, and thus vegetation is generally poor, particularly near the crater (i.e. at higher elevations). Arbuscular mycorrhizal fungi play a significant role in the establishment and resilience of pioneer vegetation in harsh environments. In this study, AM fungal communities associated with *Miscanthus sinensis*, a pioneer grass species, in a volcanic slope of Mt. Tarumae was characterized with respect to soil disturbance.
- In Chapter 1 I hypothesized that the fungi that are more tolerant to soil disturbance are selected at higher elevations based on the idea that levels of disturbance is are more severe at higher elevations. Paired-soil-core samples were collected from 10 individuals of *M. sinensis* grown in each of the three types of habitats grassland, gully, and slope between the vegetation limit and forest limit on a volcanic slope of Mt. Tarumae and used for trap culture with *M. sinensis* seedlings. One of the paired samples was sieved to destruct hyphal networks (disturbance treatment), while the other was not (intact treatment). After two months, DNA was extracted from the roots, and fungal rDNA was amplified and sequenced to determine community compositions. AM fungal diversity was decreased with increasing elevation, where nested structure was observed. Compositional dissimilarity between the disturbed and intact communities was decreased with increasing elevation, suggesting that communities at higher elevations were more robust against soil disturbance. These observations suggest that AM fungi that are more tolerant to soil

disturbance are more widely distributed across the ecosystem, that is, they are generalists. The wide distribution of disturbance-tolerant fungi may have significant implications for rapid resilience of vegetation after disturbance in the ecosystem.

- In Chapter 2 the impact of periodic soil disturbance on the fungal community was evaluated in a one-year-pot experiment. Three soil-core samples containing rhizosphere soil and roots were collected from each of four *M. sinensis* plants grown in a grassland above the boarder of forest limit in Mt. Tarumae. DNA was extracted from the roots in one of the three samples. The second sample was sieved on a 2-mm mesh to destruct hyphal networks of the fungi (disturbance treatment), while the third one was not (intact treatment). *M. sinensis* was grown in these soils in a greenhouse for 12 months, during which the soils in the disturbance treatment were sieved once a month. A small part of roots was collected every two months for DNA extraction. Fungal rDNA was amplified from the DNA and sequenced. AM fungal richness was consistently decreased during the culture period in both treatments. Community compositions diverged and converged periodically between the disturbance and intact treatment, in which different taxa responded differently. For example, one of *Rhizophagus* sp. disappeared after several months in both treatments, but reappeared only in the intact treatment in later stages. Whereas the most abundant taxon that belongs to the genus *Acaulospora* was unresponsive to disturbance, gradually increased and dominated in both treatments. These observations suggest that not only soil disturbance but also soil stability act as selection pressure for the fungi. The results indicated that community succession (i.e. periodic divergence and

convergence of the composition) occurred within a year, and the impact of disturbance on the community is larger during the time of community divergence.

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Table S1.1 Geographic data and chemical properties of soil samples collected from rhizosphere of *Miscanthus sinensis* grown in Mt. Tarumae volcano.

Sample ID	Habitat	Elevation (m)	Colony diameter (cm)	pH	Truog-P (mg kg ⁻¹)	ex. Ca (mg kg ⁻¹)	ex.Mg (mg kg ⁻¹)	ex.K (mg kg ⁻¹)	CEC (me kg ⁻¹)	NH ₃ – N (mg kg ⁻¹)	NO ₃ – N (mg kg ⁻¹)	Total N (%)
1	Grassland	660	105	4.71	67.10	467.50	107.40	186.60	381.50	98.00	2.25	0.56
2	Grassland	644	60	4.73	32.40	111.60	22.90	74.90	120.40	18.76	0.56	0.19
3	Grassland	630	90	4.79	36.30	121.40	24.70	91.80	158.50	20.09	0.50	0.27
4	Grassland	646	50	4.96	39.20	70.60	7.70	26.20	25.00	13.14	0.46	0.07
5	Grassland	650	130	5.05	25.10	78.50	9.50	34.30	24.80	14.95	0.48	0.07
6	Gully	645	30	5.29	52.00	53.30	2.80	9.30	4.10	13.45	0.54	0.02
7	Slope	648	6	4.48	38.70	495.90	91.60	86.40	209.40	23.17	0.48	0.25
8	Gully	693	65	5.31	57.70	51.30	4.80	14.40	5.80	6.03	0.42	0.02
9	Slope	697	50	4.82	34.10	55.10	8.30	33.90	31.90	9.19	0.51	0.08
10	Slope	762	12	4.44	33.00	290.90	49.30	96.60	148.60	14.48	0.44	0.17
11	Slope	790	54	4.98	44.50	142.90	14.20	32.00	22.20	10.25	0.39	0.05
12	Gully	796	8	4.94	59.60	107.70	8.30	26.50	15.30	11.59	0.44	0.05
13	Slope	848	40	4.43	41.10	348.70	47.70	77.10	86.60	9.93	0.41	0.12
14	Slope	855	30	4.63	30.50	133.10	13.40	33.50	50.90	9.53	0.41	0.13
15	Grassland	639	20	5.37	39.00	80.40	6.20	23.10	17.60	5.77	0.48	0.06
16	Grassland	644	10	5.21	31.40	88.30	7.00	28.90	20.90	4.87	0.43	0.07
17	Grassland	647	18	4.96	23.50	282.90	43.90	116.10	122.30	9.51	0.69	0.17
18	Grassland	640	14	5.32	28.80	137.00	11.60	51.70	49.00	8.12	0.45	0.12
19	Grassland	638	60	5.11	59.90	117.50	5.30	24.10	57.70	10.63	0.46	0.17

Table S1.1. Continued.

20	Gully	639	90	5.14	43.10	92.10	5.00	17.00	10.00	3.32	0.45	0.041
21	Gully	642	45	5.39	41.80	152.70	15.30	30.00	14.80	4.03	0.40	0.037
22	Slope	650	40	4.44	28.50	386.80	66.70	83.70	287.60	20.37	0.42	0.436
23	Gully	658	12	5.07	51.40	156.60	9.80	31.20	20.90	4.54	0.58	0.05
24	Gully	670	60	5.3	36.80	146.80	10.90	25.30	18.90	3.48	0.44	0.035
25	Slope	675	30	5.31	52.10	101.90	3.00	17.40	7.30	3.46	0.90	0.024
26	Gully	692	55	5.35	53.70	121.40	6.10	15.50	6.10	3.12	2.37	0.026
27	Slope	735	14	5.24	47.20	105.70	6.20	24.60	18.30	4.30	0.46	0.061
28	Gully	722	25	5.28	65.50	156.60	7.70	19.20	11.50	9.32	1.59	0.031
29	Slope	852	23	5.25	39.20	225.50	29.00	84.90	60.70	21.12	0.51	0.08
30	Gully	705	30	5.1	45.20	152.70	10.60	34.50	28.90	11.94	0.58	0.056

Table S1.2 Number of sequence read assigned to AM fungal operational taxonomic unit (OTU) in the intact treatment of trap culture.

