Network hubs in root-associated fungal metacommunities

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Abstract

Background: Although a number of recent studies have uncovered remarkable diversity of microbes associated with plants, understanding and managing dynamics of plant microbiomes remain major scientific challenges. In this respect, network analytical methods have provided a basis for exploring "hub" microbial species, which potentially organize community-scale processes of plant–microbe interactions.

Methods: By compiling Illumina sequencing data of root-associated fungi in eight forest ecosystems across the Japanese Archipelago, we explored hubs within "metacommunity-scale" networks of plant–fungus associations. In total, the metadata included 8,080 fungal operational taxonomic units (OTUs) detected from 227 local populations of 150 plant species/taxa.

Results: Few fungal OTUs were common across all the eight forests. However, in each metacommunity-scale network representing northern four localities or southern four localities, diverse mycorrhizal, endophytic, and pathogenic fungi were classified as "metacommunity hubs", which were detected from diverse host plant taxa throughout a climatic region. Specifically, *Mortierella* (Mortierellales), *Cladophialophora* (Chaetothyriales), *Ilyonectria* (Hypocreales), *Pezicula* (Helotiales), and *Cadophora* (incertae sedis) had broad geographic and host ranges across the northern (cool-temperate) region, while *Saitozyma*/*Cryptococcus* (Tremellales/Trichosporonales) and *Mortierella* as well as some arbuscular mycorrhizal fungi were placed at the central positions of the metacommunity-scale network representing warm-temperate and subtropical forests in southern Japan.

Conclusions: The network theoretical framework presented in this study will help us explore prospective fungi and bacteria, which have high potentials for agricultural application to diverse plant species within each climatic region. As some of those fungal taxa with broad geographic and host ranges have been known to increase the growth and pathogen resistance of host plants, further studies elucidating their functional roles are awaited.

Keywords: agriculture; biodiversity; ecosystem restoration; host specificity or preference; latitudinal gradients; metacommunities; microbial inoculation; network hubs; plant–fungus

47 interactions; mycorrhizal and endophytic symbiosis.

48

Background

Below-ground fungi in the endosphere and rhizosphere are key drivers of terrestrial ecosystem processes [1-4]. Mycorrhizal fungi, for example, are important partners of most land plant species, enhancing nutritional conditions and pathogen resistance of host plants [5-7]. In reward for the essential physiological services, they receive ca. 20% of net photosynthetic products from plants [8, 9]. Recent studies have also indicated that diverse taxonomic groups of endophytic fungi (e.g., endophytic fungi in the ascomycete orders Helotiales and Chaetothyriales) commonly interact with plant roots, providing soil nitrogen/phosphorous to their hosts [10-14], converting organic nitrogen into inorganic forms in the rhizosphere [15], and increasing plants' resistance to environmental stresses [16-18]. Because of their fundamental roles, below-ground fungi have been considered as prospective sources of ecosystem-level functioning in forest management, agriculture, and ecosystem restoration [17-20]. However, due to the exceptional diversity of below-ground fungi [21-23] and the extraordinary complexity of below-ground plant–fungus interactions [24-26], we are still at an early stage of managing and manipulating plant-associated microbiomes [27-29].

In disentangling complex webs of below-ground plant–fungus associations, network analyses, which have been originally applied to human relations and the World-Wide Web [30, 31], provide crucial insights. By using network analytical tools, we can infer how plant species in a forest, grassland, or farmland are associated with diverse taxonomic and functional groups of fungi [24, 32-34]. Such information of network structure (topology) can be used to identify "hub" species, which are placed at the center of a network depicting multispecies host–symbiont associations [35] (cf. [34, 36, 37]). Those hubs with broad host/symbiont ranges are expected to play key roles by mediating otherwise discrete ecological processes within a community [19, 24]. For example, although arbuscular mycorrhizal and ectomycorrhizal symbioses have been considered to involve distinct sets of plant and fungal lineages [38] (but see [39, 40]), hub endophytic fungi with broad host ranges may mediate indirect interactions between arbuscular mycorrhizal and ectomycorrhizal plant species through below-ground mycelial connections. As information of plant-associated fungal communities is now easily available with high-throughput DNA sequencing

technologies [1, 21, 22], finding hub microbial species out of hundreds or thousands of species within a network has become an important basis for understanding and predicting ecosystem-scale phenomena.

Nonetheless, given that fungi can disperse long distances with spores, conidia, and propagules [41-44], information of local-scale networks alone does not provide thorough insights into below-ground plant–fungus interactions in the wild. In other words, no forests, grasslands, and farmlands are free from perturbations caused by fungi immigrating from other localities [45-49]. Therefore, to consider how local ecosystem processes are interlinked by dispersal of fungi, we need to take into account "metacommunity-scale" networks of plant–fungus associations [35]. Within a dataset of multiple local communities (e.g., [25]), fungal species that occur in multiple localities may interlink local networks of plant–fungus associations. Among them, some species that not only have broad geographic ranges but also are associated with diverse host plant species would be placed at the core positions of a metacommunity-scale network [35]. Such "metacommunity hub" fungi would be major drivers of the synchronization and restructuring of local ecosystem processes (*sensu* [50]), and hence their functional roles need to be investigated with priority [35]. Moreover, in the screening of mycorrhizal and endophytic fungi that can be used in agriculture and ecosystem restoration programs [17, 20, 51], analytical pipelines for identifying metacommunity hubs will help us explore species that are potentially applied (inoculated) to diverse plant species over broad geographic ranges of farmlands, forests, or grasslands. Nonetheless, despite the potential importance of metacommunity hubs in both basic and applied microbiology, few studies have examined metacommunity-level networks of plant–symbiont associations.

By compiling Illumina sequencing datasets of root-associated fungi [52], we herein inferred a metacommunity-level network of below-ground plant–fungus associations and thereby explored metacommunity hubs. Our metadata consisted of plant–fungus association data in eight forest localities across the entire range of the Japanese Archipelago, including 150 plant species/taxa and 8,080 fungal operational taxonomic units (OTUs) in temperate and subtropical regions. Based on the information of local- and metacommunity-level networks, each of the fungal OTUs was evaluated in light of its topological positions. We then

examined whether fungal OTUs placed at the core of local-level plant–fungus networks could play key topological roles within the metacommunity-level network. Overall, this study uncover how diverse taxonomic groups of mycorrhizal and endophytic fungi can form metacommunity-scale networks of below-ground plant–fungus associations, providing a basis for analyzing complex spatial processes of species-rich host–microbe systems.

Methods

Terminology

While a single type of plant–fungus interactions is targeted in each of most mycological studies (e.g., arbuscular mycorrhizal symbiosis or ectomycorrhizal symbiosis), we herein analyze the metadata including multiple categories of below-ground plant–fungus associations [52]. Because arbuscular mycorrhizal, ectomycorrhizal, and endophytic fungi, for example, vary in their microscopic structure within plant tissue [38], it is impossible to develop a general criterion of mutualistic/antagonistic interactions for all those fungal functional groups. Therefore, we used the phrase “associations” instead of “interactions” throughout the manuscript when we discuss patterns detected based on the Illumina sequencing metadata of root-associated fungi. Consequently, our results represented not only mutualistic or antagonistic interactions but also neutral or commensalistic interactions [24, 53, 54]. Our aim in this study is to gain an overview of the metacommunity-scale plant–fungus associations, while the nature of respective plant–fungus associations should be evaluated in future inoculation experiments.

Data

We compiled the Illumina (MiSeq) sequencing data collected in a previous study [52], in which community-scale statistical properties of below-ground plant–fungus associations were compared among eight forest localities (four cool-temperate, one warm-temperate, and three subtropical forests) across the entire range of the Japanese Archipelago (45.042–24.407 ºN;

Fig. 1). In each forest, 2-cm segment of terminal roots were sampled from 3-cm below the soil surface at 1-m horizontal intervals [52]. Those root samples were collected irrespective of their morphology and mycorrhizal type: hence, the samples as a whole represented below-ground relative abundance of plant species in each forest community. Based on the sequences of the genes encoding the large subunit of ribulose-1,5-bisphosphate carboxylase (*rbcL*) and the internal transcribed spacer 1 (ITS1) of the ribosomal RNA region, host plant species were identified, although there were plant root samples that could not be identified to species with the *rbcL* and ITS1 regions [52]. The sequencing data are available through DDBJ Sequence Read Archives (accession: DRA006339).

The Illumina sequencing reads of the fungal ITS1 region were processed using the program Claidnet [55, 56] as detailed in the data-source study [52]: the Unix scripts used are available as Additional file 1. The primers used were designed to target not only Ascomycota and Basidiomycota but also diverse non-Dikarya (e.g., Glomeromycota) taxa [57]. In most studies analyzing community structure of Ascomycota and Basidiomycota fungi, OTUs of the ITS region are defined with a cut-off sequence similarity of 97% [22, 58, 59] (see also [60]). Meanwhile, Glomeromycota fungi generally have much higher intraspecific ITS-sequence variation than other taxonomic groups of fungi [61]. Consequently, we used 97% and 94% cut-off sequence similarities for defining non-Glomeromycota and Glomeromycota fungal OTUs, respectively [52]. The OTUs were then subjected to reference database search with the query-centric auto-*k*-nearest-neighbor algorithm [55, 56] and subsequent taxonomic assignment with the lowest common ancestor algorithm [62]. Based on the inferred taxonomy, the functional group of each fungal OTU was inferred using the program FUNGuild 1.0 [63].

After a series of bioinformatics and rarefaction procedures, 1,000 fungal ITS reads were obtained from each of the 240 samples collected in each forest locality (i.e., 1,000 reads × 240 samples × 8 sites). A sample (row) × fungal OTU (column) data matrix, in which a cell entry depicted the number of sequencing reads of an OTU in a sample, was obtained for each local forest ("sample-level" matrix) (Additional file 2: Data S2). Each local sample-level matrix was then converted into a "species-level" matrix, in which a cell entry represented the number of root samples from which associations of a plant species/taxa (row) and a fungal OTU

(columns) was observed: 17–55 plant species/taxa and 1,149–1,797 fungal OTUs were detected from the local species-level matrices (Additional file 3: Data S3). In total, the matrices included 150 plant species/taxa and 8,080 fungal OTUs (Additional file 4: Data S4).

