AEM Accepted Manuscript Posted Online 29 September 2017
Appl. Environ. Microbiol. doi:10.1128/AEM.01797-17

Ectomycorrhizal fungal communities in urban parks are similar to those in natural forests but shaped by vegetation and park age

Nan Hui[a]#, Xinxin Liu[a], D. Johan Kotze[a], Ari Jumpponen[b], Gaia Francini[a], Heikki Setälä[a]

Department of Environmental Sciences, University of Helsinki, Niemenkatu 73, 15240, Lahti, Finland[a]; Division of Biology, Kansas State University, Manhattan, KS 66506, USA[b]

Running Head: Ectomycorrhizal fungal communities in urban parks

#Address correspondence to Nan Hui, nan.hui@helsinki.fi.

N.H. and X.L. contributed equally to this work.

Abstract

Ectomycorrhizal (ECM) fungi are important mutualists for growth and health of most boreal trees. Forest age and its host species composition can impact the composition of ECM fungal communities. Although plentiful empirical data exist for forested environments, the effects of established vegetation and its successional trajectories on ECM fungi in urban greenspaces remain poorly understood. We analyzed ECM fungi in 5 control forests and 41 urban parks of two plant functional groups (conifer and broadleaf trees) and in three age categories (10, ~50 and >100 years old) in southern Finland. Our results show that although ECM fungal richness was marginally greater in forests than in urban parks, urban parks still hosted rich and diverse ECM communities. ECM community composition differed between the two habitats, but was driven by taxon rank order reordering, as key ECM taxa remained largely the same. In parks, the ECM communities differed between conifer and broadleaf trees. The successional trajectories of ECM fungi – as inferred in relation to the time since park construction – differed among the conifers and broadleaf trees: the ECM fungal communities changed over time under the conifers, whereas communities under broadleaf trees provided no evidence for such age related effects. Our data show that plant-ECM interactions in urban parks, in spite of being constructed environments, are surprisingly similar in richness to those in natural forests. This suggests that the presence of host trees, rather than soil characteristics or even disturbance regime of the system, determine ECM fungal community structure and diversity.

Importance

In urban environments soil and trees improve environmental quality and provide essential ecosystem services. Ectomycorrhizal (ECM) fungi enhance plant growth and performance, increasing plant nutrient acquisition and protecting plants against toxic compounds. Recent evidence indicates that soil-inhabiting fungal communities - including ECM and saprotrophic fungi - in urban parks are affected by plant functional type and park age. However, ECM fungal diversity and its responses to urban stress, plant functional type or park age remain

unknown. The significance of our study is in identifying - in greater detail – the responses of ECM fungi in the rhizospheres of conifer and broadleaf trees in urban parks. This will greatly enhance our knowledge of ECM fungal communities under urban stresses, and can be utilized by urban planners to improve urban ecosystem services.

Introduction

Soils and trees in urban parks improve environmental quality and provide essential ecosystem services (1). Healthy urban trees facilitate rainwater storage, mitigate urban heat island effects, support biodiversity, and provide an aesthetically appealing environment for urban residents (2). Ectomycorrhizal (ECM) fungi, necessary mutualists of most boreal trees, are important for host performance as well as for nutrient cycling at an ecosystem level (3). ECM fungi enhance plant growth and performance (4), increase plant nutrient acquisition (5) and protect plants against toxic compounds (6).

In urban parks, soil organisms are subject to anthropogenic disturbances, such as pollution, fertilization, trampling and the removal of plant litter. ECM are sensitive to urban disturbance (7) and their richness and abundance lower in urban ecosystems compared to rural areas (8, 9). Therefore, ECM fungi may be useful indicators reflecting the disturbance status of the below- and aboveground communities in urban areas, particularly where disturbances influence both soil properties and plant health. Although many factors influence ECM fungal community composition in boreal forest ecosystems such as edaphic factors, host plant species composition and stand age (10-12), factors that impact urban ECM fungi and their succession remain unclear (9).

Most ECM species typically have broad plant host ranges (13) and different hosts associate with divergent ECM fungal communities (14). ECM fungal communities can differ among urban and non-urban trees (9, 15), even among conspecific hosts (16). Recent evidence indicates that soil-inhabiting fungal communities - including ECM and saprotrophic fungi - in urban parks are affected by plant functional type and park age (17). However, ECM fungal diversity and its responses to urban stress, plant functional type or park age remain unknown.