OTU (accession no.)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
001_Rhz_AB369921	79	92	301	12	771	3126	294	674	19	80	32	0	27	1	551
002_Rhz_HQ895800	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2663
003_Rhz_AB640749	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
006_Rhz_FM865608	64	41	373	77	804	1955	449	1157	39	133	28	26	11	26	272
007_Rhz_AB369745	3	29	1	0	33	252	2	29	0	0	0	0	0	0	11334
008_Rhz_AB640745	0	0	0	0	0	216	86	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	47
011_Rhz_AB643805	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1654
013_Rhz_FR750132	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
015_Rhz_AJ510243	0	117	308	92	67	123	5	72	0	0	42	4	56	9	0
018_Rhz_JN937265	0	0	0	0	0	0	14	0	0	0	0	0	0	0	0
019_Rhz_AB640747	42	0	64	102	179	1626	273	347	78	125	34	25	4	0	0
023_Rhz_AB370888	32	0	371	1072	265	792	379	1198	48	68	88	17	120	104	1
033_Fun_AJ510239	5	0	12	0	0	0	0	30	0	0	0	0	0	0	0
034_Fun_FN547491	5	0	0	0	0	0	0	103	0	0	0	0	0	0	0
038_Rhz_AB812612	123	0	386	162	268	1849	582	1347	0	98	70	0	0	142	368
041_Rhz_LC176573	390	35236	22	0	0	0	0	0	0	9	4	0	0	0	0
042_Rhz_LC176574	60	0	85	13	1	0	61	22	65	84	0	0	76	0	2
043_Rhz_LC176575	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
050_Rhz_AB640741	0	0	0	0	0	0	11	0	0	0	0	0	0	0	0
052_Rhz_LC176579	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
053_Rhz_AB369736	363	21519	22	0	0	0	0	0	0	3	22	0	0	0	2
054_Rhz_LC176580	3750	0	8304	1859	122	0	6483	2756	4323	6837	37	0	8438	1	40
055_Rhz_JF717521	7685	5	18144	2480	101	0	12982	5750	9269	16607	138	2	18000	22	0

Table S1.2 Continued.

OTU (accession no.)	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
001_Rhz_AB369921	0	452	12	2	0	132	13	4	9	9	0	4	18	0	29
002_Rhz_HQ895800	0	2224	133	450	15	99	115	292	0	0	0	0	117	8	1
003_Rhz_AB640749	0	7	1	0	0	0	0	0	0	0	47	0	0	0	13
006_Rhz_FM865608	0	13	6	51	7	28	35	22	7	0	0	0	4	30	15
007_Rhz_AB369745	269	7902	266	683	25	1077	666	1581	41	5	101	12	0	6	2
008_Rhz_AB640745	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
009_Rhz_FM992381	0	744	0	56	0	107	0	2	0	0	0	0	0	1	0
011_Rhz_AB643805	0	1569	0	119	0	84	30	195	0	1	0	1	1	1	0
013_Rhz_FR750132	0	0	0	0	0	0	9	0	0	0	0	0	0	0	0
015_Rhz_AJ510243	0	0	0	0	2	0	0	0	5	0	0	0	0	0	0
018_Rhz_JN937265	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
019_Rhz_AB640747	0	174	0	0	0	0	0	59	0	0	0	0	0	0	0
023_Rhz_AB370888	37	0	2	7	0	12	9	1	10	1	0	1	23	0	39
033_Fun_AJ510239	0	0	11	0	0	0	0	0	0	0	0	55	0	0	9
034_Fun_FN547491	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
038_Rhz_AB812612	0	0	4	3	4	0	0	14	6	2	0	0	0	0	9
041_Rhz_LC176573	3	0	1	1827	0	0	0	13547	52	0	3	1	0	0	3
042_Rhz_LC176574	31	0	28	26	8	24	21	3	29	25	11	0	3	0	43
043_Rhz_LC176575	0	0	0	0	0	0	0	0	782	0	611	0	0	0	0
050_Rhz_AB640741	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
052_Rhz_LC176579	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0
053_Rhz_AB369736	3	0	3	1244	0	0	1	8620	15	0	0	0	0	0	0
054_Rhz_LC176580	3891	0	3129	2436	629	2845	1542	271	2640	2893	1042	0	239	1	3760
055_Rhz_JF717521	8255	323	7783	5031	1272	5090	4407	670	5420	5995	1525	2	11077	0	7082

Table S1.2 Continued.

OTU (accession no.)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
056_Rhz_AB369738	179	153	0	0	0	0	0	0	0	2	0	0	1	0	1
057_Rhz_LC176581	610	40548	11	0	0	0	0	0	0	4	39	0	0	96	0
058_Rhz_LC176582	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0
059_Rhz_AB369739	27526	11	62742	10184	363	0	43974	14602	27765	52409	368	18	67644	26	705
060_UnG_LC176583	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
062_UnG_LC176566	36	46	79	0	0	876	72	232	73	0	0	0	0	0	0
064_UnG_AB561101	8	0	64	0	0	86	52	21	0	0	31	0	0	8	0
067_UnG_AB710222	4229	0	2908	0	12872	0	21	496	26	0	183	0	0	1016	749
068_UnG_AB369764	7960	0	3242	512	18884	2	50	851	45	1	952	2	2	317	2792
069_UnG_AB369759	13	0	0	0	26	56	0	0	0	0	0	0	0	0	0
070_UnG_HE794042	6038	0	3715	376	13410	2	8	398	83	1	739	1	0	824	4394
072_UnG_AB643635	72	0	3137	4	143	0	0	12	1	0	9	0	0	2	40
073_UnG_AB369762	0	0	0	0	0	133	0	63	0	0	0	0	0	0	0
084_Glo_JX096584	0	0	0	0	0	26	21	0	0	0	0	0	0	0	0
088_Rhz_AY639221	110	0	1158	240	1507	1542	241	771	0	71	56	12	10	0	416
103_Fun_AB665499	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
107_UnG_AB369767	11	0	0	0	0	682	0	111	21	0	0	0	0	0	0
115_UnG_FR693683	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
120_UnG_AB369765	68	0	687	0	0	0	0	1	0	0	0	0	0	0	0
133_Cla_AB812593	0	0	0	0	0	0	0	20	0	0	0	0	0	0	0
137_Cla_AB665521	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
145_Cla_AY639200	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
150_Cla_JN937518	0	0	0	0	0	83	0	0	0	0	0	0	0	0	0
152_Scu_FM876838	33	0	0	0	0	886	49	368	23	0	0	7	0	0	0

Table S1.2 Continued.