Local networks

Among the eight forest localities, variation in the order-level taxonomic compositions were examined with the permutational analysis of variance (PERMANOVA; [64]) and the permutational analysis for the multivariate homogeneity of dispersions (PERMDISP; [65]) with the "adonis" and "betadisper" functions of the vegan 2.4-3 package [66] of R 3.4.1 [67], respectively. The β-diversity values used in the PERMANOVA and PERMDISP analyses were calculated with the "Bray-Curtis" metric based on the sample-level matrices (Additional file 2: Data S2). Note that the "Raup-Crick" β-diversity metric [68], which controls α-diversity in community data but requires computationally intensive randomization, was not applicable to our large metadata. Geographic variation in the compositions of fungal functional groups was also evaluated by PERMANOVA and PERMDISP analyses. The R scripts for the PERMANOVA and PERMDISP analyses are available as Additional file 5.

For each of the eight local forests, the network structure of below-ground plant–fungus associations was visualized based on the species-level matrix (Additional file 3: Data S3) using the program GePhi 0.9.1 [69] with the "ForceAtlas2" layout algorithm [70]. Within the networks, the order-level taxonomy of fungal OTUs was highlighted.

To evaluate host ranges of each fungal OTU in each local forest, we first calculated the *d'* metric of interaction specificity [71]. However, estimates of the *d'* metric varied considerably among fungal OTUs observed from small numbers of root samples (Additional file 6; Figure S1) presumably due to overestimation or underestimation of host preferences for those rare OTUs. Therefore, we scored each fungal OTU based on their topological positions within each local network by calculating network centrality indices (degree, closeness, betweenness, and eigenvector centralities metrics of network centrality; [31]). Among the centrality metrics, betweenness centrality, which measures the extent to which a given nodes (species) is located

191 within the shortest paths connecting pairs of other nodes in a network [72], is often used to
192 explore organisms with broad host or partner ranges [35]. Thus, in each local network, fungal
193 OTUs were ranked based on their betweenness centrality scores (local betweenness).

194

195 **Metacommunity-scale network**

196 By compiling the species-level matrices of the eight local forests, the topology of the
197 metacommunity-scale network of plant–fungus associations was inferred. In general, species
198 interaction (association) networks of local communities can be interconnected by species that
199 appear in two or more local networks, thereby merged into a metacommunity-scale network
200 [35]. In our data across the eight local forests, 2,109 OTUs out of the 8,080 fungal OTUs
201 appeared in two or more localities. Therefore, we could infer the topology of a
202 metacommunity-scale network, in which the eight local networks were combined by the
203 2,109 fungal OTUs. In the metacommunity-scale network, plant species/taxa observed in
204 different localities were treated as different network nodes because our purpose in this study
205 was to explore fungi that potentially play key roles in synchronizing local ecosystem
206 processes [35]. In total, 227 plant nodes representing local populations of 150 plant
207 species/taxa were included in the metacommunity-scale network.

208 We then screened for fungal OTUs with broad geographic and host ranges based on the
209 betweenness centrality scores of respective fungal OTUs within the metacommunity network
210 (metacommunity betweenness, B_{meta}). In general, species with highest metacommunity
211 betweenness scores not only occur in local communities over broad biotic/abiotic
212 environmental conditions but also are associated with broad ranges of host/partner species
213 [35]. Possible relationship between local- and metacommunity-scale topological roles was
214 then examined by plotting local and metacommunity betweenness scores (B_{local} and B_{meta}) of
215 each fungal OTUs on a two-dimensional surface. To make the betweenness scores vary from
216 0 to 1, betweenness centrality of a fungal OTU *i* was standardized in each of the local- and
217 metacommunity-scale networks as follows:

$$B'_{\mathrm{local},i} = \frac{B_{\mathrm{local},i} - \min(B_{\mathrm{local}})}{\max(B_{\mathrm{local}}) - \min(B_{\mathrm{local}})} \quad \text{and} \quad B'_{\mathrm{meta},i} = \frac{B_{\mathrm{meta},i} - \min(B_{\mathrm{meta}})}{\max(B_{\mathrm{meta}}) - \min(B_{\mathrm{meta}})},$$

where $B_{\mathrm{local},i}$ and $B_{\mathrm{meta},i}$ were raw estimates of local- and metacommunity-scale betweenness of a fungal OTU *i*, and min() and max() indicated minimum and maximum values, respectively. For local betweenness of each OTU, a mean value across local networks was subsequently calculated ($\overline{B}'_{\mathrm{local},i}$): the local communities from which a target OTU was absent was omitted in the calculation of mean local betweenness. On the two-dimensional surface, the OTUs were then classified into four categories: metacommunity hubs having high betweenness in both local- and metacommunity-scale networks ($\overline{B}'_{\mathrm{local},i} \geq 0.5$; $B'_{\mathrm{meta},i} \geq 0.5$), metacommunity connectors that had broad geographic ranges but displayed low local betweenness ($\overline{B}'_{\mathrm{local},i} < 0.5$; $B'_{\mathrm{meta},i} \geq 0.5$), local hubs that had high betweenness in local networks but not in the metacommunity-scale network ($\overline{B}'_{\mathrm{local},i} \geq 0.5$; $B'_{\mathrm{meta},i} < 0.5$), and peripherals with low betweenness at both local and metacommunity levels ($\overline{B}'_{\mathrm{local},i} < 0.5$; $B'_{\mathrm{meta},i} < 0.5$) [35]. Approximately, 1–2% of fungal OTUs show betweenness scores higher than 0.5 in each local or metacommunity network, while the threshold value can be changed depending on the purpose of each study [35].

In addition to metacommunity hubs within the metacommunity-scale network representing all the eight localities, those within the metacommunity-scale network representing northern (sites 1–4) or southern (sites 5–8) four localities were also explored. This additional analysis allowed us to screen for fungal OTUs that potentially adapted to broad ranges of biotic and abiotic environments within northern (cool-temperate) or southern (warm-temperate or subtropical) part of Japan.

Results

Local networks

Among the eight forest localities, order-level taxonomic compositions of fungi varied significantly (PERMANOVA; $F_{\mathrm{model}} = 35.7$, $P < 0.001$), while the differentiation of

244 community structure was attributed at least partly to geographic variation in among-sample
245 dispersion (PERMDISP; $F = 13.2$, $P < 0.001$) (Fig. 2a). Compositions of fungal functional
246 groups were also differentiated among the eight localities (PERMANOVA; $F_{model} = 34.9$, $P <$
247 0.001), while within-site dispersion was significantly varied geographically (PERMDISP; $F =$
248 9.2, $P < 0.001$) (Fig. 2b). The proportion of ectomycorrhizal fungal orders, such as Russulales,
249 Thelephorales, and Sebacinales, was higher in temperate forests than in subtropical forests,
250 while that of arbuscular mycorrhizal fungi increased in subtropical localities (Fig. 2). The
251 proportion of the ascomycete order Helotiales, which has been known to include not only
252 ectomycorrhizal but also endophytic, saprotrophic, and ericoid mycorrhizal fungi [73], was
253 higher in northern localities. In contrast, Diaporthales, which has been considered as
254 predominantly plant pathogenic taxon [74] (but see [75]), was common in subtropical forests
255 but not in others.

256 In each of the eight local networks depicting plant–fungus associations, some fungal
257 OTUs were located at the central positions of the network, while others are distributed at
258 peripheral positions (Additional file 7; Figure S2). Specifically, fungal OTUs belonging to the
259 ascomycete orders Chaetothyriales (e.g., *Cladophialophora* and *Exophiala*) and Helotiales
260 (e.g., *Rhizodermea*, *Pezicula*, *Rhizoscyphus*, and *Leptodontidium*) as well as some *Mortierella*
261 OTUs had high betweenness centrality scores in each of the cool-temperate forests (Fig. 3a-b).
262 In contrast, arbuscular mycorrhizal fungi (Glomeromycota) were common among OTUs with
263 highest betweenness scores in subtropical forests (Fig. 3a-c). Some fungi in the ascomycete
264 order Hypocreales (e.g., *Trichoderma*, *Ilyonectria*, *Simplicillium*, and *Calonectria*) also had
265 high betweenness scores in some temperate and subtropical forests (Fig. 3b).

266

267 **Metacommunity-scale network**

268 In the metacommunity-scale network representing the connections among the eight local
269 networks, not only arbuscular mycorrhizal but also saprotrophic/endophytic fungi were placed
270 at the central topological positions (Fig. 4; Additional file 8; Figure S3). Among
271 non-Glomeromycota OTUs, *Mortierella* (Mortierellales), *Cryptococcus* (Trichosporonales;

the Blast top-hit fungus in the NCBI database was recently moved to *Saitozyma* (Tremellales); [76]), *Malassezia* (Malasseziales), *Oidiodendron* (incertae sedis), *Trichoderma* (Hypocreales), and a fungus distantly allied to *Melanconiella* (Diaporthales) displayed highest metacommunity betweenness (Table 1). Among the OTUs with high metacommunity betweenness, only a *Mortierella* OTU was designated as a metacommunity hub (i.e., $\overline{B}'_{\mathrm{local},i} \geq 0.5$; $B'_{\mathrm{meta},i} \geq 0.5$) and others had low betweenness scores at the local community level ($\overline{B}'_{\mathrm{local},i} < 0.5$; Fig. 5a).

In the metacommunity-scale network representing the four cool-temperate forests (sites 1–4), many saprotrophic/endophytic fungal OTUs were associated with diverse plant species/taxa, located at the central topological positions within the network topology (Additional file 9; Figure S4; Fig. 5b). The list of these fungi with high metacommunity betweenness involved OTUs in the genera *Mortierella*, *Cladophialophora* (Chaetothyriales), *Pezicula* (Helotiales), and *Oidiodendron* as well as OTUs allied to *Ilyonectria protearum* (Nectriales) and *Cadophora orchidicola* (Helotiales) (Table 1). Most of those fungal OTUs also had high metacommunity betweenness, designated as metacommunity hubs (Fig. 5b).

In the metacommunity-scale network consisting of the warm-temperate and subtropical forests (sites 5–8), arbuscular mycorrhizal and saprotrophic/endophytic fungi were placed at the hub positions (Additional file 10; Figure S5; Fig. 5c). The list of non-Glomeromycota OTUs with highest metacommunity betweenness included *Saitozyma* (*Cryptococcus*), *Mortierella*, *Trichoderma*, and *Tomentella* as well as OTUs allied to *Cladophialophora*, *Scleropezicula* (Helotiales), *Melanconiella* (Diaporthales), and *Rhexodenticula* (incertae sedis) (Table 1). Among the taxa, *Saitozyma* and *Mortierella* included OTUs classified as metacommunity hubs (Fig. 5c; Table 1). In an additional analysis of a metacommunity-scale network including only the three subtropical forests (sites 6-8), similar sets of fungal taxa were highlighted (Additional file 11; Table S1). The detailed information of the network index scores examined in this study is provided in Data S3 (Additional file 4: Data S4).

