Original article

Reassembly of soil fungal communities under reforestation and herbivore exclusion

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Abstract

Soil fungi can help improve ecosystem restoration, yet our understanding of how fungi reassemble in degraded land is limited. Here, we studied fungal community structure using DNA metabarcoding in reforested sites following agricultural abandonment and overgrazing. We used a natural experiment in which reforestation with different numbers of tree species and deer exclusion have been applied for multiple decades. We found that local fungal richness (alpha diversity) was 1.9 to 2.9 times greater in reforested stands than in natural forests and total fungal richness (gamma diversity) was 1.3 to 1.9 times greater. These results were regardless of the number of tree species planted in the reforested stands. Conversely, reforested stands had a homogenized community structure with relatively lower degrees of compositional dissimilarity among sites within each stand (beta diversity). These findings were attributable to lower environmental heterogeneity, stronger dispersal limitation, and a comparatively shorter time since the onset of community assembly in reforested stands. Deer exclosures had no detectable effect on fungal community structure. Overall, the agricultural legacy in fungal community structure appears to have persisted for decades, even under proactive restoration of aboveground vegetation. Direct human intervention belowground may therefore be necessary for the recovery of soil biota once homogenized, which may facilitate ecosystem restoration.

Keywords: Biodiversity, Biotic homogenization, Community assembly, DNA metabarcoding, Ecosystem restoration, Soil microbes

Introduction

Given the extent of the human-induced damage to the biosphere, ecosystem restoration has become increasingly relevant in today's world (Hobbs and Harris, 2001). While restoration ecology has typically focused on aboveground vegetation (Brudvig 2011; Perring et al., 2015; Young 2000), less attention has been paid to belowground biota (Kardol and Wardle 2010). The diversity and composition of soil organisms, particularly fungi, can be largely altered in degraded land due to the decreased availability of organic substrates and their symbionts (Kardol and Wardle, 2010). Understanding how soil fungi respond to restoration is of ecosystem-level significance, because they can regulate key belowground functions and create feedbacks that support the aboveground recovery (Young et al. 2005).

Recently, there are increasing social concerns on reforestation using mixed-species plantations over monocultures (Verheyen et al., 2016; Tatsumi 2020). Plant diversity has often shown to have positive effects on soil fungal diversity in grassland experiments (Milcu et al., 2013; Scherber et al., 2010). In forests, however, experimental tests on the relationships between tree and fungal diversity remain scarce (Weißbecker et al., 2018). Particularly, compared with local fungal richness (alpha diversity), we still know little about the spatial variation in fungal composition (beta diversity), as influenced by tree-species mixing. Assessing both alpha and beta diversity can inform ecosystem recovery at local scales and larger scales that are relevant to land management and policy making (e.g., forest stands, landscapes).

Human-induced increases in herbivore density can drive ecosystem degradation and hamper vegetative recovery (Opperman and Merenlender, 2000). The changes in vegetation caused by herbivory are known to subsequently impact soil organisms, including fungi (Kardol et al., 2014). Thus far, such indirect effects have typically been tested by sole manipulations of herbivores (Bardgett and Wardle, 2003; Kardol et al., 2014). It is also possible, however, for the impacts of herbivores to be mediated by plant diversity. Specifically, the rates of herbivory can be reduced in plant mixtures compared to monocultures because of associational protection among neighbouring species (Cook-Patton et al., 2014). There has yet been little experiment on whether herbivores and plant diversity have individual or interactive effects on soil fungi.

In this study, we quantified the effects of aboveground forest restoration on soil fungal communities. As a study system, we selected a restoration site in northern Japan. We used a natural experiment in which the following treatments were applied in a fully-crossed design: 'tree planting

with different numbers of species' and 'deer exclusion' (Fujii et al., 2017; Mori et al., 2016). Our specific objectives were to test (1) whether the community structure of soil fungi — namely, their alpha and beta diversity — differ among monocultures and mixtures as well as nearby grasslands and natural forests, and (ii) whether tree diversity and deer herbivory have individual or interactive effects on soil fungi. Addressing these questions provides a step towards ecosystem restoration grounded on above- and belowground linkages.