OTU (accession no.)	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
056_Rhz_AB369738	0	0	0	6	0	1	0	63	2	0	0	0	0	0	0
057_Rhz_LC176581	3	0	2	2388	0	1	0	16046	21	1	0	2	0	0	0
058_Rhz_LC176582	0	0	0	1	0	0	0	46	0	0	0	0	0	0	0
059_Rhz_AB369739	24908	385	23410	15799	4595	16115	14192	2469	13572	18296	5109	60	6051	5	22488
060_UnG_LC176583	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
062_UnG_LC176566	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
064_UnG_AB561101	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
067_UnG_AB710222	373	1732	791	371	4605	4391	16	273	0	12	2	5361	0	5462	20
068_UnG_AB369764	573	1647	1513	335	6133	3305	62	166	2	24	27	11110	0	10186	90
069_UnG_AB369759	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
070_UnG_HE794042	1003	2264	2066	370	7636	5658	156	204	36	4	6	16094	0	13845	1
072_UnG_AB643635	6	21	20	3	43	2301	1	0	0	0	1	174	0	118	0
073_UnG_AB369762	0	0	0	0	11	0	0	0	0	0	0	0	0	0	0
084_Glo_JX096584	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
088_Rhz_AY639221	0	0	8	0	2	4	10	18	17	10	11	15	10	31	20
103_Fun_AB665499	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
107_UnG_AB369767	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0
115_UnG_FR693683	0	0	1	0	0	0	10	11	8	11	4	10	4	0	0
120_UnG_AB369765	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
133_Cla_AB812593	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0
137_Cla_AB665521	0	0	0	2	0	22	0	0	0	0	0	8	2	0	0
145_Cla_AY639200	0	0	0	0	0	0	0	0	0	0	0	10	0	0	0
150_Cla_JN937518	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
152_Scu_FM876838	0	0	0	0	5	0	0	0	0	0	0	28	0	0	0

Table S1.2 Continued.

[illegible]

Table S1.2 Continued.

OTU (accession no.)	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
153_Scu_AB561124	0	0	0	48	0	2	0	3	9	0	18	89	0	0	0
154_Scu_FN547618	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
155_Scu_JF816949	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0
156_Scu_FR750142	0	0	0	0	1	0	24	0	0	0	0	0	0	8	0
157_UnG_AB665510	0	0	0	1	0	0	0	0	0	0	0	0	3	0	0
163_Scu_AB665514	0	1	0	2	1	7	1	0	11	0	0	7	0	16	25
164_Scu_GQ229230	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
165_Gig_FN547559	0	263	0	20	0	0	49	0	0	2	6	4	0	24	5
176_Arc_JX848923	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0
180_Arc_AB812597	0	0	0	20	0	0	0	0	0	0	0	0	0	0	0
182_Aca_HE775276	0	0	0	0	0	0	0	0	0	0	0	0	7387	0	0
183_Aca_AF389017	93	6809	317	727	107	707	887	2079	267	79	350	212	129	367	649
187_Aca_FR692354	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
193_Aca_AB935539	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
195_Aca_AB369793	0	123	0	42	3	77	3	16	277	76	1170	39	9171	0	1
196_Amb_KC166276	19295	22	9203	24078	1	0	32	0	17391	25768	27917	27	75	138	136
197_Amb_AB369803	155	0	69	158	0	2	0	0	115	202	236	0	2	2	1
202_Div_AY842573	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
203_Div_LC176564	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
219_Par_AB369810	0	0	0	0	3	0	0	0	0	0	0	15	0	0	16
220_Par_NG_027567	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
230_Rhz_JN937313	0	576	0	4	0	0	1	184	0	0	0	1	0	0	2
231_Rhz_FR750064	0	0	0	0	0	10	0	8	13	0	0	0	1	2	45
232_Rhz_HE775295_S003318578	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0

Table S1.2 Continued.

OTU (accession no.)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
241_UnG_KF568281_S004099839	0	0	0	0	0	71	24	0	0	0	0	0	0	0	0
244_UnG_AB710223_S003308327	0	0	8	0	0	0	0	0	0	0	14	0	0	0	0
256_Rhz_FR772327_S002473136	0	0	0	0	0	0	0	0	0	0	1042	0	0	0	0
275_Rhz_EU692293_S001092514	0	0	0	0	0	0	0	0	0	0	0	15	0	0	0
276_Rhz_KF565152_S004097322	0	0	0	0	23	0	0	0	0	0	52	150	9	36	1
296_Scu_FM876834_S003844418	0	0	0	0	0	0	0	21	0	0	0	0	0	0	0
298_Scu_AJ510231_S003824973	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0
301_Scu_JF717509_S003056723	0	0	0	0	0	0	0	0	0	0	9	1	0	226	0
303_Unc_KF206548_S003698605	7	0	0	0	0	0	0	0	0	0	0	0	36	0	0
307_Unc_HE775279_S003318562	0	0	0	0	0	4066	0	0	0	0	0	0	0	0	0
327_Aca_AB369790_S002040113	0	0	0	0	0	1208	0	3613	1722	24	21551	17770	1175	129	7
328_Aca_AJ510228_S003824970	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0
348_Scu_LC191590	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
355_Scu_LC191597	0	0	0	0	65	0	0	10	0	0	0	0	0	0	0
358_Scu_LC191600	0	0	0	0	0	57	0	77	0	0	0	0	0	0	0
361_Aca_LC191603	1	0	0	0	0	1135	0	4033	1794	14	20048	19358	2168	0	125
366_Scu_LC191606	0	0	0	0	0	0	0	43	0	0	0	0	0	0	0
367_Rhz_LC191607	1	0	0	0	5	22	2	6	0	4	0	0	0	0	0
372_Unc_LC191611	0	0	0	0	0	0	0	66	0	0	0	0	0	0	0
373_Rhz_LC191612	1	0	1	63	5	345	2	17	0	0	0	0	0	1	0
374_Rhz_LC191613	8	1	64	0	97	458	79	0	0	0	0	0	0	22	0
375_Rhz_LC191614	17	0	0	0	61	283	0	0	0	26	0	0	0	0	0
379_Rhz_LC191618	11	0	48	1	128	307	19	109	0	3	0	0	1	0	0
380_Rhz_LC191619	1	0	0	0	175	288	75	277	0	73	0	0	0	36	0

Table S1.2 Continued.