Discussion

Based on the metadata of root-associated fungi across the Japanese Archipelago, we herein inferred the structure of a network representing metacommunity-scale associations of 150 plant species/taxa and 8,080 fungal OTUs. Our analysis targeted diverse functional groups of fungi such as arbuscular mycorrhizal, ectomycorrhizal, ericoid-mycorrhizal, saprotrophic/endophytic, and pathogenic fungi, which have been analyzed separately in most previous studies on plant–fungus networks. The comprehensive analysis of below-ground plant–fungus associations allowed us to explore metacommunity hub fungi, which not only occurred over broad geographic ranges but also had broad host ranges in respective local communities. Consequently, this study highlights several taxonomic groups of fungi potentially playing key roles in synchronizing metacommunity-scale processes of temperate and/or subtropical forests.

In the metacommunity-scale network representing all the eight local forests (Fig. 4), fungi in several saprotrophic or endophytic taxa showed higher betweenness centrality scores than other fungi (Table 1). *Mortierella* is generally considered as a saprotrophic lineage [77] but it also includes fungi contributing to the growth and pathogen resistance of plants [78-80]. A phosphate solubilizing strain of *Mortierella*, for example, increases shoot and root growth of host plants under salt stress, especially when co-inoculated with an arbuscular mycorrhizal fungus [78]. In addition, polyunsaturated fatty acids produced by some *Mortierella* species are known to increase resistance of plants against phytopathogens [79, 80]. Fungi in the genus *Trichoderma* are commonly detected and isolated from the rhizosphere [77, 81]. Many of them inhibit the growth of other fungi, often used in the biological control of phytopathogens [82-84]. Some of them are also reported to suppress root-knot nematodes [85] or to promote root growth [86]. The analysis also highlighted basidiomycete yeasts in the genus *Saitozyma* or *Cryptococcus* (teleomorph = *Filobasidiella*), which are often isolated from soil [22, 87] as well as both above-ground and below-ground parts of plants [88-91].

Along with those possibly saprotrophic or endophytic taxa, ericoid mycorrhizal and phytopathogenic taxa of fungi displayed relatively high betweenness scores within the metacommunity-scale network representing all the eight local forests (Table 1). Specifically, *Oidiodendron* (teleomorph = *Myxotrichum*) is a taxon represented by possibly ericoid

329 mycorrhizal species (*O. maius* and *O. griseum*) [92, 93], although fungi in the genus are found
330 also from roots of non-ericaceous plants and soil [94]. On the other hand, fungi in the family
331 Nectriaceae are known to cause black foot disease [95], often having serious damage on
332 economically important woody plants [96, 97]. Although we collected seemingly benign roots
333 in the study forests, some samples may be damaged by those pathogens. Alternatively, some
334 lineages of Nectriaceae fungi may be associated with plant hosts non-symptomatically,
335 having adverse effects context-dependently.

336 Although these fungi were candidates of metacommunity hubs, which are characterized
337 by broad geographic ranges and host plant ranges, none except but a *Mortierella* OTU had
338 high betweenness scores at both local and metacommunity levels (Fig. 5a). This result
339 suggests that even if some fungi have broad geographic ranges across the Japanese
340 Archipelago, few played important topological roles in each of the local networks
341 representing plant–fungus associations. In other words, fungi that can adapt to biotic and
342 abiotic environments in forest ecosystems throughout cool-temperate, warm-temperate, and
343 subtropical regions are rare.

344 Therefore, we also explored fungi with broad geographic and host ranges within the
345 metacommunities representing northern (cool-temperate) and southern (warm-temperate and
346 subtropical) regions of Japan. In the metacommunity consisting of the four cool-temperate
347 forests (Additional file 9; Figure S4), fungal OTUs in the genera *Mortierella*,
348 *Cladophialophora*, and *Pezicula* as well as those allied to *Ilyonectria* and *Cadophora* had
349 highest betweenness at both local and metacommunity levels, classified as metacommunity
350 hubs (Fig. 5b; Table 1). Among them, *Cladophialophora* is of particular interest because it
351 has been known as a lineage of "dark septate endophytes" [98-100] (*sensu* [14, 15, 101]). A
352 species within the genus, *C. chaetospira* (= *Heteroconium chaetospira*), to which
353 high-betweenness OTUs in our data were closely allied, has been known not only to provide
354 nitrogen to host plants but also to suppress pathogens [12, 16, 102]. Likewise, the Helotiales
355 genus *Pezicula* (anamorph = *Cryptosporiopsis*) includes endophytic fungi [103-105], some of
356 which produce secondary metabolites suppressing other microbes in the rhizosphere [106,
357 107]. Our finding that some of *Cladophialophora* and *Pezicula* fungi could be associated with

various taxonomic groups of plants over broad geographic ranges highlights potentially important physiological and ecological roles of those endophytes at the community and metacommunity levels.

In the southern metacommunity networks consisting of warm-temperate and subtropical forests (Additional file 10; Figure S5), some arbuscular mycorrhizal OTUs and *Saitozyma* (*Cryptococcus*) and *Mortierella* OTUs had high betweenness scores at both local and metacommunity levels, designated as metacommunity hubs (Fig. 5c; Table 1). Given the above-mentioned prevalence of fungal OTUs allied to *Cladophialophora chaetospira* in the cool-temperate metacommunity, the contrasting list of metacommunity hubs in the southern (warm-temperate–subtropical) metacommunity implies that different taxonomic and functional groups of fungi play major metacommunity-scale roles in different climatic regions. This working hypothesis is partially supported by previous studies indicating endemism and vicariance in the biogeography of fungi and bacteria [108, 109], promoting conceptual advances beyond the classic belief that every microbe is everywhere but the environment selects microbes colonizing respective local communities [110].

The roles of those metacommunity hubs detected in this study are of particular interest from the aspect of theoretical ecology. Hub species connected to many other species in an ecosystem often integrate "energy channels" [111] within species interaction networks, having great impacts on biodiversity and productivity of the ecosystems [35]. The concept of "keystone" or "foundation" species [112, 113] can be extended to the metacommunity level, thereby promoting studies exploring species that restructure and synchronize ecological (and evolutionary) dynamics over broad geographic ranges [35]. Given that below-ground plant–fungus symbioses are key components of the terrestrial biosphere [1, 2], identifying fungal species that potentially have great impacts on the metacommunity-scale processes of such below-ground interactions will provide crucial insights into the conservation and restoration of forests and grasslands. We here showed that the list of metacommunity hubs could involve various lineages of endophytic fungi, whose ecosystem-scale functions have been underappreciated compared to those of mycorrhizal fungi. As those endophytic fungi are potentially used as inoculants when we reintroduce plant seedlings in ecosystem restoration

programs [20, 51], exploring fungi with highest potentials in each climatic/biogeographic region will be a promising direction of research in conservation biology.

The finding that compositions of metacommunity hubs could vary depending on climatic regions also gives key implications for the application of endophytes in agriculture. Although a number of studies have tried to use endophytic fungi and/or bacteria as microbial inoculants in agriculture [17, 18, 114], such microbes introduced to agroecosystems are often outcompeted and replaced by indigenous (resident) microbes [115, 116]. Moreover, even if an endophytic species or strain increases plant growth in pot experiments under controlled environmental conditions, its effects in the field often vary considerably depending on biotic and abiotic contexts of local agroecosystems [17] (see also [117]). Therefore, in the screening of endophytes that can be used in broad ranges of biotic and abiotic environmental conditions, the metacommunity-scale network analysis outlined in this study will help us find promising candidates out of thousands or tens of thousands microbial species in the wild. Consequently, to find promising microbes whose inocula can persist in agroecosystems for long time periods, exploration of metacommunity hubs needs to be performed in respective climatic or biogeographic regions.

For more advanced applications in conservation biology and agriculture, continual improvements of methods for analyzing metacommunity-scale networks are necessary. First, while the fungal OTUs in our network analysis was defined based on the cut-off sequence similarities used in other studies targeting "species-level" diversity of fungi [59, 61], physiological functions can vary greatly within fungal species or species groups [14, 118]. Given that bioinformatic tools that potentially help us detect single-nucleotide-level variation are becoming available [119], the resolution of network analyses may be greatly improved in the near future. Second, although some computer programs allow us to infer functions of respective microbial OTUs within network data [63, 120], the database information of microbial functions remains scarce. To increase the coverage and accuracy of automatic annotations of microbial functions, studies describing the physiology, ecology, and genomes of microbes should be accelerated. With improved reference databases, more insights into the metacommunity-scale organization of plant–fungus associations will be obtained by

reanalyzing the network data by compiling enhanced information of fungal functional groups. Third, as the diversity and compositions of plant–fungus associations included in a network can depend on how we process raw samples, special care is required in the selection of methods for washing and preparing root (or soil) samples. By sterilizing root samples with NaClO [121], for example, we may be able to exclude fungi or bacteria that are merely adhering to root surfaces. Meanwhile, some of those fungi and bacteria on root surfaces may play pivotal physiological roles in the growth and survival of plants [122]. Accordingly, it would be productive to compare network topologies of plant–microbe associations among different source materials by partitioning endosphere, rhizoplane, and rhizosphere microbial samples with a series of sample cleaning processes using ultrasonic devices [123]. Fourth, although this study targeted fungi associated with roots, our methods can be easily extended to network analyses involving other groups of microbes. By simultaneously analyzing the prokaryote 16S rRNA region [123-125] with the fungal ITS region, we can examine how bacteria, archaea, and fungi are involved in below-ground webs of symbioses. Fifth, not only plant–microbe associations but also microbe–microbe interactions can be estimated with network analytical frameworks. Various statistical pipelines have been proposed to infer how microbes interact with each other in facilitative or competitive ways within host macroorganisms [37, 126, 127]. Overall, those directions of analytical extensions will enhance our understanding of plant microbiome dynamics in nature.

Conclusions

By compiling datasets of below-ground plant–fungus associations in temperate and subtropical forest ecosystems, we explored metacommunity-hub fungi, which were characterized by broad geographic and host ranges. Such metacommunity-scale analyses are expected to provide bird's-eye views of complex plant–microbe associations, highlighting plant-growth-promoting microbes that can be applied to diverse plant taxa in various environments. Given that endophytic fungi promoting the growth and pathogen resistance of host plants can be isolated from forest soil (e.g., *Cladophialophora chaetospira* [99]), the list

444 of metacommunity-hub endophytic fungi featured in this study itself may include prospective
445 species to be used in agriculture. By extending the targets of such network analyses to diverse
446 types of plant-associated microbes (e.g., phyllosphere fungi and bacteria [75, 124, 128]) in
447 various climatic/biogeographic regions, a solid basis for managing plant microbiomes will be
448 developed.

