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Brief Report

Different patterns of belowground fungal diversity along altitudinal gradients with respect to microhabitat and guild types

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Summary

Fungi are key components of belowground ecosystems with various ecological roles in forests. Although the changes in the richness and composition of belowground fungi across altitudinal gradients have been widely reported, only a few studies have focused on the microhabitat types along altitudinal gradients. Here, we analysed the effect of altitude on the ectomycorrhizal and non-ectomycorrhizal fungal communities in belowground microhabitats. We collected root and soil samples from 16 *Pinus densiflora* forests at various altitudes across Korea, and measured the soil properties as potential factors. Fungal communities were analysed by high-throughput sequencing of the internal transcribed spacer 2 (ITS2) region. We found that altitude negatively affected the species richness of root-inhabiting fungi but did not influence that of soil-inhabiting fungi. In addition, the composition of ectomycorrhizal (ECM) fungi was less influenced by altitude than non-ECM fungi. Most of the soil properties did not show a significant relationship with altitude, but the effect of soil properties was different across microhabitat types and ecological roles of fungi. Our results reveal that microhabitat types and altitudinal gradients differently affect the richness and composition of fungal communities associated with *P. densiflora*, providing a better understanding of plant-associated fungal communities.

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Introduction

Belowground fungi play an important role in nutrient cycle of forest ecosystems (Baldrian, 2016; Peay *et al*., 2016). Distinct fungal communities widely interact along a continuum of mutualism to parasitism with vascular plants and other microbes in root and soil microhabitats (Johnson *et al*., 1997; Baldrian, 2016). Previous studies have reported that species with different functions inhabit different microhabitats in reliant to exudates from plants (Clemmensen *et al*., 2013; Wang *et al*., 2020). For example, in roots, mycorrhizal fungi establish associations with the majority of plants in the form of ectomycorrhizal fungi (ECM), arbuscular mycorrhizal fungi, and ericoid mycorrhizal fungi (Smith and Read, 2008; Baldrian, 2016). Fungal endophytes and potentially endophytic saprotrophs are also commonly observed in the roots, facilitating the nutrient acquisition of their plant host and improving their tolerance to stress (Hiruma *et al*., 2016; Almario *et al*., 2017). In addition, soils harbour saprotrophic fungi that decompose dead wood and redistribute nutrients into the soil (Lindahl *et al*., 2007). Mycorrhizal fungi also spread mycelia around the soil (Prescott and Grayston, 2013) to mobilize limiting nutrients (Lindahl and Tunlid, 2015), link multiple trees and understory vegetation, and transport carbon through the mycelial network (Högberg *et al*., 1999). Because of their essential roles in ecosystem processes, belowground fungi are important for forest management and ecosystem restoration (Mandyam and Jumpponen, 2005; Porras-Alfaro and Bayman, 2011). However, our understanding of the belowground ecosystem of forests is still limited due to the complexity of belowground fungal species (Porras-Alfaro and Bayman, 2011; Tedersoo *et al*., 2014), diverse forms of plant–fungi interactions (Toju and Sato, 2018), and the variable spatial structure within belowground forest.

The distinct distribution patterns of organisms along altitudinal gradients have been studied by ecologists for long. Previous studies on altitudinal patterns have focused on various macroorganisms (Ohsawa and

Ide, 2008; Rowe, 2009; Sundqvist *et al*., 2013). Recently, owing to advances in molecular ecology techniques, altitudinal patterns in belowground bacterial communities have been revealed (Bryant *et al*., 2008; Fierer *et al*., 2011; Sundqvist *et al*., 2013). However, only a few studies on the changes in fungal community composition along altitudinal gradients have been reported. Intriguingly, the altitudinal patterns of alpha diversity showed inconsistent or non-significant patterns (Bahram *et al*., 2012; Coince *et al*., 2014; Matsuoka *et al*., 2016; Geml, 2017). Furthermore, in some studies on multiple vegetation with different hosts, the identity of vegetation had a greater effect on the community than abiotic factors (Bahram *et al*., 2012; Miyamoto *et al*., 2014). Only a few studies have focused on a single host that minimize the effect of host identity on the altitudinal patterns of the fungal community (Coince *et al*., 2014; Scattolin *et al*., 2014; Jarvis *et al*., 2015; Rincón *et al*., 2015). However, while the composition changes in the fungal community were found, significant changes in species richness were not observed in in those studies (Coince *et al*., 2014; Jarvis *et al*., 2015).