OTU (accession no.)	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
241_UnG_KF568281_S004099839	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
244_UnG_AB710223_S003308327	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
256_Rhz_FR772327_S002473136	0	0	0	0	49	5830	0	0	0	0	0	0	3536	0	0
275_Rhz_EU692293_S001092514	0	2144	4	0	0	0	0	0	0	0	0	0	0	0	0
276_Rhz_KF565152_S004097322	0	17987	5	58	0	0	0	0	1	2	0	0	2600	353	0
296_Scu_FM876834_S003844418	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
298_Scu_AJ510231_S003824973	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
301_Scu_JF717509_S003056723	0	0	0	0	0	0	0	1	0	0	0	19	0	6	15
303_Unc_KF206548_S003698605	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
307_Unc_HE775279_S003318562	0	0	0	0	0	6	0	0	0	0	0	0	0	0	0
327_Aca_AB369790_S002040113	0	177	1	12	0	0	0	2	80	10	775	0	5565	0	0
328_Aca_AJ510228_S003824970	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
348_Scu_LC191590	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
355_Scu_LC191597	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
358_Scu_LC191600	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
361_Aca_LC191603	0	3	0	0	4	5	0	16	69	71	731	0	5282	30	15
366_Scu_LC191606	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
367_Rhz_LC191607	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
372_Unc_LC191611	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
373_Rhz_LC191612	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
374_Rhz_LC191613	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0
375_Rhz_LC191614	0	0	0	0	0	8	0	0	0	0	0	0	0	0	0
379_Rhz_LC191618	0	0	0	0	0	3	0	0	0	0	0	0	0	0	1
380_Rhz_LC191619	0	0	7	0	9	5	10	0	8	5	2	9	13	19	9

Table S1.2 Continued.

OTU (accession no.)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
384_Scu_LC191622	0	0	0	0	24	0	0	0	0	0	0	0	0	0	0
388_Rhz_LC191626	0	0	0	0	0	4	1	0	0	0	0	0	0	0	0
391_Rhz_LC191628	0	0	0	0	0	71	0	0	0	0	0	0	0	0	0
392_Rhz_LC191629	0	0	0	0	23	40	0	19	0	0	0	0	0	0	0
395_Rhz_LC191631	58	39	104	0	120	911	255	390	2	34	42	8	19	73	0
399_Scu_LC191634	21	0	121	0	35	2601	205	455	72	147	45	0	11	0	0
402_Unc_LC191636	18	0	0	0	0	141	31	41	0	12	0	0	0	0	0
407_Amb_LC191640	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
410_Rhz_LC191641	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
415_Rhz_LC191645	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
416_Rhz_LC191646	0	0	0	157	0	0	0	0	0	0	0	0	0	0	0
417_Rhz_LC191647	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0
418_Rhz_LC191648	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
422_Par_LC191651	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0
427_Aca_LC191656	0	0	0	0	0	0	0	0	0	0	1	2	0	0	0
429_Cla_LC191658	0	0	0	0	0	51	0	0	0	0	0	0	0	0	0
430_Rhz_LC191659	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total read	60090	98297	107646	18347	52151	39789	68771	80839	69793	77893	74736	60364	100383	5590	37470
Richness	47	19	36	23	41	51	45	53	28	35	37	20	25	25	30

Table S1.2 Continued.

OTU (accession no.)	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
384_Scu_LC191622	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
388_Rhz_LC191626	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
391_Rhz_LC191628	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
392_Rhz_LC191629	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
395_Rhz_LC191631	0	0	0	0	0	0	0	84	0	0	0	12	12	0	0
399_Scu_LC191634	0	0	0	0	0	7	0	0	0	0	0	15	0	0	0
402_Unc_LC191636	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
407_Amb_LC191640	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
410_Rhz_LC191641	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
415_Rhz_LC191645	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
416_Rhz_LC191646	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
417_Rhz_LC191647	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
418_Rhz_LC191648	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0
422_Par_LC191651	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
427_Aca_LC191656	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
429_Cla_LC191658	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
430_Rhz_LC191659	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total read	58898	47562	48799	56383	25173	47970	22302	46971	40919	53506	39705	33401	51325	30659	34544
Richness	16	24	31	36	27	34	26	33	32	25	23	31	25	23	30

Table S1.3 Number of sequence read assigned to AM fungal operational taxonomic unit (OTU) in the disturbed treatment of trap culture.

OTU (accession no.)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
001_Rhz_AB369921	9	1352	0	88	162	131	1	55	167	28	0	6	3	4	405
002_Rhz_HQ895800	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2447
003_Rhz_AB640749	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5
006_Rhz_FM865608	1	687	72	0	198	0	31	0	171	45	1	127	4	1	197
007_Rhz_AB369745	0	0	1	0	0	0	0	0	0	0	0	0	0	0	3503
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	113
011_Rhz_AB643805	0	0	0	0	0	0	0	0	0	0	0	0	0	0	975
013_Rhz_FR750132	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
015_Rhz_AJ510243	33	0	0	0	0	0	0	0	0	6	0	0	0	0	0
018_Rhz_JN937265	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
019_Rhz_AB640747	1	0	0	0	94	81	0	35	0	0	0	0	0	91	0
023_Rhz_AB370888	76	282	47	190	359	149	0	28	88	50	51	2	2	305	35
033_Fun_AJ510239	0	0	0	0	0	0	0	0	0	0	0	0	0	77	64
034_Fun_FN547491	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
038_Rhz_AB812612	1	348	147	104	73	0	48	23	0	16	1	1	129	0	146
041_Rhz_LC176573	9716	1	37346	1	359	0	0	19	1	2	408	0	8	0	5
042_Rhz_LC176574	5	95	0	92	1	0	62	0	65	39	0	1	108	0	0
043_Rhz_LC176575	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
050_Rhz_AB640741	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
052_Rhz_LC176579	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
053_Rhz_AB369736	7013	0	23604	185	301	0	0	0	1	0	240	1	0	0	1
054_Rhz_LC176580	427	9711	0	10491	101	0	5738	0	5313	4281	98	240	9388	0	113
055_Rhz_JF717521	800	16495	3	19509	496	0	11660	0	11310	8350	107	840	21229	0	189

Table S1.3 Continued.