449

450 **Abbreviations**

451 DDBJ: DNA Data Bank of Japan; ITS: internal transcribed spacer; OTU: Operational
452 taxonomic unit; PERMANOVA: permutational analysis of variance; PERMDISP:
453 permutational analysis for the multivariate homogeneity of dispersions; rRNA: ribosomal
454 ribonucleic acid.

455

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466

467 **Availability of data and materials**

468 The Illumina sequencing data were deposited to DNA Data Bank of Japan (DDBJ Sequence
469 Read Archive: DRA006339). The raw data of fungal community structure and the fungal
470 community matrices analyzed are available with the source study [52] and Additional files
471 2-4, respectively. The Unix scripts for the bioinformatic pipeline and the R scripts for the

PERMANOVA and PERMDISP analyses are available as Additional files 1 and 4, respectively.

Authors' contributions

HT designed the work. HT, AST, and HS conducted fieldwork. HT performed the molecular experiments. HT wrote the manuscript with AST and HS.

Competing interests

The authors declare that they have no competing interests.

Consent for publication

Not applicable

Ethics approval and consent to participate

Not applicable

Additional files

Additional file 1: Data S1. Unix scripts for the bioinformatic pipeline.

Additional file 2: Data S2. Sample-level matrices of the eight forests examined.

Additional file 3: Data S3. Species-level matrices of plant–fungus associations.

Additional file 4: Data S4. Information of 8080 fungal OTUs analyzed.

Additional file 5: Data S5. R scripts for the PERMANOVA and PERMDISP analyses.

494 **Additional file 6: Figure S1.** Number of sequencing reads, interaction specificity, and local
495 betweenness.

496 **Additional file 7: Figure S2.** Structure of plant–fungus networks in each local forest.

497 **Additional file 8 Figure S3.** Locality information within the full metacommunity-scale
498 network.

499 **Additional file 9: Figure S4.** Metacommunity-scale network of cool-temperate forests.

500 **Additional file 10: Figure S5.** Metacommunity-scale network of warm-temperate and
501 subtropical forests.

502 **Additional file 11: Table S1.** Top-10 list of non-Glomeromycota OTUs with highest
503 betweenness within the subtropical metacommunity network.

504

505 ## References

506 1. Peay KG, Kennedy PG, Talbot JM: Dimensions of biodiversity in the Earth
507 mycobiome. Nat. Rev. Microbiol. 2016;14:434-447.

508 2. van der Heijden MG, Bardgett RD, van Straalen NM: The unseen majority: soil
509 microbes as drivers of plant diversity and productivity in terrestrial ecosystems. Ecol.
510 Lett. 2008;11:296-310.

511 3. van der Heijden MG, Klironomos JN, Ursic M, Moutoglis P, Streitwolf-Engel R,
512 Boller T et al: Mycorrhizal fungal diversity determines plant biodiversity, ecosystem
513 variability and productivity. Nature 1998;396:69-72.

514 4. Wardle DA, Bardgett RD, Klironomos JN, Setälä H, van der Putten WH, Wall DH:
515 Ecological linkages between aboveground and belowground biota. Science
516 2004;304:1629-1633.

517 5. Nara K: Ectomycorrhizal networks and seedling establishment during early primary
518 succession. New Phytol. 2006;169:169-178.

519 6. Finlay R, Read D: The structure and function of the vegetative mycelium of
520 ectomycorrhizal plants. II. The uptake and distribution of phosphorus by mycelial
521 strands interconnecting host plants. New Phytol. 1986;103:157-165.

522 7. Klironomos JN, McCune J, Hart M, Neville J: The influence of arbuscular
523 mycorrhizae on the relationship between plant diversity and productivity. Ecol. Lett.
524 2000;3:137-141.

525 8. Högberg MN, Högberg P: Extramatrical ectomycorrhizal mycelium contributes
526 one-third of microbial biomass and produces, together with associated roots, half the
527 dissolved organic carbon in a forest soil. New Phytol. 2002;154:791-795.

528 9. Högberg P, Nordgren A, Buchmann N, Taylor AF, Ekblad A, Högberg MN et al:
529 Large-scale forest girdling shows that current photosynthesis drives soil respiration.
530 Nature 2001;411:789-792.

531 10. Almario J, Jeena G, Wunder J, Langen G, Zuccaro A, Coupland G et al:
532 Root-associated fungal microbiota of nonmycorrhizal *Arabis alpina* and its
533 contribution to plant phosphorus nutrition. Proc. Natl. Acad. Sci. USA.
534 2017;114:E9403–E9412.

535 11. Hiruma K, Gerlach N, Sacristán S, Nakano RT, Hacquard S, Kracher B et al: Root
536 endophyte *Colletotrichum tofieldiae* confers plant fitness benefits that are phosphate
537 status dependent. Cell 2016;165:464-474.

538 12. Hashiba T, Narisawa K: The development and endophytic nature of the fungus
539 *Heteroconium chaetospira*. FEMS Microbiol. Lett. 2005;252:191-196.

540 13. Addy H, Piercey M, Currah R: Microfungal endophytes in roots. Can. J. Bot.
541 2005;83:1-13.

542 14. Jumpponen A: Dark septate endophytes-are they mycorrhizal? Mycorrhiza
543 2001;11:207-211.

544 15. Newsham KK: A meta-analysis of plant responses to dark septate root endophytes.
545 New Phytol. 2011;190:783-793.

546 16. Narisawa K, Tokumasu S, Hashiba T: Suppression of clubroot formation in Chinese
547 cabbage by the root endophytic fungus, *Heteroconium chaetospira*. Plant Pathol.

1998;47:206-210.

17. Calvo P, Nelson L, Kloepper JW: Agricultural uses of plant biostimulants. Plant Soil 2014;383:3-41.

18. Porras-Alfaro A, Bayman P: Hidden fungi, emergent properties: endophytes and microbiomes. Ann. Rev. Phytopathol. 2011;49:291-315.

19. Mandyam K, Jumpponen A: Seeking the elusive function of the root-colonising dark septate endophytic fungi. Studies Mycol. 2005;53:173-189.

20. Fuchs B, Haselwandter K: Red list plants: colonization by arbuscular mycorrhizal fungi and dark septate endophytes. Mycorrhiza 2004;14:277-281.

21. Tedersoo L, Bahram M, Põlme S, Kõljalg U, Yorou NS, Wijesundera R et al: Global diversity and geography of soil fungi. Science 2014;346:1256688.

22. Buée M, Reich M, Murat C, Morin E, Nilsson R, Uroz S et al: 454 Pyrosequencing analyses of forest soils reveal an unexpectedly high fungal diversity. New Phytol. 2009;184:449-456.

23. Öpik M, Vanatoa A, Vanatoa E, Moora M, Davison J, Kalwij J et al: The online database MaarjAM reveals global and ecosystemic distribution patterns in arbuscular mycorrhizal fungi (Glomeromycota). New Phytol. 2010;188:223-241.

24. Toju H, Guimarães PR, Jr, Olesen JM, Thompson JN: Assembly of complex plant–fungus networks. Nat. Commun. 2014;5:5273.

25. Toju H, Guimarães PR, Jr, Olesen JM, Thompson JN: Below-ground plant–fungus network topology is not congruent with above-ground plant–animal network topology. Sci. Adv. 2015;1:e1500291.

26. Kadowaki K, Yamamoto S, Sato H, Tanabe AS, Hidaka A, Toju H: Plant−soil feedbacks between arbuscular- and ecto-mycorrhizal communities. bioRxiv 2017:https://doi.org/10.1101/228387

27. Schlaeppi K, Bulgarelli D: The plant microbiome at work. Mol. Plant-Microbe Int. 2015;28:212-217.

28. Berendsen RL, Pieterse CM, Bakker PA: The rhizosphere microbiome and plant

576 health. Trends Plant Sci. 2012;17:478-486.

577 29. Busby PE, Soman C, Wagner MR, Friesen ML, Kremer J, Bennett A et al: Research
578 priorities for harnessing plant microbiomes in sustainable agriculture. PLOS Biol.
579 2017;15:e2001793.

580 30. Albert R, Jeong H, Barabási AL: Error and attack tolerance of complex networks.
581 Nature 2000;406:378-382.

582 31. Newman MEJ: Networks: an introduction. Oxford: Oxford University Press; 2010.

583 32. Toju H, Tanabe A, Ishii H: Ericaceous plant–fungus network in a harsh alpine–
584 subalpine environment. Mol. Ecol. 2016;25:3242-3257.

585 33. Bennett AE, Daniell TJ, Öpik M, Davison J, Moora M, Zobel M et al: Arbuscular
586 mycorrhizal fungal networks vary throughout the growing season and between
587 successional stages. PLoS One 2013;8:e83241.

588 34. Bahram M, Harend H, Tedersoo L: Network perspectives of ectomycorrhizal
589 associations. Fungal Ecol. 2014;7:70-77.

590 35. Toju H, Yamamichi M, Guimarães Jr. PR, Olesen J, Mougi A, Yoshida T et al:
591 Species-rich networks and eco-evolutionary synthesis at the metacommunity level.
592 Nat. Ecol. Evol. 2017;1.

593 36. Agler MT, Ruhe J, Kroll S, Morhenn C, Kim S-T, Weigel D et al: Microbial hub taxa
594 link host and abiotic factors to plant microbiome variation. PLOS Biol.
595 2016;14:e1002352.

596 37. Toju H, Yamamoto S, Tanabe AS, Hayakawa T, Ishii HS: Network modules and hubs
597 in plant-root fungal biome. J. R. Soc. Interface 2016;13:20151097.

598 38. Smith SE, Read DJ: Mycorrhizal symbiosis. New York: Academic press; 2008.

599 39. Dickie IA, Koide RT, Fayish AC: Vesicular-arbuscular mycorrhizal infection of
600 *Quercus rubra* seedlings. New Phytol. 2001;151:257-264.

601 40. Plattner I, Hall I: Parasitism of non-host plants by the mycorrhizal fungus *Tuber*
602 *melanosporum*. Mycol. Res. 1995;99:1367-1370.