Methods

Study site

We conducted a natural experiment in lowland coastal areas of the Shiretoko National Park, northern Japan (44°08'–11' N, 145°03'–08' E, elevation 140–220 m). The area has been designated a World Heritage Site by the United Nations Educational, Scientific and Cultural Organization (UNESCO) on account of it being home to one of the most species rich, northern temperate ecosystems in the world (http://whc.unesco.org/en/list/1193). The mean monthly temperature ranges from −6.1°C in February to 20.8°C in August. The mean annual precipitation is 1149 mm. According to the soil classification system of Japan (Obara et al., 2011) and the Japan soil inventory (https://soil-inventory.dc.affrc.go.jp/), the soil type in our study sites are low-humic allophanic Andosols, which corresponds to Typic Hapludands and Hydric Hapludands in the USDA soil taxonomy (Soil Survey Staff, 2010) (Supplementary Fig. S1).

Approximately 90% of the park's terrestrial area is covered by pristine natural vegetation, most of which is composed of mixed conifer–broadleaf forests. Parts of the remaining area had been used for agriculture from the early twentieth century until the Government of Japan ordered the settlers to abandon the land, a process that was completed by the late 1960s. Since then, a number of reforestation initiatives has been conducted in the area to restore the arable land to mixed conifer–broadleaf forests (>861 ha; the Shiretoko National Trust Movement Area). Such activities included reforestation with different numbers of tree species and the establishment of fences to prevent overgrazing and browsing by sika deer (*Cervus nippon yesoensis*). Deer density has increased rapidly from the late 1980s to the late 1990s in the park, with a current density of 6.1–13.6 individuals km^{-2}. Currently, the landscape is composed of mosaics of multiple vegetation types, including monoculture and mixture stands, grasslands, and natural forests.

Study design and sampling

The design and sampling methods are explained in detail in Mori et al. (2016) and Fujii et al. (2017). Although we used the same study setting and a partly overlapping dataset with Mori et al. (2016) and Fujii et al. (2017), this study completely differs from them. Specifically, while the previous studies investigated the effects of fungal richness on ecosystem functioning, we instead focused on the fungal community structure under different restoration treatments.

We used a natural experiment with a 4 × 2 factorial design — namely, four habitat types and the inside/outside of deer exclosures. The four habitat types were (i) monoculture stands reforested with *Larix kaempferi*, (ii) mixture stands reforested with *Abies sachalinensis*, *Picea glehnii*, and *Betula ermanii*, (iii) grasslands dominated by a dwarf bamboo species (*Sasa cernua*) as a negative control group (i.e., the initial state of restoration), and (iv) mixed conifer–broadleaf natural forests dominated by *A. sachalinensis*, *Quercus crispula*, and *Kalopanax septemlobus* as a positive control group (i.e., the reference state of restoration).

The deer-exclusion and control sites were established adjacent to each other (i.e., inside and outside of deer fences) in each habitat type. Each site was ~1 ha in size. All the sites were distributed within an area of 2 km × 5 km (Supplementary Fig. S1). Given the restricted number of reforested stands and deer fences in the region, our experiment consisted of eight sites with one replication for each factorial combination. Reforested stands and deer fences were respectively established >30 years and ca. 10 years prior to our field sample collection (Fujii et al., 2017). The present vegetation inside and outside deer exclosures show significant structural differences (Fujii et al., 2017; Nishizawa et al., 2016) (Supplementary Fig. S2).

The collection of soil samples and chemical analyses were conducted as previously described (Fujii et al., 2017; Mori et al., 2016). Briefly, in May 2013 we established three 10 m × 10 m plots in each of the eight factorial combinations. In each plot, we randomly selected three points and collected topsoil from a 0–5 cm depth at each point (i.e., totalling 72 soil samples). Soil samples were transported from the field on ice and kept at −20°C until further analysis. Soil water content, pH, total carbon (C) and nitrogen (N) content, and inorganic N (ammonium and nitrate) content were measured to characterize soil properties. Total C and N content were measured using an organic elemental analyzer (Macro Coder JM1000CN, J-Science Lab Co., Ltd., Kyoto, Japan). Ammonium and nitrate were extracted from soil using a 2-M KCL solution and then measured with an auto-analyzer (AACS-4, BL-TEC Co., Ltd., Osaka, Japan).