OTU (accession no.)	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
001_Rhz_AB369921	102	345	134	53	144	2	207	29	41	0	0	1	70	0	0
002_Rhz_HQ895800	724	1779	448	334	531	230	860	285	255	92	0	0	328	0	0
003_Rhz_AB640749	20	139	25	0	0	0	45	2	13	1	0	0	1	0	0
006_Rhz_FM865608	84	197	42	21	52	0	71	34	75	54	0	1	81	0	1
007_Rhz_AB369745	2613	4352	1049	780	1548	540	2905	604	1108	332	0	14	1259	2	3
008_Rhz_AB640745	0	0	0	0	0	0	0	0	0	0	0	0	6	0	0
009_Rhz_FM992381	46	281	62	20	166	57	145	15	39	2	0	0	64	1	0
011_Rhz_AB643805	259	785	359	56	312	58	494	58	119	27	1	0	185	1	0
013_Rhz_FR750132	0	0	0	0	12	0	0	0	0	0	0	0	0	0	0
015_Rhz_AJ510243	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
018_Rhz_JN937265	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
019_Rhz_AB640747	0	0	4	0	3	0	2	2	0	0	0	0	4	0	0
023_Rhz_AB370888	0	31	11	0	13	0	2	2	6	0	3	0	1	0	0
033_Fun_AJ510239	0	0	3	0	0	0	4	0	7	0	0	0	0	0	0
034_Fun_FN547491	0	0	0	0	0	0	0	0	2	0	0	0	6	0	0
038_Rhz_AB812612	76	172	0	10	0	21	52	26	19	15	18	0	11	0	0
041_Rhz_LC176573	1	0	1	7643	99	0	0	580	5477	0	0	1	0	0	1
042_Rhz_LC176574	17	1	36	23	0	4	9	1	1	1	0	0	2	0	44
043_Rhz_LC176575	0	0	0	0	0	0	0	0	0	0	19	0	0	0	0
050_Rhz_AB640741	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
052_Rhz_LC176579	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
053_Rhz_AB369736	3	132	3	4198	51	0	0	389	5011	0	0	0	0	0	0
054_Rhz_LC176580	1464	61	2654	1028	41	814	844	275	110	20	30	0	295	1	4942
055_Rhz_JF717521	2922	731	6713	3188	52	1626	1857	571	308	121	26	0	512	2	11602

Table S1.3 Continued.

OTU (accession no.)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
056_Rhz_AB369738	5618	1	118	3	162	0	0	1	0	0	2	0	0	0	0
057_Rhz_LC176581	14202	33	47754	1	773	0	0	0	0	0	442	0	10	0	1
058_Rhz_LC176582	226	0	6	0	0	0	0	0	0	0	0	0	0	0	0
059_Rhz_AB369739	3603	57551	219	62126	353	0	37609	0	37439	23151	170	1586	73072	1	517
060_UnG_LC176583	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
062_UnG_LC176566	0	338	0	0	34	0	0	0	0	0	0	51	0	0	0
064_UnG_AB561101	0	76	0	0	43	0	0	0	0	0	0	0	0	0	0
067_UnG_AB710222	315	1794	0	1618	1510	2	0	0	0	0	2323	12	8	21521	1279
068_UnG_AB369764	547	1149	1	2599	2211	3	0	0	0	0	3324	30	23	37324	1982
069_UnG_AB369759	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
070_UnG_HE794042	465	2951	1	1953	1915	1	0	37	0	0	2969	17	29	32545	3844
072_UnG_AB643635	4	1260	0	12	27	0	0	0	0	0	43	0	0	414	26
073_UnG_AB369762	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
084_Glo_JX096584	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
088_Rhz_AY639221	0	418	86	0	28	0	88	93	0	8	54	42	0	0	185
103_Fun_AB665499	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
107_UnG_AB369767	0	203	27	0	0	0	0	0	0	0	0	0	0	0	0
115_UnG_FR693683	0	0	0	0	0	0	0	0	0	0	0	0	0	0	33
120_UnG_AB369765	1463	3050	46	0	135	0	18	0	0	0	1	0	1	0	0
133_Cla_AB812593	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
137_Cla_AB665521	0	0	0	0	0	0	0	0	0	0	0	0	0	0	41
145_Cla_AY639200	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
150_Cla_JN937518	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
152_Scu_FM876838	0	0	0	0	80	0	0	0	0	12	0	0	128	0	0

Table S1.3. Continued.

OTU (accession no.)	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
056_Rhz_AB369738	1	0	1	52	0	0	0	6	2762	0	0	0	0	0	2
057_Rhz_LC176581	4	0	3	8120	32	0	0	655	9185	0	0	0	1	0	0
058_Rhz_LC176582	0	0	0	67	0	0	0	1	90	0	0	0	0	0	0
059_Rhz_AB369739	8868	371	24636	9277	482	5358	6047	2418	768	409	128	0	1348	41	32056
060_UnG_LC176583	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
062_UnG_LC176566	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
064_UnG_AB561101	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0
067_UnG_AB710222	244	275	212	79	8686	89	203	168	69	0	2786	4645	117	8688	275
068_UnG_AB369764	223	583	277	152	9526	76	268	307	83	96	5571	8173	274	15841	312
069_UnG_AB369759	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
070_UnG_HE794042	502	1077	469	205	11515	166	418	528	186	219	6063	10346	271	19231	404
072_UnG_AB643635	7	13	37	1	113	1	7	3	2	2	55	94	13	111	4
073_UnG_AB369762	0	0	0	0	0	0	7	0	1	13	0	0	0	0	0
084_Glo_JX096584	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
088_Rhz_AY639221	47	0	47	0	0	13	15	3	19	56	0	1	16	0	0
103_Fun_AB665499	0	0	0	0	0	0	0	1	0	0	0	0	10	0	0
107_UnG_AB369767	0	109	0	0	0	0	0	0	0	0	0	0	0	0	0
115_UnG_FR693683	15	39	0	0	0	0	0	0	0	0	0	0	0	0	0
120_UnG_AB369765	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
133_Cla_AB812593	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
137_Cla_AB665521	4	0	0	0	0	0	3	0	0	0	0	0	0	0	0
145_Cla_AY639200	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
150_Cla_JN937518	0	0	0	0	0	0	0	0	0	7	0	0	0	0	0
152_Scu_FM876838	0	0	0	0	7	0	0	1	4	0	0	0	0	0	0

Table S1.3 Continued.

[illegible]

Table S1.3 Continued.

[illegible]

Table S1.3 Continued.

OTU (accession no.)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
241_UnG_KF568281_S004099839	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
244_UnG_AB710223_S003308327	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
256_Rhz_FR772327_S002473136	0	0	0	0	0	0	0	0	0	0	4420	0	169	121	0
275_Rhz_EU692293_S001092514	0	0	0	0	0	0	0	0	0	0	19	0	0	0	0
276_Rhz_KF565152_S004097322	0	0	0	0	0	0	0	0	0	0	0	0	0	2	1
296_Scu_FM876834_S003844418	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
298_Scu_AJ510231_S003824973	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0
301_Scu_JF717509_S003056723	0	0	0	0	0	0	0	0	0	0	234	0	0	1594	0
303_Unc_KF206548_S003698605	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
307_Unc_HE775279_S003318562	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
327_Aca_AB369790_S002040113	2	0	2	2	0	25150	29	1326	3173	1	19191	13240	509	238	4159
328_Aca_AJ510228_S003824970	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
348_Scu_LC191590	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
355_Scu_LC191597	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
358_Scu_LC191600	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
361_Aca_LC191603	2	0	5	0	0	30062	0	1905	3259	1	21996	15792	130	269	6100
366_Scu_LC191606	0	0	0	0	0	0	0	0	0	0	0	0	106	0	0
367_Rhz_LC191607	0	9	0	0	0	6	0	0	0	0	0	0	0	0	0
372_Unc_LC191611	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
373_Rhz_LC191612	0	0	74	110	0	0	0	0	0	0	1	0	0	0	0
374_Rhz_LC191613	0	27	77	0	0	0	0	0	0	0	0	0	0	0	0
375_Rhz_LC191614	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
379_Rhz_LC191618	0	14	0	0	0	4	2	0	1	0	0	5	0	0	28
380_Rhz_LC191619	20	391	0	93	0	1	4	14	2	0	0	3	1	78	28

Table S1.3 Continued.