To the best of our knowledge, there are only a few reports on how altitudinal gradients are related to fungi in different belowground microhabitats (Bernard *et al*., 2020) or different guilds (Yao *et al*., 2017). Although these studies provided valuable insights of the relationship between altitudinal gradients and fungal communities, the relationship between altitudinal gradients and the fungal community of different microhabitats or guilds remain unknown.

To this end, we examined how altitudinal gradients affect belowground fungal communities in different microhabitats (root and soil) and different guilds (ECM and other non-ECM guilds). We selected the widely distributed ectomycorrhizal tree *Pinus densiflora* Sieb. et Zucc. (red pine) as a target species to minimize the host effect and focus on altitude and microhabitat. In the present study, the composition and diversity of fungal communities were compared between microhabitats and altitudinal gradients, and the results were interpreted according to the fungal guilds and soil properties. We expected a significant change in community compositions across altitudinal gradients in both microhabitats and guilds, especially in the fungal communities that are not directly associated with *P. densiflora*. In species richness level, we hypothesized that plant-associated fungi (i.e. ECM or root-inhabiting fungi) would be more affected by altitudinal gradients due to change in host fitness along the altitude. Our findings would offer a more comprehensive understanding of the fungal interactions between root and soil microhabitats. A clear understanding of these changes can help to predict the reactions of microbial diversity to changing environments and climate (Bryant *et al*., 2008).

Results

The root and soil samples from 16 *P. densiflora* forests were collected (Fig. 1A, Table 1). Five healthy-looking trees were randomly selected from each forest. 5.8S and ITS2 regions of fungal rRNA were successfully amplified using 5.8S-Fun and ITS4-Fun primers (Taylor *et al*., 2016), then performed high-throughput sequencing of Illumina MiSeq platform. Five soil samples of each sampling site were mixed, then following soil properties were measured (Supporting Information Table S1): pH, total organic carbon (TOC), total nitrogen (TN), total

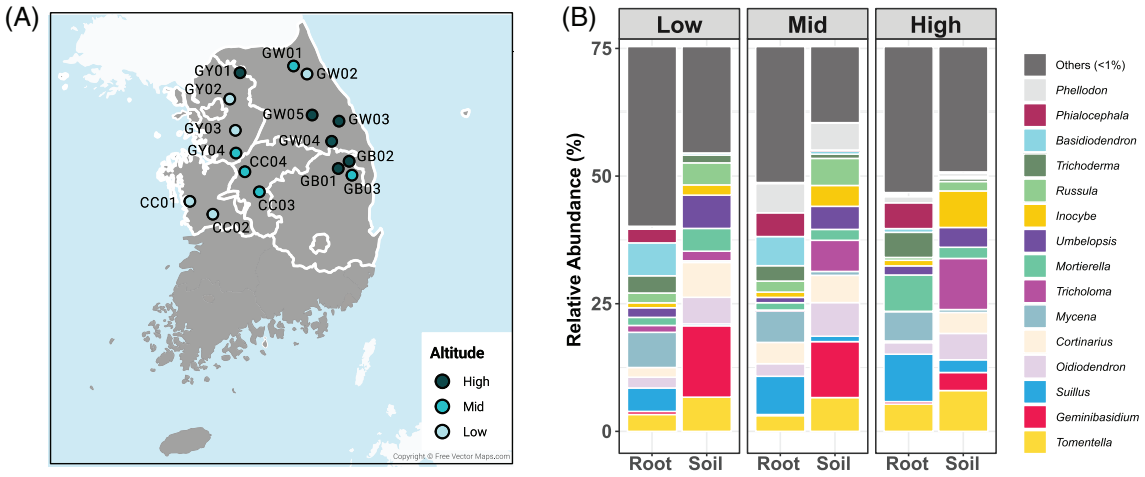

Fig 1. Sampling sites and composition of fungal communities in this study.A. Circles indicate the sampling locations, and the various colours indicate the range of altitude.B. Taxonomic assignments of the major genera (>1% in relative abundance) at the genus level (altitude ranges: high >650 m, mid 300–650 m, low <300 m).

Table 1. Characteristics of sampling sites.