In the current study, we extracted ECM fungal sequence data from a broader dataset analyzed in Hui et al. (17). This allowed for exclusive analyses ECM fungal responses in the rhizospheres of conifer and broadleaf trees in 41 parks in the cities of Lahti and Helsinki, Finland. The selected sites represent different park ages (*i.e.*, time since park construction) and thus provide a means to dissect how ECM fungal communities are modified over time in an urban environment. To compare these park communities to those in a more natural and less disturbed environment, we included 5 minimally disturbed rural forests dominated by *Picea abies* and *Tilia cordata* as non-urban controls.

Here, we focus on 1) the response of ECM fungal communities to plant functional type and park age in urban park soils; and 2) potential differences in ECM fungal communities between non-urban control forests and disturbed urban parks (land-use type). Further, we investigated 3) which ECM fungal genera are particularly responsive to land-use, plant functional type, and park age. We hypothesized that: i) ECM fungal communities under conifer and broadleaf trees in urban parks differ from those in control forests. This is because urban soils often have high pH, high concentrations of organic and inorganic pollutants (18) and their microbial communities may be affected by urban management (9). We also predicted that in the forest soil, ECM fungi are more diverse than in urban park soils. This is because of the positive relationship between canopy tree diversity and ECM diversity (9, 11). ii) ECM fungal community structure in urban parks depend on plant functional type. This is because plant functional types differ fundamentally in terms of effects on soil properties (18), allocation of recent photosynthate (19), litter and root exudates (14), upon which ECM fungi depend. iii) ECM fungal communities respond to park age. This is due to the different abilities of early- and late-stage fungi to form symbioses with host roots (20).

Results

96

97 Comparisons of ECM fungal communities between urban parks and control forests

98

99 The control forests and old parks in Lahti, representing roughly similarly aged trees, differed
100 in ECM fungal diversity. Diversity was generally greater in control forests than in the old
101 parks (Fig. 1, Table S1). OTU richness and diversity were lower under conifer trees than
102 broadleaf trees both in old parks and control forests (Fig. 1a, b), whereas evenness showed an
103 opposite trend (Fig. 1c). OTU richness, diversity and evenness correlated positively with soil
104 OM. Soil pH correlated with ECM fungal community richness positively and with evenness
105 negatively (Table S1).

106

107 ECM fungal community composition differed between (i) the old parks and control forests (r^2
108 = 0.425, $p < 0.001$) and (ii) the two tree functional types ($r^2 = 0.587$, $p < 0.001$; Fig. 2a). ECM
109 OTUs were classified into 51 genera throughout the dataset. *Inocybe* was the most dominant
110 genus (13.8% of the ECM fungal sequences, 49 OTUs), followed by *Cenococcum* (11.7%, 21
111 OTUs) and *Wilcoxina* (10.1%, 4 OTUs). To explore the ECM fungal community distinctions
112 between control forests and old parks in Lahti, we conducted GLMM analyses on the ten
113 most abundant genera. Five genera (*Amphinema*, *Piloderma*, *Russula*, *Tomentella* and
114 *Tylospora*) were more abundant in control forests than in old parks (Fig. 3, Table S1), while
115 none of the most abundant genera occurred more frequently in the parks. *Cenococcum* and
116 *Cortinarius* were constantly more abundant under broadleaf trees than conifer, whereas
117 *Wilcoxina* showed an opposite trend. *Russula* and *Tylospora* showed significant plant
118 functional type x land-use type (control forest vs. old Lahti parks) interactions. In addition to
119 these analyses, we also included a set of environmental variables in the GLMM analyses: four
120 ECM genera were correlated with soil N (one positively and three negatively), three with soil
121 C (one positively and two negatively), three with soil OM (all positively), four with

122 percentage sand (two positively and two negatively) and three with soil pH (two positively
123 and one negatively) (Table S1).
124
125 Effects of plant functional group and park age on ECM fungi in urban parks
126
127 In parks, ECM OTU richness and evenness had significant plant functional group x park age
128 interactions (Table S2). In young parks, both ECM fungal richness and diversity were
129 indistinguishable between the two plant functional groups. However, ca. 50 years after park
130 establishment, soils under broadleaf trees tended to host more diverse ECM fungal
131 communities than under conifer trees (Fig. 1 a, b). Diversity and evenness of the ECM fungal
132 communities in parks were negatively correlated with soil pH. In our case, all soils were
133 acidic with maximum pH ~ 6.9 (18). As a result, the diversity and evenness declined as pH
134 approached neutral. All diversity indices correlated negatively with soil C (Table S2).
135
136 ECM fungal community composition differed clearly between conifer and broadleaf trees in
137 old parks in Lahti. As a result, we conducted analyses separately for the two tree functional
138 groups in parks: ECM fungal communities under broadleaf trees responded to park age (r^2 =
139 0.110, p = 0.029, Fig. 2b), but this was not the case under conifer trees (r^2 = 0.041, p = 0.697).
140
141 To study the effects of plant functional type and park age on common ECM fungi in parks,
142 we analyzed the ten most abundant ECM fungal genera using GLMM. The abundances of
143 *Inocybe*, *Wilcoxina* and *Cenococcum* responded similarly and the differences among the two
144 plant functional types became more pronounced in intermediate and old parks than in young
145 parks (Fig. 4). In intermediate and old parks, *Wilcoxina* was more abundant under conifer
146 trees than under broadleaf trees, whereas *Cenococcum* and *Tuber* showed the opposite trend.
147 *Tuber* was also highly abundant under conifer trees in young parks but not under broadleaf