OTU (accession no.)	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
241_UnG_KF568281_S004099839	0	0	0	8	0	0	0	0	0	0	0	0	0	0	0
244_UnG_AB710223_S003308327	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
256_Rhz_FR772327_S002473136	0	0	35	26	0	463	24	3	9	31	0	0	381	0	0
275_Rhz_EU692293_S001092514	0	136	68	0	0	0	0	0	0	0	0	0	0	0	0
276_Rhz_KF565152_S004097322	0	0	175	0	0	0	0	8	0	2	0	0	5	0	0
296_Scu_FM876834_S003844418	0	0	0	0	0	0	18	0	0	0	0	0	0	0	0
298_Scu_AJ510231_S003824973	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
301_Scu_JF717509_S003056723	0	0	3	0	52	0	0	0	0	0	0	12	0	0	0
303_Unc_KF206548_S003698605	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
307_Unc_HE775279_S003318562	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
327_Aca_AB369790_S002040113	50	166	82	26	609	14864	61	8815	18	7452	4651	297	10979	1	1
328_Aca_AJ510228_S003824970	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
348_Scu_LC191590	0	0	0	0	16	0	0	0	0	0	0	0	0	0	0
355_Scu_LC191597	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
358_Scu_LC191600	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
361_Aca_LC191603	73	892	143	71	1114	15178	105	12315	50	10814	4820	237	16626	3	1
366_Scu_LC191606	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
367_Rhz_LC191607	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
372_Unc_LC191611	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
373_Rhz_LC191612	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0
374_Rhz_LC191613	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
375_Rhz_LC191614	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0
379_Rhz_LC191618	0	4	19	0	0	0	0	0	1	0	0	0	0	0	0
380_Rhz_LC191619	29	75	21	4	0	0	3	0	0	0	1	0	50	0	0

Table S1.3 Continued.

OTU (accession no.)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
384_Scu_LC191622	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
388_Rhz_LC191626	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
391_Rhz_LC191628	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
392_Rhz_LC191629	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
395_Rhz_LC191631	0	0	79	0	28	0	48	48	0	0	1	0	0	0	48
399_Scu_LC191634	0	0	0	0	79	219	27	0	98	47	0	0	0	1	11
402_Unc_LC191636	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
407_Amb_LC191640	0	0	0	0	0	0	0	1	2	0	0	0	0	0	0
410_Rhz_LC191641	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
415_Rhz_LC191645	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
416_Rhz_LC191646	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
417_Rhz_LC191647	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
418_Rhz_LC191648	0	0	0	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	0	0	0	0	0
427_Aca_LC191656	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
429_Cla_LC191658	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
430_Rhz_LC191659	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total read	44558	101236	110041	99841	10215	110037	55589	106339	100565	36267	88556	85600	105504	100077	38798
Richness	26	32	26	24	32	19	18	22	23	21	30	25	27	24	42

Table S1.3 Continued.

OTU (accession no.)	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
384_Scu_LC191622	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
388_Rhz_LC191626	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
391_Rhz_LC191628	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
392_Rhz_LC191629	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
395_Rhz_LC191631	0	38	15	14	6	0	43	17	8	0	16	0	15	32	0
399_Scu_LC191634	0	171	0	0	0	0	18	0	4	0	0	0	8	0	0
402_Unc_LC191636	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
407_Amb_LC191640	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
410_Rhz_LC191641	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
415_Rhz_LC191645	28	0	0	0	0	0	0	0	0	0	0	0	0	0	0
416_Rhz_LC191646	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
417_Rhz_LC191647	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
418_Rhz_LC191648	0	0	0	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	0	0	0	0	0
427_Aca_LC191656	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
429_Cla_LC191658	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
430_Rhz_LC191659	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Total read	58714	20258	60902	53795	38758	56814	17144	47812	50711	56204	44406	24468	56056	43988	49806
Richness	37	34	41	38	38	22	37	42	46	28	22	16	41	15	18

Table S1.4 Correlation coefficients and probabilities between the environmental factors.

	Elevation	Colony diameter	pH	Truog-P	Ca	Mg	K	CEC	NH ₃	NO ₃	Total N
Elevation	-	-0.271	-0.256	0.074	0.116	0.010	0.021	-0.127	-0.057	-0.076	-0.174
Colony diameter	0.147	-	-0.037	0.047	-0.096	0.058	0.152	0.193	0.379	0.229	0.263
pH	0.171	0.845	-	0.294	-0.652	-0.694	-0.611	-0.692	-0.386	0.229	-0.656
Truog-P	0.697	0.804	0.114	-	-0.039	-0.058	-0.149	0.071	0.268	0.527	-0.067
Ca	0.540	0.613	<0.001	0.837	-	0.955	0.794	0.831	0.059	0.233	0.733
Mg	0.957	0.758	<0.001	0.758	<0.001	-	0.883	0.928	0.748	0.258	0.846
K	0.91	0.423	<0.001	0.430	<0.001	<0.001	-	0.896	0.791	0.267	0.848
CEC	0.502	0.306	<0.001	0.709	<0.001	<0.001	<0.001	-	0.804	0.273	0.977
NH ₃	0.764	0.039	0.034	0.151	<0.001	<0.001	<0.001	<0.001	-	0.497	0.803
NO ₃	0.688	0.223	0.223	0.002	0.214	0.169	0.153	0.144	0.005	-	0.262
Total N	0.356	0.159	<0.001	0.724	<0.001	<0.001	<0.001	<0.001	<0.001	0.160	-

The values in the upper right are correlation coefficients, and those in the lower left are their probabilities.

Table S1.5 Averages of the environmental parameters in the three habitats.