Molecular analyses and bioinformatics

Molecular analysis and bioinformatics were conducted as previously described (Fujii et al., 2017; Mori et al., 2016) and are explained in detail in Matsuoka et al. (2016b). Briefly, total DNA was extracted from each of the 72 soil samples (0.25 g sample^{-1}) using the Soil DNA Isolation kit (Norgen Biotek Corp., Thorold, ON, Canada). A semi-nested polymerase chain reaction (PCR) was then performed to amplify the nuclear internal transcribed spacer 1 region. The pooled amplicons were sequenced with a GS Junior sequencer (454 Life Sciences, Branford, CT, USA).

The reads were clustered with a cut-off sequence similarity of 97% (Osono, 2014) using the Minimus genome assembler (Sommer et al., 2007). Consensus sequences were used as molecular operational taxonomic units (OTUs). A total of 389 OTUs were obtained. For each OTU, taxonomic identification was conducted using the QCauto method implemented in Claident (Tanabe and Toju, 2013). Hereafter, we refer to 'OTUs' as 'species' for simplicity, bearing in mind that OTUs defined by a fixed sequence similarity do not necessarily represent species in a biological sense. The functional group of each species was determined based on the FUNGuild database (Nguyen et al., 2016) and an intensive literature review. See Supplementary Materials for details of molecular analyses and bioinformatics. Raw sequence data files are available at the DNA Data Bank of Japan (DRA003024).

Community structure analyses

We defined fungal alpha diversity as the number of species within a community (i.e., a soil sample) and beta diversity as the extent of community dissimilarity within a treatment combination. We tested the effects of habitat types, deer fences, and their interactions on fungal alpha diversity using two-way analysis of variance (ANOVA) and Tukey's HSD test with 'plot' as a random effect. Variation among samples in sequencing depths (i.e., read counts), which can bias alpha diversity estimations, was standardized by rarefying read numbers. We used two rarefaction methods, namely sample size-based and coverage-based rarefactions (Chao and Jost, 2012), to confirm the robustness of results. In addition to habitat types, deer fences, and their interactions, we also included soil properties (pH, total C, total N, C:N ratio, inorganic N, and water content) as explanatory variables in separate ANOVA and linear regression analyses to account for the potential confounding effects these variables could have on alpha diversity. The effects of habitat types, deer fences, and their interactions on soil properties were tested by two-way ANOVA and

Tukey's HSD tests.

To detect the types of rank-frequency distribution of the species in each treatment combination, four models — preemption, log-normal, Zipf, and null models (Wilson, 1991) — were fitted to the distribution of occurrence by species rank (i.e., the number samples in which species occurred vs. species rank). The best model was selected based on the Akaike information criterion (AIC).

The effects of habitat types, deer fences, and their interactions on fungal community composition were tested using two-way permutational multivariate analysis of variance (PERMANOVA). The compositional dissimilarity between each treatment pair was tested using pairwise PERMANOVA where *P*-values were adjusted using the Bonferroni correction method. We compared beta diversity among the treatment combinations using the homogeneity of multivariate dispersions test (Anderson, 2006). The communities were ordinated using nonmetric multidimensional scaling (NMDS). The effects of soil properties on fungal community composition were tested by fitting their vectors onto the NMDS ordination. For all the above community dissimilarity analyses, we used two dissimilarity measurements — the Jaccard (Jaccard, 1912) and Raup–Crick indices (Raup and Crick, 1979) — to confirm the robustness of results. We used the indices based on presence/absence information in order to minimize the possible influence of read-count biases resulting from interspecific variation in the number of ribosomal DNA tandem repeats and from PCR processes (Toju, 2015). The habitat preferences of each fungal species and functional group were tested based on the association between their occurrence patterns and treatment combinations (De Cáceres et al., 2010). All statistical analyses were implemented in R 3.5.1 (R Core Team, 2018). The R packages used are listed in Supplementary Table S1.