trees. *Hebeloma* and *Tomentella* abundances differed across park age, with a higher count in young parks than in intermediate and old parks. *Laccaria* and *Cortinarius* were consistently more abundant under broadleaf trees than under conifer trees, especially in old parks. *Cenococcum*, *Scleroderma* and *Tomentella* showed plant functional type x park age interactions (Table S2). Our GLMM results showed that six ECM fungal genera were correlated with soil N (all negatively), five with C (four positively and one negatively), three with OM (all negatively), seven with percentage sand (one positively and six negatively), and two with pH (one positively and one negatively) (Table S2).

Discussion

Our previous research showed that vegetation and park age drive changes in soil properties in urban parks (18) leading to distinct microbial communities (bacteria and fungi) (17). Here, we focused exclusively on ECM fungi and addressed how they respond to land-use type (forest vs urban park), plant functional type and park age under northern climatic conditions. Since different fungal groups have distinct life history strategies (21), we expected that ECM responses would differ from those of the general soil-inhabiting fungi (17).

Differences in ECM fungal communities between old parks and forests

Urbanization likely has negative effects on soil properties (18), microbial communities (22) and soil fauna (23). Our data indicate that ECM OTU richness and diversity were greater in control forests than in urban parks, supporting our first hypothesis and corroborating previous observations. Urban anthropogenic disturbance can reduce ECM diversity and richness (8, 9, 24, 25), particularly in boreal regions where these fungi are most diverse. Our data are in contrast to predictions that increasing urbanization and the concomitant loss of natural forests

will lead to the dramatic suppression of ECM fungi in urbanized ecosystems (22). Instead, we conclude that boreal hosts recruit quite diverse ECM fungi in urban greenspaces, suggesting ECM fungal community resistance and resilience to urbanization and co-occurring anthropogenic disturbances.

Despite the possible resistance and resilience, the ECM fungal communities in urban parks and control forests differed. This result was driven by taxon rank reordering, not taxon replacement. Urban disturbances (litter removal and raking, trampling, and mowing) are absent in forests, which likely result in alterations between the relative proportions of ECM taxa between urban park and forest communities (9). Furthermore, unlike natural boreal forests typified by podzol soils with organic matter layer developed on top of the soil, urban parks - even the oldest ones in our study - lacked such a clear pedogenesis. The direct and indirect effects of pedogenesis on soil physical-chemical parameters are factors that likely affect ECM fungal communities between the two land-use types. However, despite the absence of visible pedogenesis in the urban settings, ECM fungal richness and diversity were surprisingly similar between the natural and urban environments.

At the genus level, the ten most abundant ECM general were present in both urban parks and forest stands, but the abundance of many of these genera differed between urban parks and forests. These observations are in line with previous reports (9). For example, the abundance of *Russula* and *Tylospora* were low in urban parks compared to control forests. This is in accordance with Hartmann et al. who showed that the abundance of *Russula* is negatively related with soil compaction (26). *Tylospora* occurs in decaying wood (27) which is scarce or absent in urban parks. *Tuber* was more frequent in urban parks than in control forests. This result is in agreement with (28) who predicted that *Tuber* may be “pre-adapted” to environmental conditions associated with human activities. *Tuber* species tend to prefer

alkaline soils (28). A potential explanation for the observed greater abundance is that the acidic soil in control forest largely suppresses *Tuber* species, while they may survive in the neutral or weakly acid soils, which indeed typify urban environments (29).

Generally our results suggest that ECM fungal richness and diversity were comparable in urban parks and control forests, albeit minimally different. ECM fungal communities shared a number of taxa between the land-use types, but were reordered and dominants replaced as indicated by our genus level analyses.