ID	Latitude (°)	Longitude (°)	Altitude (m)	Altitude classification	Sampling date
CC01	36.422933	126.614875	205	Low	17 June 2019
CC02	36.2879	126.96868	185	Low	17 June 2019
CC03	36.538444	127.763163	465	Mid	18 June 2019
CC04	36.811423	127.533476	330	Mid	18 June 2019
GY01	37.980825	127.456962	649	Mid	10 June 2019
GY02	37.682968	127.293288	220	Low	10 June 2019
GY03	37.282817	127.371432	230	Low	14 June 2019
GY04	37.02884	127.391773	425	Mid	05 June 2019
GW01	38.062439	128.306534	618	High	12 June 2019
GW02	37.965	128.520828	229	Low	11 June 2019
GW03	37.394307	129.028686	673	High	20 May 2020
GW04	37.150021	128.912155	1333	High	20 May 2020
GW05	37.468468	128.601944	742	High	11 June 2020
GB01	36.820801	129.016922	657	High	19 May 2020
GB02	36.9081	129.189484	825	High	20 May 2020
GB03	36.739689	129.234329	618	Mid	19 May 2020

phosphorus (P), soil moisture, replaceable potassium (K), and ratios C:N (based on TOC/TN). Detailed information of experimental procedures was described in the Supporting data 1.

Overall characteristics of fungal communities

The fungal community structure associated with *P. densiflora* was determined for 16 root and soil samples in South Korea (Fig. 1A; Table 1). In total, 11 150 431 rRNA sequence reads were obtained after quality filtering, and 10 712 967 reads were further analysed after filtering singletons and non-fungal sequences. The average length of sequence reads was 386.01 $\pm$ 48.18 base pairs. After post-clustering, filtering rare OTUs (≤10 reads or ≤5 samples) and rarefaction process, 2206 OTUs were found in total dataset. From each microhabitat, 1831 OTUs and 1,928 OTUs were found in the roots and soil, respectively. On average, 195.86 $\pm$ 53.13 OTUs and 222.76 $\pm$ 71.42 OTUs were found in each root and soil sample, respectively. Good's coverage ranged from 0.991 to 0.998 with an average of 0.996 $\pm$ 0.001, indicating that our sequencing depth was sufficient to represent most of the fungal OTUs (Supporting Information Table S2).

Fungal community composition

The composition of belowground fungi varied between the root and soil microhabitats. At the genus level, *Tomentella*, *Geminibasidium*, *Suillus*, *Oidiodendron*, and *Cortinarius* were the most abundant taxa (Fig. 1B; Supporting Information Table S3). Among the ectomycorrhizal genera, *Suillus* and *Phellodon* showed higher relative abundance (sequence reads count of specific OTU(s)/sequence reads count of all OTUs in the sample) in roots than in soil. In contrast, the relative abundance of *Tomentella* and *Cortinarius* was higher in the soil than in root. Meanwhile, non-ectomycorrhizal fungi also showed different relative abundances according to the microhabitats. The genera *Mycena*, *Trichoderma*, and *Basidiodendron* were more abundant in roots than in soil, whereas *Gemnibasidium*, *Umbelopsis*, and *Oidiodendron* were more abundant in soil (Supporting Information Table S3). Among the plant pathogenic fungi, *Venturia* was mostly found in root microhabitats but in relatively low abundance compared to other guilds. The relative abundance of *Geminibasidium*, *Umbelopsis*, and *Mycena* showed large differences across microhabitats (Fig. 1B).

The composition of belowground fungal communities also changed across altitudinal gradients. *Suillus* was more abundant in root samples collected at high altitudes, whereas *Geminibasidium* was more abundant in soil samples from low altitudes. Several genera, such as *Mortierella* and *Tricholoma*, showed opposite altitudinal patterns between root and soil samples (Fig. 1B, Supporting Information Table S3). Across all the sampling sites and microhabitats, 20 core genera (=genera that were present in all sampling sites and microhabitats) were identified (Supporting Information Fig. S1). Despite the relatively low number, they accounted for 37.50% (root) and 46.10% (soil) of total sequence reads. Most of them were saprotrophic fungi, but ectomycorrhizal fungi (*Cenococcum*, *Cortinarius*, *Rhizopogon*, *Russula*, and *Tomentella*), ericoid mycorrhizal fungi (*Oidiodendron*), endophytic fungi (*Phialocephala*, *Mortierella*) were also included in the list. However, their proportions were not stable across the sampling sites and altitudes.

In terms of ecological function, saprotrophs and ECM were the major guilds in both microhabitats. Saprotroph ($P < 0.01$), endophytes ($P < 0.001$), and saprotroph + plant pathogens ($P < 0.001$) were more abundant in roots,

whereas the ECM ($P < 0.001$) and ericoid mycorrhiza ($P < 0.001$) were more abundant in the soil (Fig. 2A). The relative abundance of saprotroph + endophytes was not significantly different between the microhabitats. Relative abundance of saprotroph + endophyte guild was higher in the soil than in the root at low altitudes, and the pattern was opposite at high altitudes ($P < 0.05$). Similarly, the relative abundance of ECM was significantly higher in the soil than in the roots only at high altitudes (Fig. 2B; $P < 0.001$).