Results

Fungal alpha diversity was 1.9 to 2.9 times greater in grasslands, monocultures, and mixtures than in natural forests (Fig. 1). It was confirmed that the sample size- and coverage-based rarefactions, as well as the case without rarefaction, yielded qualitatively consistent results; therefore, the results of the last two are provided in Supplementary Fig. S3. The effect of deer exclosures on fungal alpha diversity was not significant (Figs. 1, S3). The interactions between habitat types and deer exclosures also had no detectable effect on alpha diversity (Figs. 1, S3). Soil properties (i.e.,

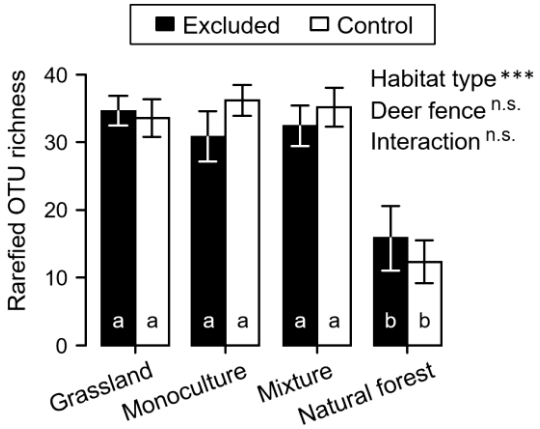


Figure 1. Effects of habitat type and deer fence on rarefied fungal operational taxonomic unit (OTU) richness (i.e., alpha diversity). Results from two-way ANOVA is shown in the upper right; *** $P < 0.001$; n.s. $P \geq 0.05$. Different letters indicate significant differences ($P < 0.05$) among treatments (Tukey's test). Error bars indicate standard errors.

pH, total C, total N, C:N ratio, inorganic N, and water content) varied among the treatment combinations (Fig. S4). Habitat type was the most significant predictor of fungal alpha diversity even when we included the soil properties as explanatory variables in ANOVA (Table S2). Regression analyses which controlled for the effect of soil pH (which was found to be a significant variable in ANOVA) also showed that alpha diversity was lower in natural forests than in grasslands (Table S3; Fig. S5).

The frequency of occurrence against species rank decreased less steeply in grasslands, monocultures, and mixtures than in natural forests (Fig. 2). Based on AIC, the preemption model was selected as the best model for habitats other than natural forests. The Zipf model was selected for natural forests. Gamma diversity (i.e., the total number of species present in each treatment combination) was 1.3 to 1.9 times greater in grasslands, monocultures, and mixtures (>130 species) than in natural forests (≤100 species) (Fig. 2).

Fungal community composition differed significantly among the habitat types (Fig. 3a, b). We confirmed that the Jaccard and Raup–Crick indices qualitatively yielded the same results; therefore, results from the Raup–Crick index are provided in Supplementary Fig. S6. The effect of deer exclosures on community composition was not significant (Figs. 3a, S6a). The interaction

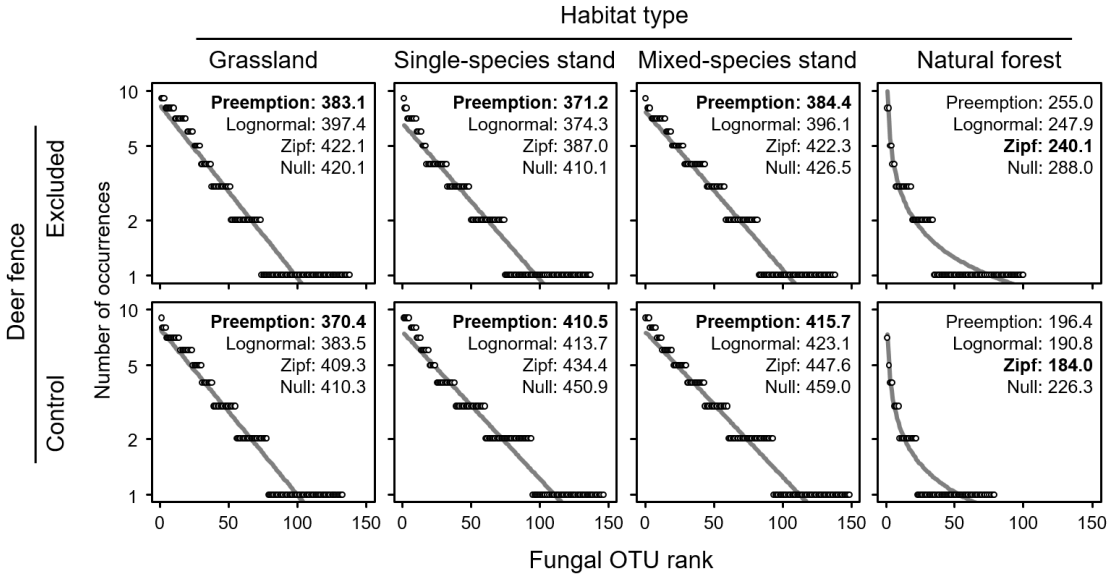
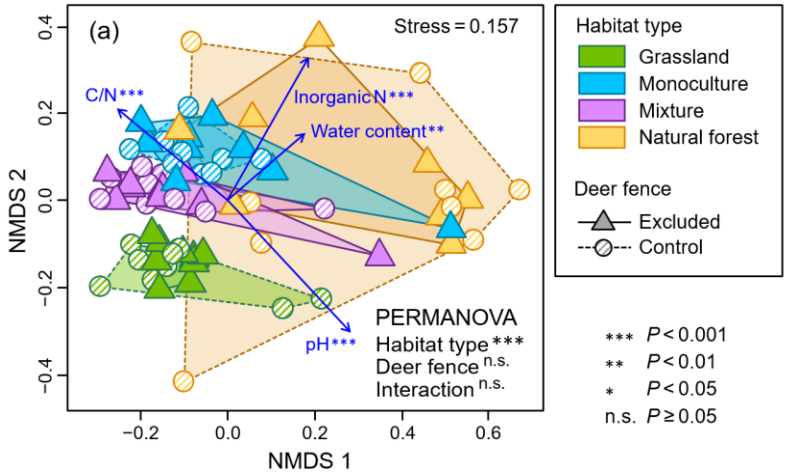