603 41. Hallenberg N, Kuffer N: Long - distance spore dispersal in wood - inhabiting

basidiomycetes. Nordic J. Bot. 2001;21:431-436.

42. Nagarajan S, Singh D: Long-distance dispersion of rust pathogens. Ann. Rev. Phytopathol. 1990;28:139-153.

43. Brown JK, Hovmøller MS: Aerial dispersal of pathogens on the global and continental scales and its impact on plant disease. Science 2002;297:537-541.

44. Hovmøller M, Justesen A, Brown J: Clonality and long - distance migration of *Puccinia striiformis* f. sp. tritici in north - west Europe. Plant Pathol. 2002;51:24-32.

45. Jousimo J, Tack AJ, Ovaskainen O, Mononen T, Susi H, Tollenaere C et al: Ecological and evolutionary effects of fragmentation on infectious disease dynamics. Science 2014;344:1289-1293.

46. Parker MA: Mutualism in metapopulations of legumes and rhizobia. Am. Nat. 1999;153:S48-S60.

47. Thrall PH, Burdon JJ: Evolution of virulence in a plant host-pathogen metapopulation. Science 2003;299:1735-1737.

48. Lekberg Y, Koide RT, Rohr JR, ALDRICH - WOLFE L, Morton JB: Role of niche restrictions and dispersal in the composition of arbuscular mycorrhizal fungal communities. J. Ecol. 2007;95:95-105.

49. Peay KG, Schubert MG, Nguyen NH, Bruns TD: Measuring ectomycorrhizal fungal dispersal: macroecological patterns driven by microscopic propagules. Mol. Ecol. 2012;21:4122-4136.

50. Gouhier TC, Guichard F, Gonzalez A: Synchrony and stability of food webs in metacommunities. Am. Nat. 2010;175:E16-E34.

51. Miller R, Jastrow J: The application of VA mycorrhizae to ecosystem restoration and reclamation. New York: Springer; 1992.

52. Toju H, Sato H, Yamamoto S, Tanabe AS: Structural diversity across arbuscular mycorrhizal, ectomycorrhizal, and endophytic plant–fungus networks. bioRxiv 2018:https://doi.org/10.1101/269563

53. Caruso T, Rillig MC, Garlaschelli D: On the application of network theory to

arbuscular mycorrhizal fungi–plant interactions: the importance of basic assumptions. New Phytol. 2012;194:891-894.

54. Thompson JN: The geographic mosaic of coevolution. Chicago: University of Chicago Press; 2005.

55. Tanabe AS: Claident v0.2.2016.07.05, a software distributed by author at http://www.fifthdimension.jp/. 2016.

56. Tanabe AS, Toju H: Two new computational methods for universal DNA barcoding: a benchmark using barcode sequences of bacteria, archaea, animals, fungi, and land plants. PLOS ONE 2013;8:e76910.

57. Toju H, Tanabe AS, Yamamoto S, Sato H: High-coverage ITS primers for the DNA-based identification of ascomycetes and basidiomycetes in environmental samples. PLOS ONE 2012;7:e40863.

58. Smith DP, Peay KG: Sequence depth, not PCR replication, improves ecological inference from next generation DNA sequencing. PLOS ONE 2014;9:e90234.

59. O'Brien HE, Parrent JL, Jackson JA, Moncalvo J-M, Vilgalys R: Fungal community analysis by large-scale sequencing of environmental samples. Appl. Env. Microbiol. 2005;71:5544-5550.

60. Kõljalg U, Nilsson RH, Abarenkov K, Tedersoo L, Taylor AF, Bahram M et al: Towards a unified paradigm for sequence-based identification of fungi. Mol. Ecol. 2013;22:5271-5277.

61. Thiéry O, Vasar M, Jairus T, Davison J, Roux C, Kivistik PA et al: Sequence variation in nuclear ribosomal small subunit, internal transcribed spacer and large subunit regions of *Rhizophagus irregularis* and *Gigaspora margarita* is high and isolate - dependent. Mol. Ecol. 2016;25:2816-2832.

62. Huson DH, Auch AF, Qi J, Schuster SC: MEGAN analysis of metagenomic data. Genome Res. 2007;17:377-386.

63. Nguyen NH, Song Z, Bates ST, Branco S, Tedersoo L, Menke J et al: FUNGuild: an open annotation tool for parsing fungal community datasets by ecological guild. Fungal Ecol. 2016;20:241-248.

64. Anderson MJ: A new method for non-parametric multivariate analysis of variance. Austral Ecol. 2001;26:32-46.

65. Anderson MJ: Distance-based tests for homogeneity of multivariate dispersions. Biometrics 2006;62:245-253.

66. Oksanen J, Blanachet FG, Kindt R, Legendre P, Minchin PR, O'Hara RB et al: Vegan: community ecology package. R package version 2.0-3 available at http://CRAN.R-project.org/package=vegan. 2012;10:2008.

67. R-Core-Team: R 3.4.1: A language and environment for statistical computing available at http://www.R-project.org/. In. Vienna, Austri: R Foundation for Statistical Computing; 2015.

68. Chase JM, Kraft NJ, Smith KG, Vellend M, Inouye BD: Using null models to disentangle variation in community dissimilarity from variation in α-diversity. Ecosphere 2011;2:1-11.

69. Bastian M, Heymann S, Jacomy M: Gephi: an open source software for exploring and manipulating networks. Proceedings of the 3rd International AAAI Conference on Weblogs and Social Media 2009;8:361-362.

70. Jacomy M, Heymann S, Venturini T, Bastian M: ForceAtlas2, a continuous graph layout algorithm for handy network visualization designed for the Gephi Software. PLOS ONE 2011;96:e98679.

71. Blüthgen N, Menzel F, Blüthgen N: Measuring specialization in species interaction networks. BMC Ecol. 2006;6:9.

72. Freeman LC: A set of measures of centrality based on betweenness. Sociometry 1977;40:35-41.

73. Tedersoo L, Pärtel K, Jairus T, Gates G, Põldmaa K, Tamm H: Ascomycetes associated with ectomycorrhizas: molecular diversity and ecology with particular reference to the Helotiales. Env. Microbiol. 2009;11:3166-3178.

74. Rossman AY, Farr DF, Castlebury LA: A review of the phylogeny and biology of the Diaporthales. Mycoscience 2007;48:135-144.

75. Sieber TN: Endophytic fungi in forest trees: are they mutualists? Fungal Biol. Rev.

2007;21:75-89.

76. Liu X-Z, Wang Q-M, Göker M, Groenewald M, Kachalkin A, Lumbsch HT et al: Towards an integrated phylogenetic classification of the Tremellomycetes. Studies Mycol. 2015;81:85-147.

77. Watanabe T: Pictorial atlas of soil and seed fungi: morphologies of cultured fungi and key to species. Boca Raton: CRC press; 2010.

78. Zhang H, Wu X, Li G, Qin P: Interactions between arbuscular mycorrhizal fungi and phosphate-solubilizing fungus (Mortierella sp.) and their effects on Kostelelzkya virginica growth and enzyme activities of rhizosphere and bulk soils at different salinities. Biol. Fertil. Soils 2011;47:543.

79. Eroshin V, Dedyukhina E: Effect of lipids from *Mortierella hygrophila* on plant resistance to phytopathogens. World J. Microbiol. Biotechnol. 2002;18:165-167.

80. Melo IS, Santos SN, Rosa LH, Parma MM, Silva LJ, Queiroz SC et al: Isolation and biological activities of an endophytic *Mortierella alpina* strain from the Antarctic moss *Schistidium antarctici*. Extremophiles 2014;18:15-23.

81. Papavizas G: *Trichoderma* and *Gliocladium*: biology, ecology, and potential for biocontrol. Ann. Rev. Phytopathol. 1985;23:23-54.

82. Datnoff L, Nemec S, Pernezny K: Biological control of *Fusarium* crown and root rot of tomato in Florida using *Trichoderma harzianum* and *Glomus intraradices*. Biologi. Control 1995;5:427-431.

83. Bailey B, Bae H, Strem M, Crozier J, Thomas S, Samuels G et al: Antibiosis, mycoparasitism, and colonization success for endophytic *Trichoderma* isolates with biological control potential in Theobroma cacao. Biologi. Control 2008;46:24-35.

84. Bae H, Roberts DP, Lim H-S, Strem MD, Park S-C, Ryu C-M et al: Endophytic *Trichoderma* isolates from tropical environments delay disease onset and induce resistance against Phytophthora capsici in hot pepper using multiple mechanisms. Mol. Plant-Microbe Int. 2011;24:336-351.

85. AL-Shammari TA, Bahkali AH, Elgorban AM, El-Kahky MT, Al-Sum BA: The use of Trichoderma longibrachiatum and *Mortierella alpina* against root-knot nematode,

719 *Meloidogyne javanica* on tomato. J. Pure Appl. Microbiol. 2013;7:199-207.

720 86. Ming Q, Su C, Zheng C, Jia M, Zhang Q, Zhang H et al: Elicitors from the endophytic
721 fungus *Trichoderma atroviride* promote *Salvia miltiorrhiza* hairy root growth and
722 tanshinone biosynthesis. J. Exp. Bot. 2013;64:5687-5694.

723 87. Mašínová T, Bahnmann BD, Větrovský T, Tomšovský M, Merunková K, Baldrian P:
724 Drivers of yeast community composition in the litter and soil of a temperate forest.
725 FEMS Microbiol. Ecol. 2016;93:fiw223.

726 88. Nutaratat P, Srisuk N, Arunrattiyakorn P, Limtong S: Plant growth-promoting traits of
727 epiphytic and endophytic yeasts isolated from rice and sugar cane leaves in Thailand.
728 Fungal Biol. 2014;118:683-694.

729 89. Renker C, Blanke V, Börstler B, Heinrichs J, Buscot F: Diversity of *Cryptococcus* and
730 *Dioszegia* yeasts (Basidiomycota) inhabiting arbuscular mycorrhizal roots or spores.
731 FEMS Yeast Res. 2004;4:597-603.

732 90. Gai CS, Lacava PT, Maccheroni W, Glienke C, Araújo WL, Miller TA et al: Diversity
733 of endophytic yeasts from sweet orange and their localization by scanning electron
734 microscopy. J. Basic Microbiol. 2009;49:441-451.

735 91. Cloete KJ, Valentine AJ, Stander MA, Blomerus LM, Botha A: Evidence of symbiosis
736 between the soil yeast *Cryptococcus laurentii* and a sclerophyllous medicinal shrub,
737 *Agathosma betulina* (Berg.) Pillans. Microb. Ecol. 2009;57:624-632.