Change in fungal diversity across microhabitats and altitudinal gradients

We found that microhabitat type had a significant effect on the community composition of belowground fungal communities (Supporting Information Table S4). However, their influence differed according to the ecological groups. According to the results of the permutational multivariate analysis of variance (PERMANOVA) test, the effects of microhabitat were higher in non-ECM community ($R^2 = 0.143$) than both the ECM ($R^2 = 0.053$) and the entire fungal communities ($R^2 = 0.097$). Unweighted pair group method with arithmetic mean (UPGMA) analysis showed that the composition of fungal communities between the two microhabitats was much more similar in ECM communities than in non-ECM communities (Supporting Information Fig. S2). In contrast, the influence of altitude was similar in both microhabitats and ecological groups (Supporting Information Table S4). Variation partitioning analysis showed that sampling sites, altitude, microhabitat, soil property and climate data explained about 44% (total), 49% (ECM), and 38% (non-ECM) in variation of fungal communities (Supporting Information Fig. S3). Similar to result of UPGMA analysis, microhabitat could explain 11% (total), 5% (ECM), and 13% (non-ECM) of fungal communities after excluding the effects of sampling sites and other factors.

PERMANOVA tests showed that various types of soil properties are related with changes in fungal communities in different microhabitats and ecological groups. In both microhabitats, altitude, pH, K, and P were significant factors in all ecological groups, while TN and soil moisture were significant factors in all ecological groups from soil microhabitats (Fig. 3). TOC and MAT were only significant factor in root ECM community, while AP was significant factor in root total fungal community, root nonECM fungal community and soil ECM fungal community (Supporting Information Table S4). Similar to the results of the PERMANOVA test, those of the Mantel test revealed that altitude exhibited distance–decay patterns with fungal communities, though the differences between roots and soil samples were varied to the ecological groups. The change of community composition across altitudinal gradients was much higher in non-ECM groups than that of ECM groups (Supporting Information Fig. S4). In contrast, the similarities of fungal communities were significantly correlated with geographic distances only in roots (Supporting Information Fig. S5). Overall, microhabitat, soil properties, and altitude were significantly correlated with the fungal community composition. In most microhabitats, non-ectomycorrhizal fungal communities were more affected by altitudinal differences than the total fungal or ectomycorrhizal fungal communities.

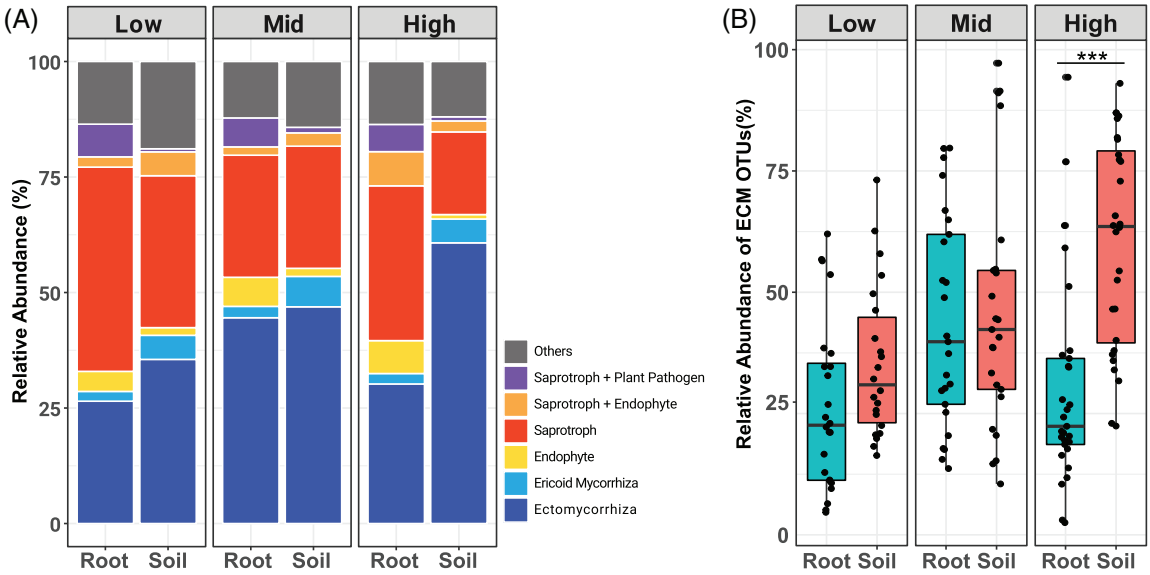

Fig 2. Composition and relative abundance of fungal communities in guilds. (A) Relative abundance of each guild and (B) the proportion of ectomycorrhizal fungi. Significance was determined using *t*-tests (*$P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$). Altitude ranges: high >650 m, mid 300–650 m, low <300 m.