Figure 2. Relationships between occurrence frequencies of fungal operational taxonomic units (OTUs) and their rank. The *y* axes indicate the number of times each OTU occurred out of the nine replications in each treatment. The OTUs are arranged in decreasing order of occurrence frequency. Note that the maximum number of rank (on the *x* axis) in each panel equals the total number of OTUs observed in each treatment (i.e., gamma diversity). The numbers next to the model names indicate the Akaike information criterion (AIC). Curves show the selected model (shown in boldface), based on AIC, which was fitted to the distribution.

between reforestation types and deer exclosures also had no significant effect on community composition. The soil properties were significantly correlated to community composition (Figs. 3a, S6a). Increases in inorganic N and water content were mainly associated with shifts in fungal composition among habitat types (i.e., arrows roughly paralleled shift direction), whereas soil pH and C:N ratio were associated with community dissimilarity within each habitat type (Figs. 3a, S6a). Fungal beta diversity was significantly higher in natural forests compared to the other three habitat types (Figs. 3c, S6c).

Most fungal species and functional groups occurred more frequently in certain habitat types (Fig. 4). For example, ectomycorrhizal fungi, ericoid mycorrhizal fungi, and other symbionts that coexist with Ericaceae species occurred more frequently in monocultures and mixtures than in the other two habitat types. Conversely, the majority of fungal species and functional groups



(b)

Pairwise-PERMANOVA	Grassland	Monoculture	Mixture
Monoculture	**	–	–
Mixture	**	**	–
Natural forest	**	**	**

(c)

Beta diversity	Grassland	Monoculture	Mixture
Monoculture	n.s.	–	–
Mixture	n.s.	n.s.	–
Natural forest	**	*	*

Figure 3. Dissimilarity of fungal communities within and among treatments. (a) Ordination of communities based on nonmetric multidimensional scaling (NMDS) and the effects of treatments (habitat type and deer fence) on community composition tested by two-way permutational multivariate analysis of variance (PERMANOVA). Community dissimilarity was measured using the Jaccard index. Arrows show the associations of soil properties with community composition. (b) Community dissimilarity between pairs of vegetation types. (c) Among-vegetation differences in the size of within-vegetation community dissimilarity (i.e., beta diversity) tested by the permutation test of homogeneity of multivariate dispersion (PERMDISP).

occurred at approximately the same frequency both inside and outside deer fences (Fig. 4). Exceptions were *Mortierella* sp. 1 and coprophilous fungi that more often occurred outside than inside fenced exclosures.