738 92. Couture M, Fortin J, Dalpe Y: Oidiodendron griseum Robak: An endophyte of ericoid
739 mycorrhiza in *Vaccinium* spp. New Phytol. 1983;95:375-380.

740 93. Vohník M, Albrechtová J, Vosátka M: The inoculation with *Oidiodendron maius* and
741 *Phialocephala fortinii* alters phosphorus and nitrogen uptake, foliar C: N ratio and root
742 biomass distribution in Rhododendron cv. Azurro. Symbiosis 2005;40:87-96.

743 94. Rice AV, Currah RS: *Oidiodendron*: A survey of the named species and related
744 anamorphs of *Myxotrichum*. Studies Mycol. 2005;53:83-120.

745 95. Lombard L, Van Der Merwe NA, Groenewald JZ, Crous PW: Lineages in
746 Nectriaceae: re-evaluating the generic status of *Ilyonectria* and allied genera.
747 Phytopathol. Mediterr. 2014;53:515-532.

748 96. Whitelaw - Weckert M, Rahman L, Appleby L, Hall A, Clark A, Waite H et al: Co -
749 infection by Botryosphaeriaceae and Ilyonectria spp. fungi during propagation causes
750 decline of young grafted grapevines. Plant Pathol. 2013;62:1226-1237.

751 97. Vitale A, Aiello D, Guarnaccia V, Perrone G, Stea G, Polizzi G: First report of root rot
752 caused by *Ilyonectria* (= *Neonectria*) macrodidyma on avocado (*Persea americana*) in
753 Italy. J. Phytopathol. 2012;160:156-159.

754 98. Usuki F, Narisawa K: Formation of structures resembling ericoid mycorrhizas by the
755 root endophytic fungus *Heteroconium chaetospira* within roots of *Rhododendron*
756 *obtusum* var. *kaempferi*. Mycorrhiza 2005;15:61-64.

757 99. Narisawa K, Hambleton S, Currah RS: *Heteroconium chaetospira*, a dark septate root
758 endophyte allied to the Herpotrichiellaceae (Chaetothyriales) obtained from some
759 forest soil samples in Canada using bait plants. Mycoscience 2007;48:274-281.

760 100. Vohník M, Albrechtová J: The co-occurrence and morphological continuum between
761 ericoid mycorrhiza and dark septate endophytes in roots of six European
762 *Rhododendron* species. Folia Geobotanica 2011;46:373-386.

763 101. Jumpponen A, Trappe JM: Dark septate endophytes: a review of facultative biotrophic
764 root-colonizing fungi. New Phytol. 1998;140:295-310.

765 102. Usuki F, Narisawa K: A mutualistic symbiosis between a dark septate endophytic
766 fungus, *Heteroconium chaetospira*, and a nonmycorrhizal plant, Chinese cabbage.
767 Mycologia 2007;99:175-184.

768 103. Wang W, Tsuneda A, Gibas CF, Currah RS: *Cryptosporiopsis* species isolated from
769 the roots of aspen in central Alberta: identification, morphology, and interactions with
770 the host, in vitro Botany 2007;85:1214-1226.

771 104. Kowalski T, Bartnik C: *Cryptosporiopsis radicicola* sp. nov. from roots of *Quercus*
772 *robur*. Mycol. Res. 1995;99:663-666.

773 105. Sigler L, Allan T, Lim SR, Berch S, Berbee M: Two new *Cryptosporiopsis* species
774 from roots of ericaceous hosts in western North America. Studies Mycol.
775 2005;53:53-62.

776 106. Schulz B, Sucker J, Aust H, Krohn K, Ludewig K, Jones P et al: Biologically active

secondary metabolites of endophytic *Pezicula* species. Mycol. Res. 1995;99:1007-1015.

107. Strobel GA, Miller RV, Martinez-Miller C, Condron MM, Teplow DB, Hess W: Cryptocandin, a potent antimycotic from the endophytic fungus *Cryptosporiopsis* cf. *quercina*. Microbiology 1999;145:1919-1926.

108. Talbot JM, Bruns TD, Taylor JW, Smith DP, Branco S, Glassman SI et al: Endemism and functional convergence across the North American soil mycobiome. Proc. Natl. Acad. Sci. USA. 2014;111:6341-6346.

109. Martiny JBH, Bohannan BJ, Brown JH, Colwell RK, Fuhrman JA, Green JL et al: Microbial biogeography: putting microorganisms on the map. Nat. Rev. Microbiol. 2006;4:102-112.

110. De Wit R, Bouvier T: 'Everything is everywhere, but, the environment selects'; what did Baas Becking and Beijerinck really say? Env. Microbiol. 2006;8:755-758.

111. Rooney N, McCann K, Gellner G, Moore JC: Structural asymmetry and the stability of diverse food webs. Nature 2006;442:265-269.

112. Ellison AM, Bank MS, Clinton BD, Colburn EA, Elliott K, Ford CR et al: Loss of foundation species: consequences for the structure and dynamics of forested ecosystems. Front. Ecol. Env. 2005;3:479-486.

113. Paine RT: Food web complexity and species diversity. Am. Nat. 1966;100:65-75.

114. Waller F, Achatz B, Baltruschat H, Fodor J, Becker K, Fischer M et al: The endophytic fungus *Piriformospora indica* reprograms barley to salt-stress tolerance, disease resistance, and higher yield. Proc. Natl. Acad. Sci. USA. 2005;102:13386-13391.

115. Castro-Sowinski S, Herschkovitz Y, Okon Y, Jurkevitch E: Effects of inoculation with plant growth-promoting rhizobacteria on resident rhizosphere microorganisms. FEMS Microbiol. Lett. 2007;276:1-11.

116. Streeter JG: Failure of inoculant rhizobia to overcome the dominance of indigenous strains for nodule formation. Can. J. Bot. 1994;40:513-522.

117. Hartman K, van der Heijden MG, Roussely-Provent V, Walser J-C, Schlaeppi K:

Deciphering composition and function of the root microbiome of a legume plant. Microbiome 2017;5:2.

118. Jumpponen A, Trappe JM: Performance of *Pinus contorta* inoculated with two strains of root endophytic fungus, *Phialocephala fortinii*: effects of synthesis system and glucose concentration. Can. J. Bot. 1998;76:1205-1213.

119. Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP: DADA2: high-resolution sample inference from Illumina amplicon data. Nat. Methods 2016;13:581-583.

120. Langille MG, Zaneveld J, Caporaso JG, McDonald D, Knights D, Reyes JA et al: Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. Nat. Biotech. 2013;31:814-821.

121. Vohník M, Mrnka L, Lukešová T, Bruzone MC, Kohout P, Fehrer J: The cultivable endophytic community of Norway spruce ectomycorrhizas from microhabitats lacking ericaceous hosts is dominated by ericoid mycorrhizal *Meliniomyces variabilis*. Fungal Ecol. 2013;6:281-292.

122. Richardson AE, Barea J-M, McNeill AM, Prigent-Combaret C: Acquisition of phosphorus and nitrogen in the rhizosphere and plant growth promotion by microorganisms. Plant Soil 2009;321:305-339.

123. Lundberg DS, Lebeis SL, Paredes SH, Yourstone S, Gehring J, Malfatti S et al: Defining the core *Arabidopsis thaliana* root microbiome. Nature 2012;488:86-90.

124. Bai Y, Müller DB, Srinivas G, Garrido-Oter R, Potthoff E, Rott M et al: Functional overlap of the *Arabidopsis* leaf and root microbiota. Nature 2015;528:364-369.

125. Edwards J, Johnson C, Santos-Medellín C, Lurie E, Podishetty NK, Bhatnagar S et al: Structure, variation, and assembly of the root-associated microbiomes of rice. Proc. Natl. Acad. Sci. USA. 2015;112:E911-E920.

126. Faust K, Raes J: Microbial interactions: from networks to models. Nat. Rev. Microbiol. 2012;10:538-550.

127. Kurtz ZD, Mueller CL, Miraldi ER, Littman DR, Blaser MJ, Bonneau RA: Sparse and compositionally robust inference of microbial ecological networks. PLOS Comp. Biol.

2015;11:e1004226.

128. Arnold AE, Mejía LC, Kyllo D, Rojas EI, Maynard Z, Robbins N et al: Fungal endophytes limit pathogen damage in a tropical tree. Proc. Natl. Acad. Sci. USA. 2003;100:15649-15654.

Table 1 Top-10 list of non-Glomeromycota OTUs with highest betweenness within the metacommunity networks. In each of the three metacommunity-scale networks examined (full, cool-temperate, and warm-temperate/subtropical), fungal OTUs were ranked based on their betweenness centrality scores. As taxonomic information of Glomeromycota OTUs with high betweenness scores was redundant (e.g., *Glomus* spp. or Glomeraceae spp.), the top-10 list of non-Glomeromycota OTUs is shown. Taxonomy information of each OTU was inferred based on the query-centric auto-*k*-nearest-neighbor algorithm of reference database search [55, 56] and subsequent taxonomic assignment with the lowest common ancestor algorithm [62]. The results of the NCBI nucleotide Blast are also shown. For simplicity, the functional groups of fungi inferred with the program FUNGuild [63] were organized into several categories. See Data S4 (Additional file 4) for details of the categories and for full results including Glomeromycota and other fungal OTUs.