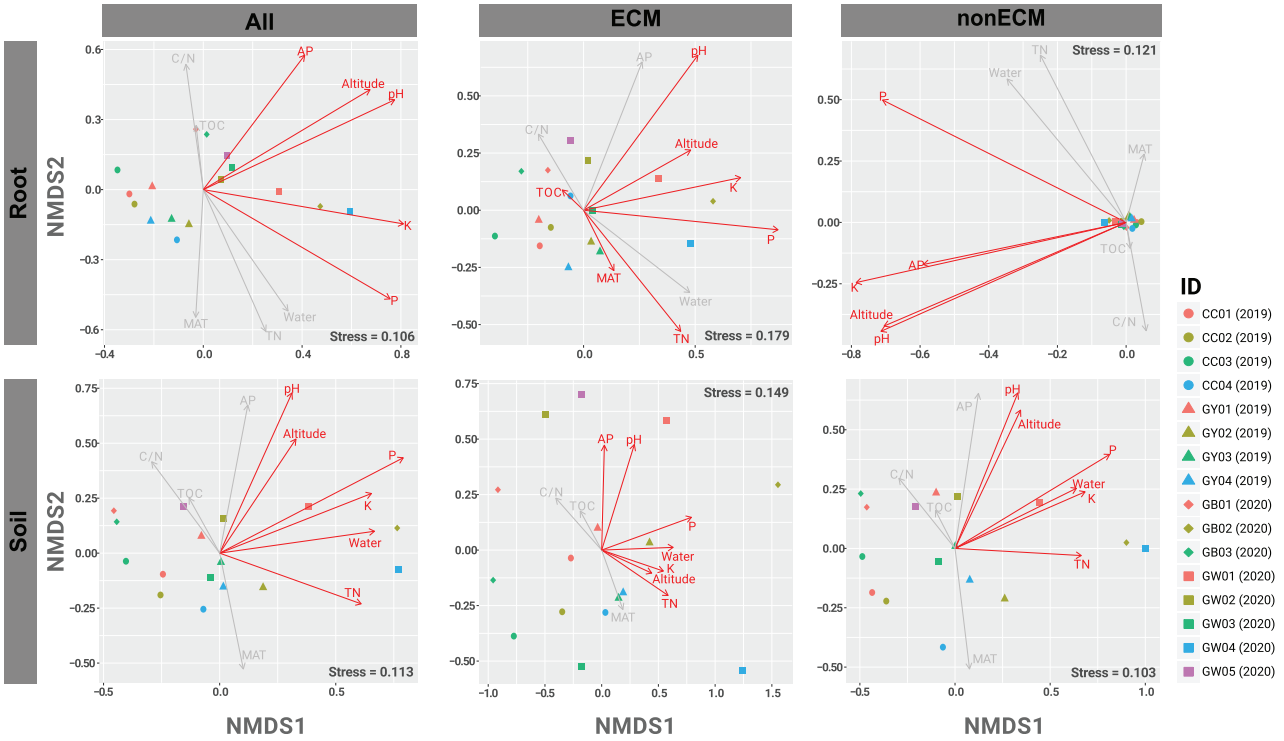

Fig 3. Non-metric multidimensional scaling plots calculated using Jaccard dissimilarity index for each ecological group and microhabitat. The sampling sites are displayed in different colours and shapes. Soil and climate properties with significant relationships ($P < 0.05$) are indicated by red arrows. TOC, total organic matter; TN, total nitrogen; K, replaceable potassium; P, total phosphorus; Water, soil moisture; C:N, carbon to nitrogen ratio; MAT, mean annual temperature; AP, annual precipitation.

The relationship of the alpha-diversity indices of fungal communities associated with *P. densiflora* and altitude varied according to microhabitats and ecological groups (Fig. 4). Both richness and diversity indices were higher in soil samples than in root samples. Species richness showed significant negative relationships with altitude in root samples, but not significant in soil samples. Diversity index was not significantly affected by altitudes in all groups, while non-ECM communities in soil exhibited different altitudinal pattern compared to other groups.