Discussion

Effects of tree diversity and herbivores on soil fungi

Tree planting and herbivore exclusion are among the globally conducted approaches in terrestrial restoration (Kardol and Wardle, 2010; Verheyen et al., 2016). In this study, we tested the effects of these restoration practices on soil fungal communities using a natural experiment. Most notably, we found that local fungal richness (alpha diversity) was 1.9 to 2.9 times greater in grasslands and monoculture and mixture stands than in natural forests (Fig. 1). This result was contrary to the often reported, positive association between aboveground and belowground diversity (Peay et al.,

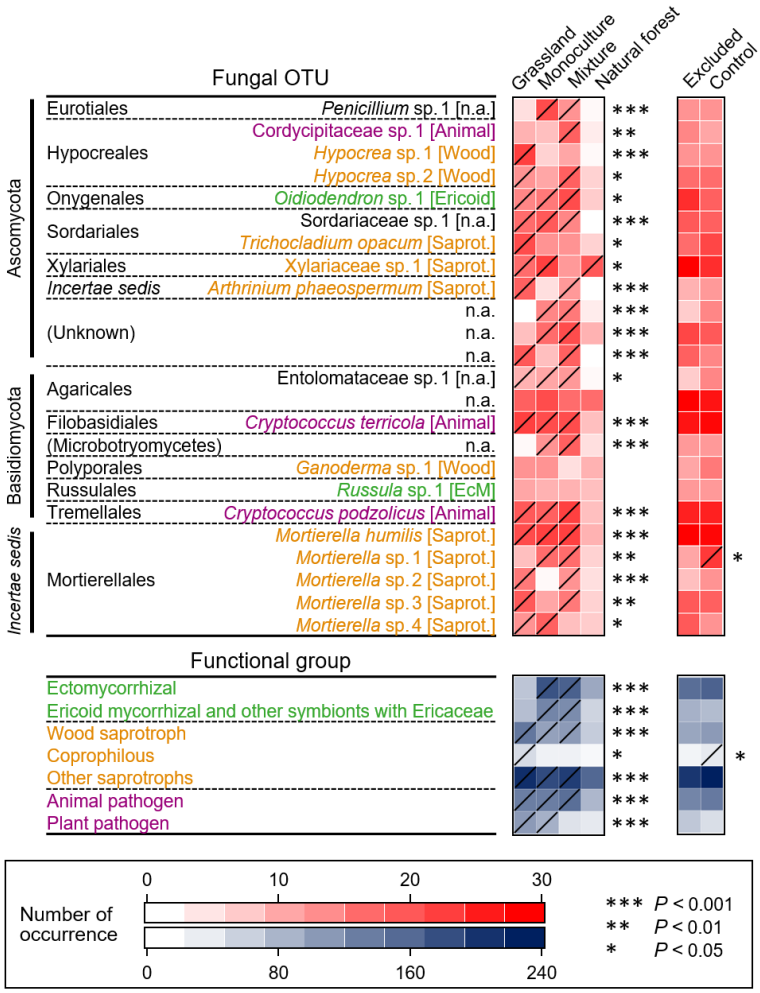


Figure 4. Differences in occurrence frequency of fungal operational taxonomic units (OTUs) and functional groups among restoration treatments. OTUs and functional groups with adequate sample sizes (OUTs occurring at >25 sampling points out of 72 points and functional groups having >3 OTUs) are presented. The transverse lines in boxes indicate treatments in which a given species or a functional group occurred significantly more often than in other treatments.

2013; Prober et al., 2015). We also found that fungal beta diversity and rank-frequency distribution in reforested stands were more similar to those of grasslands compared to natural forests, regardless of the number of tree species planted (Figs. 2, 3). The total fungal richness (gamma diversity) was 1.3 to 1.9 time greater in grasslands and reforested stands than in natural forests (Fig. 2). The reforested stands shared more indicator species and functional groups with grasslands than with natural forests (Fig. 4). These results stand in contrast with the fact that aboveground

vegetation in our restoration sites is steadily recovering (Nishizawa et al. 2016, Fujii et al. 2017; Fig. S2). Previous studies on naturally regenerating forests showed that historical effects of past land-use activity (e.g., farming, logging) on soil fungal communities can last for decades (Bachelot et al., 2016; Hartmann et al., 2012). Our results further suggest that, even after decades of proactive aboveground restoration, the way soil fungi assemble in reforested stands still differs from how communities are structured in natural forests.