OTU	Phylum	Class	Order	Family	Genus	Category	NCBI Blast top hit	Accession	Cover	Identity
Full (8sites)										
F_0042*	-	-	Mortierellales	Mortierellaceae	*Mortierella*	Saprotroph/Endophyte	*Mortierella humilis*	KP714537	100%	100%
F_0381	Basidiomycota	Tremellomycetes	Trichosporonales	Trichosporonaceae	*Cryptococcus*	Others_Unknown	*Saitozyma podzolica*†	KY320605	92%	99%
F_0079	Ascomycota	Sordariomycetes	Hypocreales	Nectriaceae	-	Saprotroph/Endophyte	*Ilyonectria protearum*	NR_152890	99%	100%
F_0489	-	-	Mortierellales	Mortierellaceae	*Mortierella*	Saprotroph/Endophyte	*Mortierella* sp.	KM113754	100%	100%
F_0010	Ascomycota	Leotiomycetes	-	Myxotrichaceae	*Oidiodendron*	Ericoid_Mycorrhizal	*Oidiodendron maius*	LC206669	100%	100%
F_0368	Basidiomycota	Malasseziomycetes	Malasseziales	Malasseziaceae	*Malassezia*	Others_Unknown	*Malassezia restricta*	KT809059	100%	100%
F_0623	-	-	Mortierellales	Mortierellaceae	*Mortierella*	Saprotroph/Endophyte	*Mortierella gamsii*	KY305027	100%	100%
F_1188	Basidiomycota	Tremellomycetes	Trichosporonales	Trichosporonaceae	*Cryptococcus*	Others_Unknown	*Saitozyma podzolica*†	KY320605	92%	99%
F_0007	Ascomycota	Sordariomycetes	Diaporthales	Melanconidaceae	*Melanconiella*	Saprotroph/Endophyte	*Melanconiella elegans*	KJ173701	100%	85%
F_0485	Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	*Trichoderma*	Saprotroph/Endophyte	*Trichoderma* sp.	HG008760	100%	100%

Northen 4 sites (cool-temperate)

F_0042*	-	-	Mortierellales	Mortierellaceae	*Mortierella*	Saprotroph/Endophyte	*Mortierella humilis*	KP714537	100%	100%
F_0034*	Ascomycota	Eurotiomycetes	Chaetothyriales	Herpotrichiellaceae	*Cladophialophora*	Saprotroph/Endophyte	*Cladophialophora chaetospira*	KF359558	100%	99%
F_0079*	Ascomycota	Sordariomycetes	Hypocreales	Nectriaceae	-	Saprotroph/Endophyte	*Ilyonectria protearum*	NR_152890	99%	100%
F_0015*	Ascomycota	-	-	-	-	Others_Unknown	*Cadophora orchidicola*	KX611558	100%	99%
F_0202*	Ascomycota	Eurotiomycetes	Chaetothyriales	Herpotrichiellaceae	*Cladophialophora*	Saprotroph/Endophyte	*Cladophialophora chaetospira*	HQ871875	100%	99%
F_0195*	Ascomycota	Eurotiomycetes	Chaetothyriales	Herpotrichiellaceae	*Cladophialophora*	Saprotroph/Endophyte	*Cladophialophora chaetospira*	EU035405	100%	100%
F_0181*	Ascomycota	Leotiomycetes	Helotiales	Dermateaceae	*Pezicula*	Endophyte	*Pezicula melanigena*	LC206665	100%	99%
F_0010	Ascomycota	Leotiomycetes	-	Myxotrichaceae	*Oidiodendron*	Ericoid_Mycorrhizal	*Oidiodendron maius*	LC206669	100%	100%
F_0103*	Ascomycota	Eurotiomycetes	Chaetothyriales	Herpotrichiellaceae	*Cladophialophora*	Saprotroph/Endophyte	*Cladophialophora chaetospira*	EU035403	100%	97%
F_0489*	-	-	Mortierellales	Mortierellaceae	*Mortierella*	Saprotroph/Endophyte	*Mortierella* sp.	KM113754	100%	100%

Southern 4 sites (warm-temperate and subtropical)

F_0381*	Basidiomycota	Tremellomycetes	Trichosporonales	Trichosporonaceae	*Cryptococcus*	Others_Unknown	*Saitozyma podzolica*†	KY320605	92%	99%
F_0042*	-	-	Mortierellales	Mortierellaceae	*Mortierella*	Saprotroph/Endophyte	*Mortierella humilis*	KP714537	100%	100%
F_0610*	Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	*Trichoderma*	Saprotroph/Endophyte	*Trichoderma spirale*	KU948158	100%	100%
F_1188*	Basidiomycota	Tremellomycetes	Trichosporonales	Trichosporonaceae	*Cryptococcus*	Others_Unknown	*Saitozyma podzolica*†	KY320605	92%	99%
F_0029	Ascomycota	Eurotiomycetes	Chaetothyriales	Herpotrichiellaceae	-	Others_Unknown	*Cladophialophora* sp.	LC189029	100%	99%
F_0017	Ascomycota	-	-	-	-	Others_Unknown	*Scleropezicula* sp.	KT809119	100%	98%
F_0007	Ascomycota	Sordariomycetes	Diaporthales	Melanconidaceae	*Melanconiella*	Saprotroph/Endophyte	*Melanconiella elegans*	KJ173701	100%	85%
F_0485	Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	*Trichoderma*	Saprotroph/Endophyte	*Trichoderma* sp.	HG008760	100%	100%
F_0112	Basidiomycota	Agaricomycetes	Thelephorales	Thelephoraceae	*Tomentella*	Ectomycorrhizal	*Tomentella stuposa*	KR019860	100%	98%
F_0073	Ascomycota	Sordariomycetes	-	-	-	Others_Unknown	*Rhexodenticula acaciae*	KY173442	94%	95%

*Fungal OTUs classified as metacommunity hubs (mean local betweenness > 0.5; metacommunity betweenness > 0.5)

†Synonym, *Cryptcoccus podzolica*

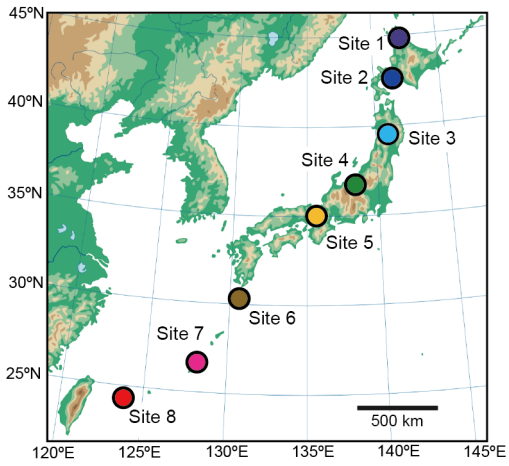

Fig. 1 Study sites examined in this study. Across the entire range of the Japanese Archipelago, root samples were collected in four cool-temperate forests (sites 1–4), one warm-temperate forest (site 5), and three subtropical forests (sites 6–8).

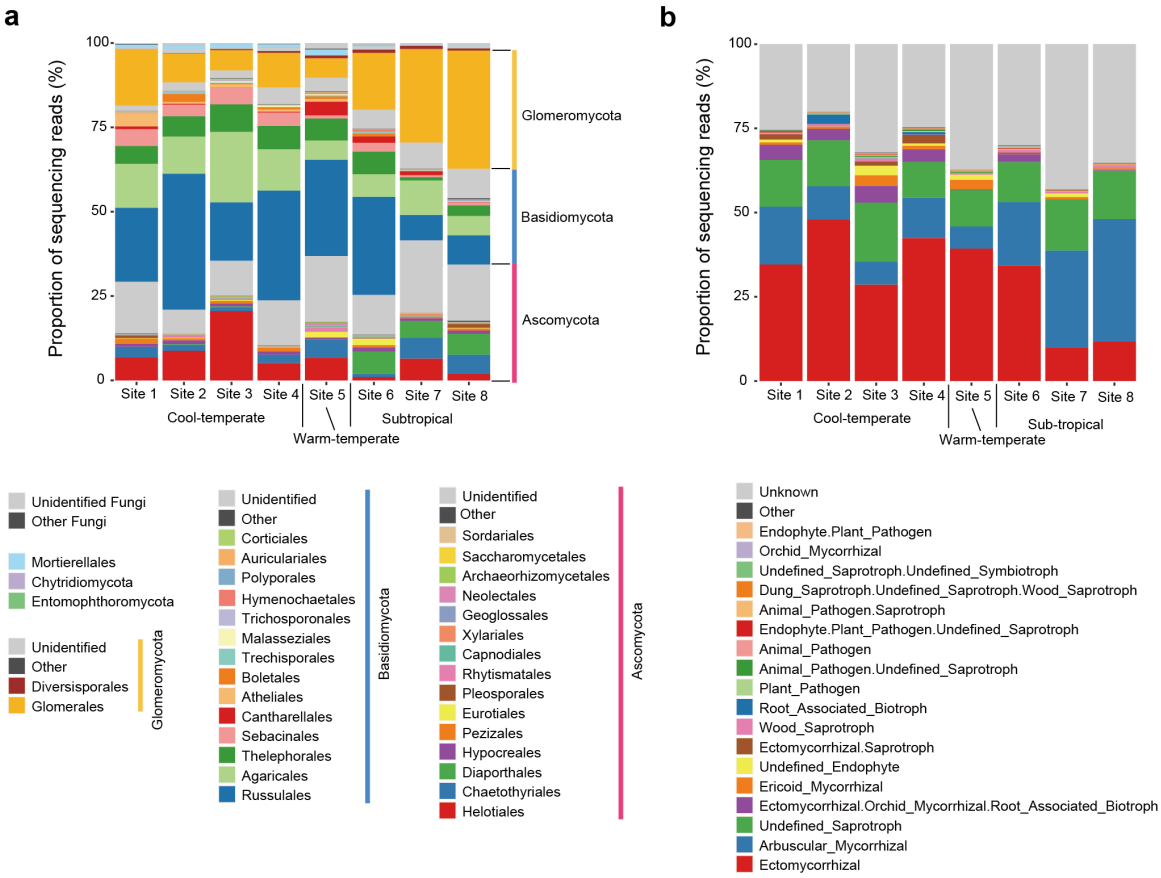

Fig. 2 Compositions of fungal taxa and functional groups in each forest. **a** Order-level taxonomic composition of fungal OTUs in each locality. The number of fungal OTUs detected is shown in a parenthesis for each forest. **b** Functional-group composition. The fungal functional groups were inferred by the program FUNGuild [63].