Discussion

We found that altitudinal gradients and microhabitats were both significant factors affecting the richness and composition of fungal communities without host vegetation change. However, their influence and pattern differed between both of microhabitats and ECM/non-ECM communities. Most of the soil properties did not significantly changed across altitudinal gradients, but ordination suggested that the effect of soil properties also influenced community variations in all microhabitats and ecological groups. To the best of our knowledge, this is one of the few studies that covered the altitudinal changes in fungal communities across different microhabitats and guilds. It is important to note that in contrast to previous studies, ECM fungal richness showed significant change along altitudinal gradients.

Similar to the findings of previous studies (Coleman-Derr *et al*., 2016; Bernard *et al*., 2020), we observed that microhabitat significantly affects belowground fungal community composition. However, in our study, the difference between microhabitats was much smaller in ECM communities than in non-ectomycorrhizal communities. The similar composition of ECM communities is likely due to the ECM mycelia in the soil as most ectomycorrhizal fungi form extraradical hyphae in the soil environment around colonized roots (Baldrian, 2016). Although ectomycorrhizal fungi in both microhabitats mostly overlapped, species richness and diversity were consistently higher in the soil than in the root. In addition, altitudinal gradients were significantly related with ECM community in soil, while not in root microhabitats. Several factors may explain the differences between microhabitats. First, inactive spore banks or relic DNA from dead mycelia may influence these differences in alpha diversity. Spore banks are formed by spores from nearby fruiting bodies that are spread by wind. Dormant spores stored in the soil can initiate associations if the right host plant is present (Taylor and Bruns, 1999) and can survive in soil for several years even in the absence of the right host (Miyamoto and Nara, 2016). Similarly, DNA

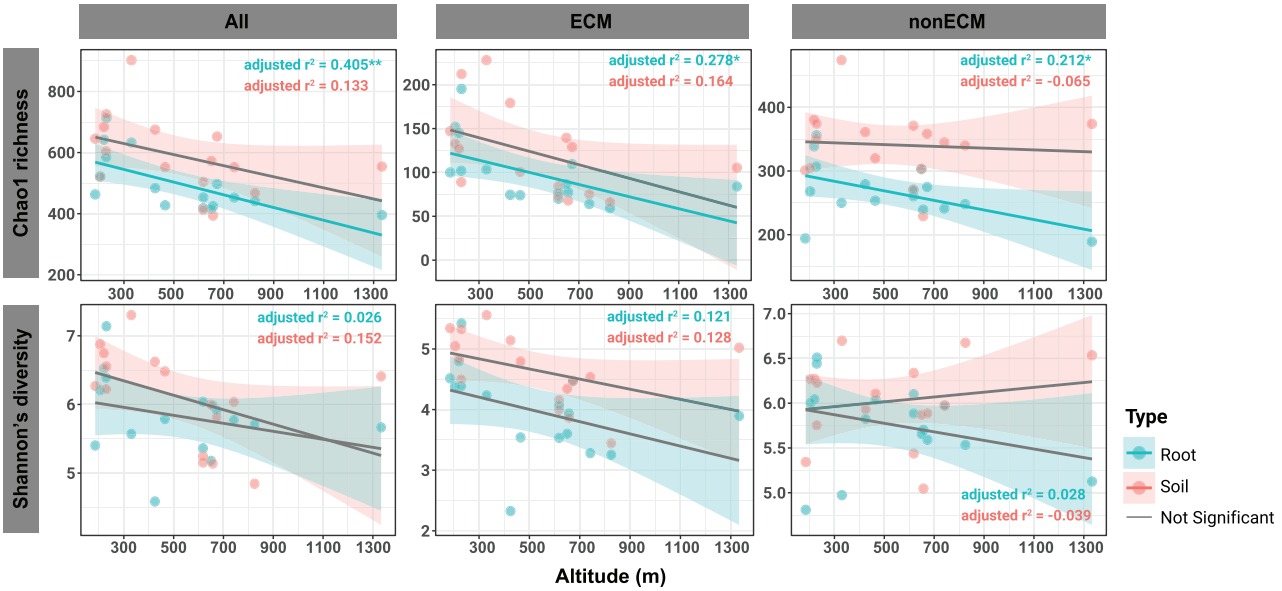

Fig 4. Relationship between altitude and alpha-diversity indices in root (blue) and soil (red) microhabitats. The line indicates linear regression fit, and the shaded band represents 95% confidence level. All: overall fungal communities; ECM: ectomycorrhizal fungal communities; non-ECM: non-ectomycorrhizal fungal communities. Non-significant relationships are marked with grey lines (*$P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$).