Deer exclusion had no detectable effect on soil fungal communities at the species level (Figs. 1, 2, 3a). Nevertheless, at the level of functional groups, coprophilous fungi occurred more frequently outside fenced exclosures than inside (Fig. 4), which is attributable to the input of deer faeces. Coprophilous fungi were also found most often in grasslands (Fig. 4), coinciding with the habitat preference of sika deer for grasslands over forests (Yabe, 1995). Such deer-induced changes in the functional composition of fungi may, in turn, affect ecosystem nutrient cycling (Kardol et al., 2014). In fact, a faeces decomposition experiment conducted at our study area (Yabe, 1995) revealed more rapid faeces decomposition (and thus nutrient release) in grasslands than forests. This indicates that, in our grassland sites, a positive feedback exists between faeces production, fungal activity, and the growth of plants on which deer feed (van der Wal et al., 2004). Conversely, we found no evidence to support the interactive effects of deer and tree diversity on soil fungi (Figs. 1, 3a). A previous study (Cook-Patton et al., 2014) found a diversity-derived reduction in seedling herbivory by white-tailed deer (*Odocoileus virginianus*) owing to the associational protection of palatable species by unpalatable species. We did not observe such associational effects presumably because sika deer unselectively feed on a wide variety of plants including unpalatable species when its population density is high (Takahashi and Kaji, 2001). Overall, the results indicate that aboveground plant–herbivore interactions in our experimental site have limited impact on the species diversity of soil fungi, yet have potential to affect their functional composition and ecosystem functioning.

Fungal community assembly

Understanding the ecological processes underlying diversity patterns can provide a critical step towards the development of theory-driven restoration (Mori et al., 2017). Here, based on a conceptual synthesis in community ecology (Vellend, 2010), we discuss three potential assembly processes by which the lower fungal alpha diversity and higher beta diversity in natural forests

than reforested stands (Figs. 1, 3a, 3c) can arise — that is, ecological selection across space and time, and dispersal limitation.

The first possibility is the selection under different degrees of spatial environmental heterogeneity. Increasing environmental heterogeneity can increase beta diversity (i.e., the dissimilarity among local communities within each treatment combination) through the selection of different species at different sites (Mouquet and Loreau, 2003). The high fungal beta diversity we found in natural forests (Fig. 3a, c) is attributable to the fact that natural forests often have higher habitat heterogeneity compared to grasslands and plantations (Mori, 2011). The greater variation in water content and inorganic N of natural forests compared to grassland and reforested stands (Fig. 3a) also coincides with previous findings that soil properties are often homogenized in ex-arable land (Bachelot et al., 2016; Fraterrigo et al., 2005). Moreover, it is often expected that environmental heterogeneity increases alpha diversity (i.e., local species richness) via source-sink effects (Pulliam, 1988). Nonetheless, a theory by Kadmon and Allouche (2007) suggests that environmental heterogeneity can reduce the available habitat size of each species and, thus, could conversely decrease overall species richness. According to this theory, the low alpha diversity in natural forests (Fig. 1) could have derived from the high environmental heterogeneity that brought some species to such low abundances that they go locally extinct.

The second possibility is the time dependency in community response to environmental conditions. Constrained rates of mortality and reproduction prevent species from going extinct immediately, even when their population growth rates are negative. The higher alpha and lower beta diversity in reforested stands compared to natural forests (Figs. 1, 3a, 3c) can be interpreted simply that the process of local species selection has not yet been completed in reforested stands, regardless of environmental variation *per se*. Moreover, the long duration of time in natural forests could have allowed the effect of species' arrival order to be amplified and thereby the communities to diverge (i.e., priority effects; Fukami, 2015). In fact, a multi-year monitoring study of fungal communities (Matsuoka et al. 2016a) found that compositional similarities among communities were largely explained by the closeness in the time of survey. This suggests that the elapsed time after the onset of community assembly can have a major control on fungal diversity patterns observed in the field. Furthermore, species occurrence patterns in grasslands and reforested stands exhibited the preemption (geometric) distribution (Fig. 2). This type of distribution is often found in the early stage of succession for various taxa, including fungi (Visser, 1995), plants (Whittaker,

1965), and soil invertebrates (Caruso and Migliorini, 2006). We note that caution is needed in comparing our study to previous studies because we used rank-frequency distributions instead of rank-abundance distributions due to the methodological concerns in high-throughput DNA data analyses (Toju, 2015). Nonetheless, we do offer a potential interpretation that fungal species in our reforested sites are still in the course of ecological selection even after decades of tree planting.