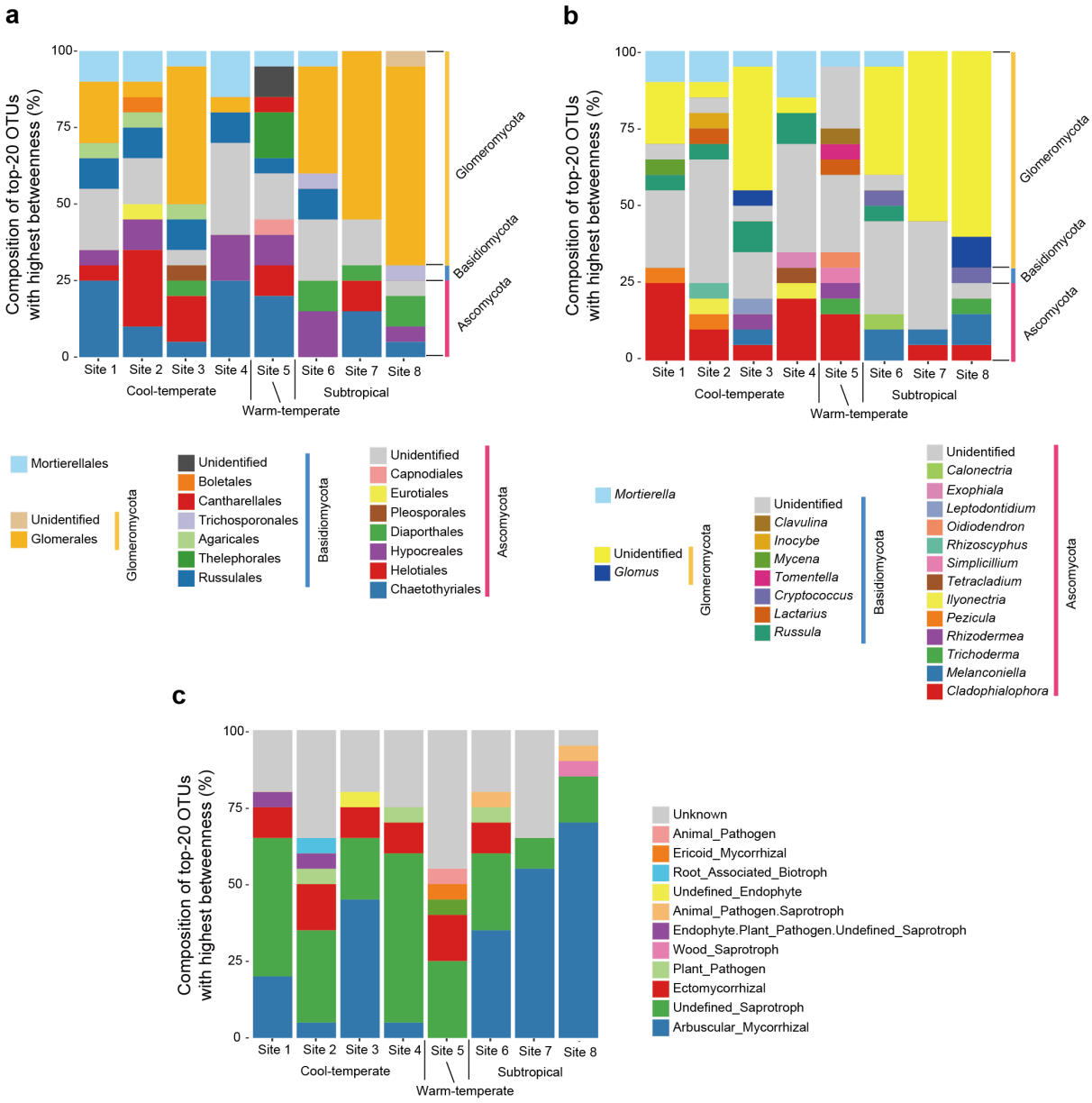

Fig. 3 Fungal OTUs with highest local betweenness. **a** Order-level taxonomic composition of top-20 OTUs with highest local betweenness in each forest. See Data S4 (Additional file 4) for betweenness scores of all fungal OTUs in respective local forests. **b** Genus-level taxonomic composition of top-20 OTUs with highest local betweenness. **c** Functional-group composition of top-20 OTUs with highest local betweenness.

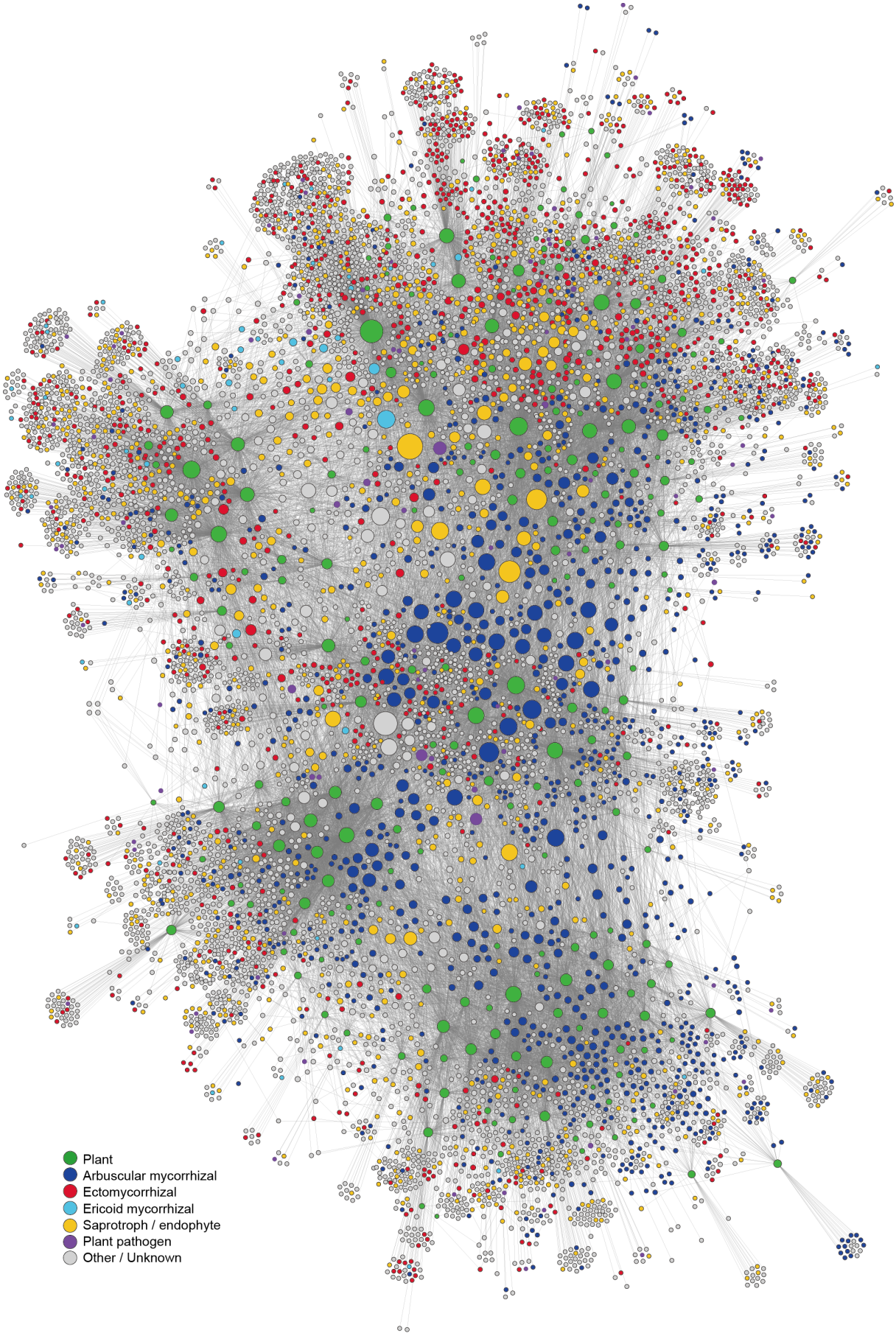

Fig. 4 Metacommunity-scale network including all the eight local forests. The size of circles roughly represents relative scores of betweenness centrality. The functional groups of fungi inferred with the program FUNGuild [63] were organized into six categories: i.e., arbuscular mycorrhizal (bue), ectomycorrhizal (red), ericoid mycorrhizal (skyblue), saprotrophic/endophytic (yellow), plant pathogenic (purple), and other/unknown fungi (grey) (Additional file 4; Data S4). For plant species/taxa (green), the geographic information of source populations is indicated in Additional file 8 (Figure S3).

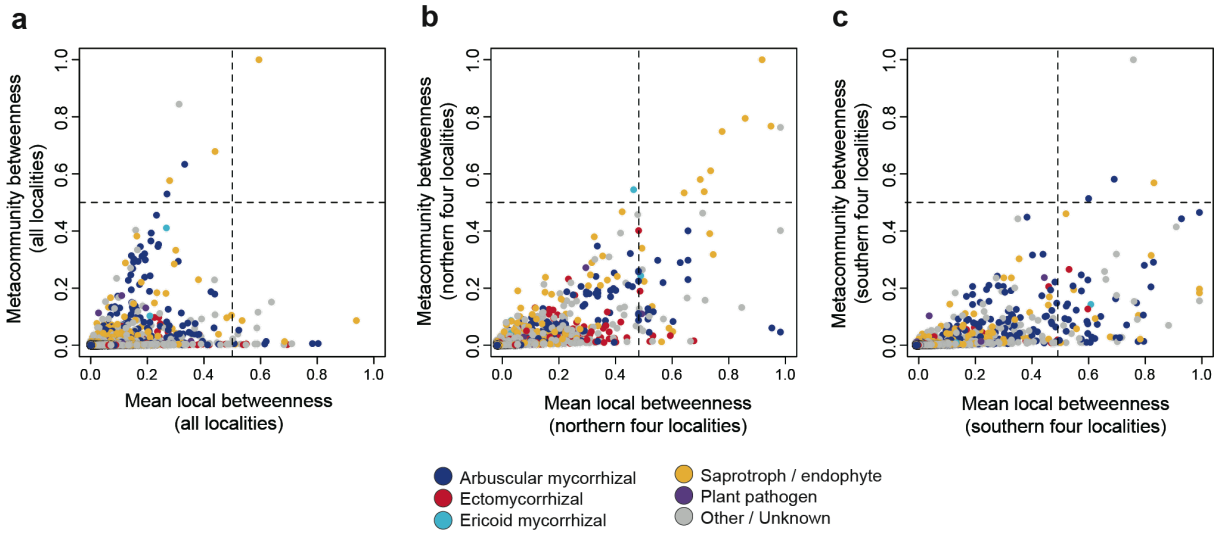

Fig. 5 Relationship between local- and metacommunity-level betweenness. **a** Full meatcommunity. On the horizontal axis, the mean values of betweenness centrality scores across all the eight local forests are shown for respective fungal OTUs. On the vertical axis, the betweenness scores within the metacommunity-scale network consisting of the eight localities (Fig. 4) are shown for respective OTUs. **b** Metacommunity of cool-temperate forests. For the sub-dataset consisting of the four cool-temperate forests (Additional file 9: Figure S4), mean local betweenness and metacommunity betweenness are shown on the horizontal and vertical axes, respectively. **c** Metacommunity of warm-temperate and subtropical forests. For the sub-dataset consisting of the warm-temperate forest and the three subtropical forests (Additional file 10: Figure S5), mean local betweenness and metacommunity betweenness are shown on the horizontal and vertical axes, respectively.