Factor	Grassland	Gully	Slope
Elevation (m)	643.8±2.53 ^a	686.2±15.17 ^a	751.2±26.21 ^b
Richness	31.75±1.85 ^a	31.15±2.41 ^a	26.95±1.66 ^a
Shannon-wiener	1.74±0.09 ^a	1.63±0.13 ^{ab}	1.32±0.07 ^b
Colony diameter (cm)	55.7±13.21 ^a	42±8.14 ^{ab}	29.9±5.14 ^b
pH	5.01±0.07 ^a	5.21±0.04 ^b	4.80±0.11 ^c
Truog-P	3.82±0.45 ^a	5.06±0.28 ^b	3.88±0.24 ^a
Ca	15.55±3.96 ^{ab}	11.91±1.31 ^b	22.86±4.62 ^a
Mg	2.46±0.99 ^{ab}	0.81±0.11 ^b	3.29±0.94 ^a
K	6.57±1.68 ^a	2.23±0.26 ^b	5.70±0.98 ^a
CEC	9.77±3.53 ^a	1.36±0.24 ^b	9.23±2.96 ^a
NH ₃	2.03±0.87 ^a	0.79±0.12 ^b	1.26±0.21 ^{ab}
NO ₃	0.07±0.02 ^a	0.08±0.02 ^a	0.05±0.01 ^a
Total N	0.17±0.04 ^a	0.03±0.01 ^b	0.14±0.03 ^a

Table S1.6 Mantel test^a for the assessment of correlations between the community compositions and the environmental factors

Factor	<i>r</i>	<i>P</i>
Elevation	0.243	0.007
Colony diameter	0.044	0.177
pH	0.08	0.081
Truog-P	0.116	0.042
Ca	-0.01	0.547
Mg	0.047	0.217
CEC	-0.004	0.476
K	0.069	0.184
NH ₃	-0.045	0.591
NO ₃	-0.064	0.769
Total N	-0.008	0.495
Elevation	0.243	0.007

^a Bray-Curtis index is employed as a measure of community dissimilarity (9,999 permutations).

Table S2.1 Geographic data soil samples collected from rhizosphere of *Miscanthus sinensis* grown in Mt. Tarumae volcano

Sample ID	Longitude	Latitude	Habitat	Collection year	Elevation (m)
1	141.384193	42.701773	Grassland	2016	639
2	141.384065	42.701566	Grassland	2016	644
3	141.383982	42.701335	Grassland	2016	638
4	141.384071	42.701712	Grassland	2016	640

Table S2.2 Number of sequence read assigned to AM fungal operational taxonomic unit (OTU) in the intact treatment of trap culture.

[illegible]

Table S2.2 Continued.

[illegible]

Table S2.2 Continued.

Month	0				2				4				6			
OTU	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
053_Rhz_AB369736	52	435	802	1054	18	60	20	72	0	28	0	0	6	0	0	0
054_Rhz_LC176580	221	962	99	105	0	0	119	0	7	0	0	0	6	0	0	0
055_Rhz_JF717521	283	2020	262	458	11	0	234	2	55	0	0	0	0	0	0	0
056_Rhz_AB369738	0	5	3	3	0	0	2	42	0	0	0	1	0	0	0	0
057_Rhz_LC176581	207	958	1875	946	28	78	23	450	0	127	0	72	0	0	0	0
058_Rhz_LC176582	0	1	0	0	0	0	0	6	0	0	0	1	0	0	0	0
059_Rhz_AB369739	1072	6935	955	1435	84	0	837	66	320	28	0	42	53	0	1	0
060_UnG_LC176583	0	0	0	0	1	0	0	2	0	0	0	0	0	0	0	0
064_UnG_AB561101	0	0	0	0	0	0	0	9	0	0	0	0	0	0	0	0
067_UnG_AB710222	292	647	698	127	6	0	24	4	31	22	0	0	0	0	0	81
068_UnG_AB369764	480	1096	1102	173	81	0	67	90	47	0	0	58	0	0	80	27
069_UnG_AB369759	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
070_UnG_HE794042	547	1051	1105	436	27	0	112	72	73	30	0	0	0	0	75	76
072_UnG_AB643635	46	5	17	2	9	0	0	0	0	1	0	0	0	0	1	0
073_UnG_AB369762	0	0	0	0	0	0	0	8	0	0	0	0	0	0	0	0
084_Glo_JX096584	0	15	0	0	0	0	192	0	0	0	0	0	0	0	0	0
088_Rhz_AY639221	9	7	7	0	26	51	55	60	6	0	21	0	0	0	0	0
089_Rhz_LC014186	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
096_Fun_AB665501	0	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0
103_Fun_AB665499	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
104_UnG_LC014191	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
115_UnG_FR693683	0	0	0	0	32	69	37	15	0	0	0	0	0	0	0	0
137_Cla_AB665521	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
143_Cla_AB935545	0	0	0	0	23	0	1	0	0	0	0	0	0	0	0	0
150_Cla_JN937518	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0

Table S2.2 Continued.

[illegible]

Table S2.2 Continued.

Month	0				2				4				6			
OTU	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
152_Scu_FM876838	0	0	0	0	8	0	2	0	0	0	0	0	0	0	0	0
153_Scu_AB561124	11	0	42	0	0	0	0	0	0	0	0	0	0	0	0	0
154_Scu_FN547618	0	0	0	0	2	0	7	22	0	0	0	0	0	0	0	0
155_Scu_JF816949	0	0	0	0	1	23	4	0	0	0	0	0	0	0	0	0
156_Scu_FR750142	0	0	0	0	4	0	2	1	0	0	0	0	0	0	0	49
163_Scu_AB665514	0	0	38	0	65	36	5	1	0	0	22	0	0	0	0	0
164_Scu_GQ229230	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
165_Gig_FN547559	0	0	19	0	4	0	43	58	0	9	0	0	0	0	0	0
180_Arc_AB812597	0	0	0	0	3	0	2	0	0	0	0	0	0	0	0	0
182_Aca_HE775276	11	0	51	1	0	0	0	0	0	0	0	0	0	0	0	0
183_Aca_AF389017	192	215	1019	323	7079	6976	5984	5115	231	64	154	274	127	233	274	215
195_Aca_AB369793	28	58	22	1	74	140	414	10	7185	7381	7595	9434	6937	9602	8710	6188
196_Amb_KC166276	117	161	467	625	12	0	2	0	53	76	0	22	0	0	9	0
197_Amb_AB369803	1	0	8	2	18	27	9	14	0	0	0	73	0	0	1	0
219_Par_AB369810	0	15	0	0	0	56	0	0	0	0	0	0	0	0	0	0
227_Par_AB812601	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
229_Par_AB547188	0	0	0	0	0	0	11	0	0	0	0	0	0	0	0	0
230_Rhz_JN937313	1	0	0	1	26	4	35	36	3	0	78	0	0	0	0	0
231_Rhz_FR750064	0	0	0	0	27	1	11	16	0	2	13	0	0	0	0	0
256_Rhz_FR772327	128	16	111	0	0	0	0	0	0	0	0	0	0	0	0	0
261_Rhz_JX683740	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
275_Rhz_EU692293	189	265	58	268	0	0	0	0	0	0	0	0	0	0	0	0
276_Rhz_KF565152	1265	1301	800	1457	0	0	0	0	0	0	0	0	0	0	0	0
296_Scu_FM876834	0	0	0	0	0	0	1	7	0	0	0	0	0	0	0	0
301_Scu_JF717509	0	0	0	0	0	0	0	0	0	31	0	0	0	0	0	0

Table S2.2 Continued.