The third possibility is dispersal limitation. Despite the small size and immense number of propagules, dispersal limitation is now acknowledged as a crucial determinant of fungal community structure (Peay et al., 2010). Fungal diversity patterns in our experiment (Figs. 1, 3a, 3c) can be explained by dispersal limitation at two conceptual scales: external dispersal from outside the set of local communities (as assumed in the mainland-island model) and internal dispersal among the local communities (as described in the metacommunity model) (Fukami, 2015). In the former scenario, the low beta diversity of grasslands and reforested stands (Fig. 3a, 3c) can be explained by limitations in the external dispersal of habitat specialists, which may have led the communities to become composed of a same suite of generalists, irrespective of local habitat conditions (Vellend et al., 2007). Indeed, a study that compared fungal community structure in primary forests and artificial pastures (Mueller et al., 2016) reported an increase in dominance of generalist fungi in pastures. Under the latter metacommunity scenario, maximal alpha and beta diversity is predicted to occur at intermediate and low levels of internal dispersal, respectively (Mouquet and Loreau 2003). This indicates that the low alpha and high beta diversity of natural forests (Figs. 1, 3a, 3c) resulted from limited within-treatment dispersal. This view is further supported by the presence of competition-dispersal tradeoffs among fungal species (Peay et al., 2007; Smith et al., 2018); that is, only species with high competitive but low dispersal abilities were able to subsist in natural forests.

Future challenges

We found clear differences in fungal community structure among restoration treatments, but uncertainty still persists regarding causal relationships among some variables. Specially, given the lack of site-level replication for each treatment in our experiment, we cannot explicitly separate the effects of human activities (i.e., restoration treatments and past agricultural practices) from the among-site environmental variation that might have existed from before (e.g., soil types). For example, we found that the C:N ratio was relatively low in the natural forests (Fig. S3), similarly

to what was found for fungal richness (Fig. 1); this similarity, however, can be due to either human activities or the original environment of the sites. Future studies with multiple site replications are thus required in order to draw conclusions about causalities among the variables (e.g., potential reductions in species richness caused by increased N availability; Cline et al., 2018).

Nevertheless, some of our results did, in fact, indicate that the fungal community structure was driven by the human activities in the study sites. Most notably, adding the measured soil properties as explanatory variables to the statistical models did not alter our result that fungal richness differs among habitat types (Table S2). Additional analyses showed that natural forests had the lowest fungal richness even when we controlled for the soil pH which negatively affected the richness (Table S3; Fig. S5). The fact that all the study sites have the same soil type (low-humic allophanic Andosols; Fig. S1) further supports the possibility that the soil environment was homogeneous across the sites prior to the treatments and land-use changes. Moving forward, accumulations of additional field data will allow us to explicitly disentangle the relationships among site conditions, human activities, and fungal diversity.

Implications for restoration

In this study, we investigated soil fungal communities in a restoration landscape and found that aboveground-oriented restoration treatments (i.e., tree planting and herbivore exclusion) do not necessarily translate into the recovery of fungal diversity. Our results suggest that in order to enhance the recovery of soil fungi, direct intervention to the soil, in conjunction with the application of vegetative treatments, may be necessary. For example, supplying organic substrates (e.g., deadwoods) to the soil surface can help create additional habitats for soil fungi (Mäkipää et al., 2017). Especially in restored sites with homogenized community structure like ours (Fig. 3a, 3c), creating mosaics of habitat patches by supplemental substrates could increase environmental heterogeneity and thus beta diversity. Another commonly applied approach in soil restoration is fungal inoculation, although caution is needed because fungal inoculation can occasionally cause negative impacts on the ecosystem (Janoušková et al., 2013). In fact, in our study site, natural forests had the lowest fungal alpha diversity (Fig. 1), indicating that simply adding multiple species to degraded land may not necessarily shift the community structure to that of natural forests. Rather, it might be effective to selectively inoculate a small number of species that could otherwise not reach the sites, considering the limited colonization of habitat specialists in our restored sites (as

indicated by low beta diversity; Fig. 3a, 3c). Furthermore, inoculating fungal species in different order at different locations can increase beta diversity via priority effect. We believe that such direct treatments to the soil and belowground biota will allow us to better enhance the recovery of degraded ecosystems.

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