fragments from dead fungi have been detected for several years (Carini *et al*., 2016). Because we could not differentiate between active and inactive fungi using NGS, DNA from inactive fungi might have contributed to higher ectomycorrhizal fungal richness in soil in our data. Second, ECM fungi colonizing non-ectomycorrhizal hosts (Schneider-Maunoury *et al*., 2020) or herbaceous plants (Smith and Read, 2008) have been reported in previous studies. Mycelia from other plant hosts could have also influenced ECM richness in soil.

On the other hand, the composition of non-ECM fungi exhibited higher variation than ECM fungi between microhabitats. The clear functional separation may explain this difference. Most non-ECM genera that were abundant in roots were endophytes or plant pathogens, such as *Phialocephala*, *Mycnea*, and *Venturia*. *Phialocephala* is a well-known endophyte found in the roots of various host trees, including *P. densiflora* (Grünig *et al*., 2008). On the other hand, several *in vitro* studies suggested that *Mycena* can colonize ECM plant roots and promote growth of host plant (Thoen *et al*., 2020). *Venturia* is a plant pathogenic fungus that is widely distributed in the Northern temperate area (González-Domínguez *et al*., 2017). However, the presence of *Venturia* in the roots of *P. densiflora* has not been reported previously; therefore, further studies are needed to understand their relationship. In the soil, ericoid mycorrhizal fungi from *Oidiodendron* were more abundant than in the root. The presence of *Oidiodendron* in soil around the roots of ectomycorrhizal trees may be attributed to nearby understory vegetation or their saprobic activity in soil (Rice and Currah, 2007).

In addition, compositional changes were observed along the altitudinal gradients. Decrease in similarity of ECM communities following altitudinal gradients has been observed in most studies, irrespective of host identity control (Talbot *et al*., 2014; Geml, 2017; Yang *et al*., 2019). Interestingly, the effect of altitudinal gradients was weaker in the ECM than in the non-ECM groups of both microhabitats in our study. As our study focused on a single host species, the ECM community may have been less affected at the regional scale. Bahram *et al*. (Bahram *et al*., 2012) have reported that host tree species was the most important factors that affected the ECM community composition. In contrast, free-living fungi, such as saprotrophs, in soil are expected to be more affected by geographic distance than host-dependent fungi because the former are relatively more functionally overlapped (Talbot *et al*., 2014) and sensitive to species pools in the area (Rillig *et al*., 2019). Similarly, belowground endophytes in roots have been reported to be relatively host-independent (Glynou *et al*., 2016) and dispersal-limited, which may account for the differences in altitudinal and geographic patterns. However, other environmental factors such as annual temperature or soil chemical properties around individual trees could have also resulted in these differences (Bahram *et al*., 2012; Jarvis *et al*., 2015). In variation partitioning and PERMANOVA test, the sampling site explained the biggest variation in fungal communities (Matsuoka *et al*., 2016).

As our sampling was conducted in multiple areas, these results could be due to the combined effect of altitude, soil property, and climate data that are directly associated with sampling site. Although results from mantel tests showed that the influence of geographic distance does not correspond to that of altitudinal gradients, further studies would be required to separate the effect of spatial distance from the overall variations.

Significant decrease in richness was found along altitudinal gradients in the root fungal communities of *P. densiflora*, and diversity indices showed a similar pattern. Whereas altitudinal patterns of alpha diversity did not show significant change in the soil fungal communities. In previous studies, a decline in ECM species richness has been observed along altitudinal gradients in both microhabitats (Kernaghan and Harper, 2001; Bahram *et al*., 2012). However, studies with controlled host species have reported no relationship between ECM richness and the altitude in the roots of *Fagus sylvatica* (Coince *et al*., 2014). This could be due to the characteristics of the host tree (*P. densiflora*) in our study. The nutrient input from leaves and roots is different between conifers and broadleaves (Awad *et al*., 2019), as well as hyphal production and nutrient demands of ECM fungi across tree host species (Bakker *et al*., 2015; Rosinger *et al*., 2020); however, further studies are needed to clarify the effect of altitude on fungal richness associated with *P. densiflora*. Although the alpha diversity of the non-ECM group decreased at high altitudes in the root microhabitat, there was no significant change in soil. The lowered productivity of host trees might have affected the richness and diversity of associated fungi, i.e., ECM and root-associated non-ECM groups (Hiiesalu *et al*., 2017), as decreased growth and photosynthetic productivity of *P. densiflora* has been reported at high altitudes (Kim *et al*., 2020). The non-significant relationship between altitude and alpha diversity of soil-inhabiting fungi may suggest that the decomposition of organic matter and/or productivity of understory vegetation is less affected by altitude (Guo *et al*., 2013).