[illegible]

Table S2.2 Continued.

Month	0				2				4				6			
OTU	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
327_Aca_AB369790	103	6	1	1	31	73	209	38	4494	5640	5582	6823	6301	7823	6987	5784
348_Scu_LC191590	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
355_Scu_LC191597	0	0	0	0	0	0	3	16	0	0	0	0	0	0	0	0
361_Aca_LC191603	29	6	113	184	46	285	1064	189	11691	13561	13332	16857	11475	15160	12711	11741
367_Rhz_LC191607	0	0	1	0	1	0	1	1	0	0	0	0	0	0	0	0
373_Rhz_LC191612	0	0	0	0	11	0	0	4	0	0	0	0	0	0	0	0
374_Rhz_LC191613	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
375_Rhz_LC191614	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
379_Rhz_LC191618	0	0	0	0	3	0	0	1	0	0	57	0	0	0	0	0
380_Rhz_LC191619	0	0	0	0	6	18	26	1	0	5	9	0	0	0	0	0
381_Rhz_LC191620	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
382_Rhz_LC191621	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
384_Scu_LC191622	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
387_Rhz_LC191625	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
388_Rhz_LC191626	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
390_Rhz_LC191627	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
391_Rhz_LC191628	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
392_Rhz_LC191629	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0
395_Rhz_LC191631	0	0	0	0	5	0	24	14	12	0	0	25	0	0	0	0
399_Scu_LC191634	0	0	0	0	12	0	0	1	0	0	0	0	0	0	0	0
402_Unc_LC191636	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0
410_Rhz_LC191641	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
411_Rhz_LC191642	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
415_Rhz_LC191645	0	0	0	0	0	0	4	1	0	0	0	0	0	0	0	0
416_Rhz_LC191646	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
417_Rhz_LC191647	0	0	0	0	0	0	0	0	0	0	0	22	0	0	0	0
418_Rhz_LC191648	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0
427_Aca_LC191656	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0
429_Cla_LC191658	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
430_Rhz_LC191659	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0

Table S2.2 Continued.

[illegible]

Table S2.3 Continued.

[illegible]

Table S2.3 Continued.

[illegible]

Table S2.3 Continued.

[illegible]

Table S2.3 Continued.

[illegible]

Table S2.3 Continued.

[illegible]

Table S2.3 Continued.

[illegible]

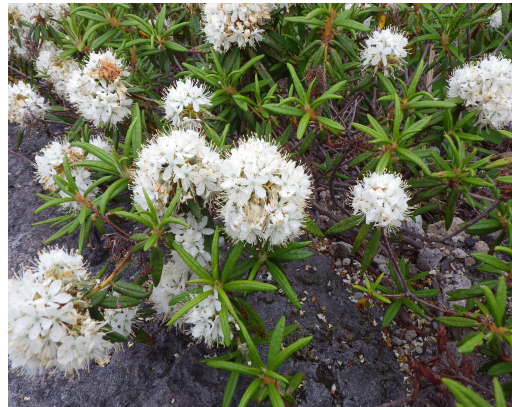
Table S2.3 Continued.

[illegible]

a)



b)



c)



d)



e)



f)



Fig. S1.1 Pioneer plant species in Mt. Tarumae *Miscanthus sinensis* (a), *Ledum palustre* (b), *Pennellianthus frutescens* (c), *Spiraea betulifolia* (d), *Alnus maximowiczii* (e), *Salix reinii* (f).

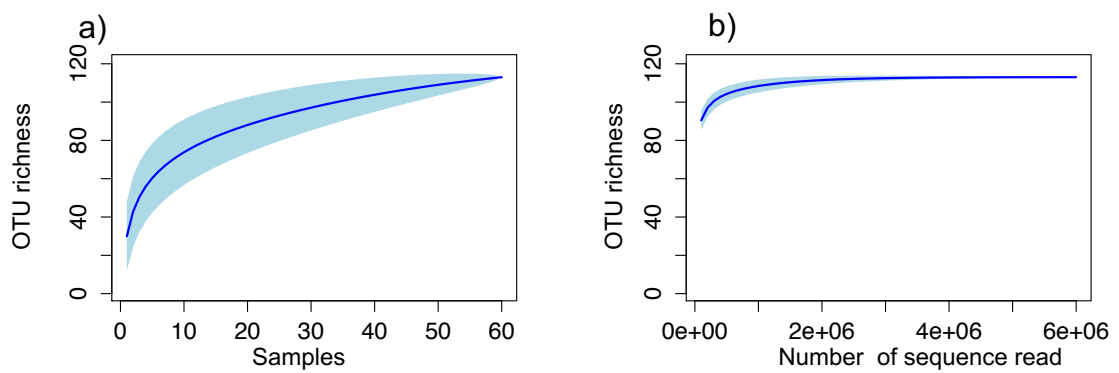


Fig. S1.2 Rarefaction curves constructed from number of sample (a) and number of sequence read (b). The grey area represents 95% confidence interval.

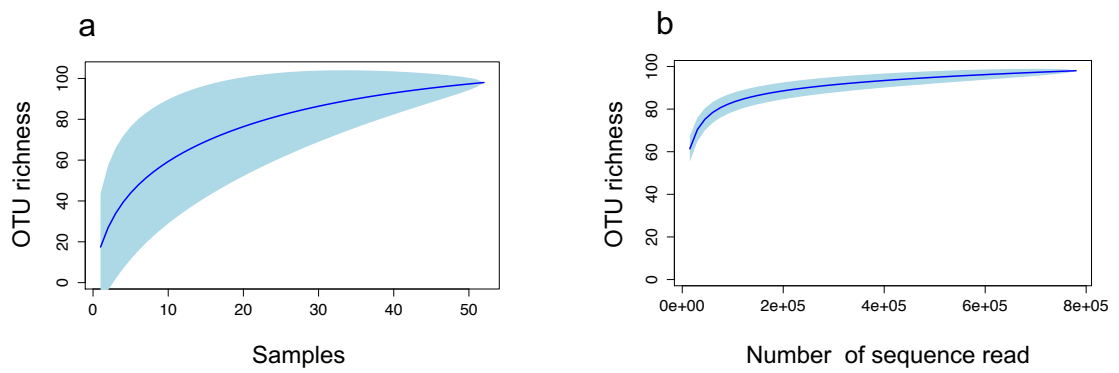


Fig. S2.1 Rarefaction curves constructed from number of sample (a) and number of sequence read (b). The grey area represents 95% confidence interval.