In case of soil properties and climate, pH, phosphorus, and potassium were associated with fungal community composition regardless of their microhabitats or ecological roles. However, it is hard to explain these results with altitude as most of soil properties were not affected by altitude, except for pH, in contrast to previous studies (Coince *et al*., 2014; Jarvis *et al*., 2015). Soil pH is an important factor in fungal communities, but the significance of pH's influence was varied by sampling sites and host identities (Rousk *et al*., 2010; Coince *et al*., 2014; Jarvis *et al*., 2015). While significant relationship was found between pH and fungal community composition in our study, it could be an indirect relationship as fungi have high tolerance to pH change (Rousk *et al*., 2010). Similarly, role of phosphorus on fungal community structure was already reported in several studies, but potassium's role in still unclear (Coince *et al*., 2014; Rosenstock *et al*., 2016; González-Domínguez *et al*., 2017). TN and soil moisture were significantly correlated with soil fungal communities. They could be important factors in soil fungal community due to fungi's high demand for nitrogen (Cox *et al*., 2010) and its narrow optimal range of soil moisture contents (Kaisermann *et al*., 2015; Erlandson *et al*., 2016). On the other hand, only ECM communities in root had correlation with MAT and the organic carbon in addition to other soil properties. Soil carbon contents (Coince *et al*., 2014; Mrak *et al*., 2020) and temperature (Cox *et al*., 2010; Coince *et al*., 2014) were both reported to have relationship with root ECM communities. Temperature can influence ECM communities by affecting the host plant, soil nutrients or physical tolerances. For organic carbon, the accumulation of photosynthetic products in soil from ectomycorrhizal fungi would be associated with this result (Smith and Read, 2008; Clemmensen *et al*., 2013; Erlandson *et al*., 2016).

Overall, our study provides a basis for understanding belowground fungal community compositions associated with *P. densiflora* in temperate forests along altitudinal gradients. Our results indicate strong correlations between richness and altitude in root-inhabiting fungal communities but not in soil-inhabiting fungal communities. Microhabitat types and guilds of fungal taxa are important factors in determining fungal community structures along altitudinal gradients, which highlights the need to study various ranges of microhabitat types to understand the factors that influence belowground fungal communities. By extending the targets to diverse types of plant hosts and microhabitats, these findings can provide a solid foundation for plant microbiome management and highlight the importance of small-scale variations in preservation of fungal diversity.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Supporting data 1. Experimental procedures.

Fig. S1. The relative abundance of core genera in each microhabitats.

Fig. S2. UPGMA dendrogram of all samples.

Fig. S3. Venn diagram of variation-partitioning analysis showing the effect of sampling sites, altitude, microhabitat, soil property (pH, total organic matter, total nitrogen, replaceable potassium, total phosphorus, soil moisture, carbon to nitrogen ratio) and climate data (mean annual temperature and annual precipitation).

Fig. S4. Mantel correlation plots calculated using Jaccard dissimilarity and the altitudinal differences between two microhabitats.

Fig. S5. Mantel correlation plots calculated using Jaccard dissimilarity and the geographical distances between two microhabitats.

Table S1. Soil properties of sampling sites. Abbreviations: MAT, mean annual temperature; AP, annual precipitation; TOC, total organic matter; TN, total nitrogen; K, replaceable potassium; P, total phosphorus; Water, soil moisture; C:N, carbon to nitrogen ratio.

Table S2. Alpha diversity indices including OTUs, chao1 richness, Shannon's diversity, equitability, and Good's coverage.

Table S3. List of genera (> 0.1% relative abundance) and their guilds.

Table S4. Detailed results of PERMANOVA tests. PERMANOVA tests were performed to identify the effects of environmental factors on fungal communities associated with the roots and soil of *P. densiflora*. The Jaccard dissimilarity index and abundance of OTUs were used for analysis with 999 permutations (*$p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$). Abbreviations: TOC, total organic matter; TN, total nitrogen; K, replaceable potassium; P, total phosphorus; MAT, mean annual temperature; AP, annual precipitation.