Effects of plant functional type on ECM fungal communities in parks

Supporting previous findings (17, 30) and our second hypothesis, the two tree functional groups hosted distinct ECM fungal communities – both in their diversity and composition. Reasons for the observed compositional differences may lie in the plant-ECM fungus interaction. Plant functional types can influence ECM fungal communities in several ways, including effects through host specificity, modulation of edaphic conditions, litter quality and quantity (labile or recalcitrant), and rhizodeposition (root exudates) (14, 31-33). A recent meta-analysis revealed that host family explained 34% of the variation in ECM fungal community composition (34). This may be a result of specific molecular signaling between the host and its fungal symbionts that determines their compatibility (35).

Our previous studies show that plant functional types modify soils differently and soils under conifer trees have lower pH, but higher %OM, %N and %C than soils under broadleaf trees in urban parks (18). In the current study, we found that six of the 10 most abundant ECM fungal genera in urban parks were negatively correlated with soil N. Nitrogen content is a major factor influencing ECM communities (35). High N supply suppresses biomass of ECM fungi,

because when the host tree can easily obtain nutrients, there is no need to form such plant-fungi symbiont (36, 37). However these chemical responses and correlations did not remarkably affect ECM fungal community richness and diversity.

Differences in the ECM fungal communities between the two tree functional types were largely attributable to shifts in the abundances of some ECM fungal genera. For example, *Cenococcum* was more common with broadleaf trees than conifers. Twieg et al. showed that the mean relative abundance of *Cenococcum* on broadleaf tree roots (paper birch, *Betula papyrifera*) was about four times greater than on conifer trees (Douglas-fir) in a mixed temperate forest (12). *Cenococcum* – one of the most common ECM species in boreal forest soil – seems to respond negatively to high nitrogen in the soil (11). *Cenococcum* is common in soils with low nitrogen content, thus, as a result of the N deposition and subsequent higher N availability, the taxon declines (38, 39). Indeed, in our study, *Cenococcum* was negatively correlated with N in park soils that accumulate traffic-derived nitrogen (40). *Wilcoxina* spp., in turn, are generalists and well adapted to a wide range of plant community types. They are often among the dominant ECM taxa in coniferous forest (41, 42). Similarly, in parks, *Wilcoxina* spp. were clearly associated with conifer trees. These results highlight that, despite the distinct environmental conditions in parks and forests, ECM fungus host preferences operate similarly regardless of land use. This highlights the pivotal role of plant identity in controlling plant-fungus symbiosis.

The effects of park age on the ECM fungal community

ECM fungal diversity seemed rather insensitive to park age. This finding supports neither our third hypothesis nor previous results by Twieg et al., who showed that ECM diversity increased as stand age increased in soils under conifer trees in natural forests (12).

252 Presumably ECM diversity would increase as plants grow older, because trees in young parks
253 lack an extensive root system for ECM colonization (43). Further, young soils may lack an
254 extensive ECM propagule bank because landfill top soils are common in park construction
255 and because of insufficient propagule dispersal to recently established habitats (44). The
256 relatively stable ECM diversity that we observed across park ages may result from the
257 minimal competition in the urban environments allowing many ECM fungi to rapidly
258 colonize roots of young trees. It appears that young trees are equally suitable hosts for these
259 ECM fungal spores to colonize their roots. Although parks soil characteristics change by age
260 (18), the modifications do not influence the colonization of ECM fungi, suggesting that ECM
261 spores are ubiquitously present in the urban environment. However, to our knowledge, studies
262 that explicitly explore the effects of host age on ECM fungal communities in urban soils are
263 non-existent.
264
265 Despite the lack of an overall park age effect on ECM fungal community diversity,
266 community composition responded to park age in the broadleaf tree rhizospheres. Similar
267 responses were absent with the conifer hosts. The lack of this response under conifer trees is
268 surprising, given that soil chemistry changes (lower pH, higher OM, C and N) were
269 particularly pronounced underneath conifers in our parks (18). Previous studies on ECM
270 succession in natural forests suggest some context dependency of community responses to
271 stand age. ECM fungal composition and diversity were insensitive to the age of oak (*Quercus*
272 *ilex*) stands (45), whereas Kyaschenko et al. reported changes in the ECM fungus species
273 composition along an age gradient of managed *Pinus sylvestris* stands (46). Taken together,
274 these suggest that ECM fungal communities are primarily shaped by host-fungus interactions
275 rather than by abiotic habitat conditions such as soil chemistry.
276
277 **Conclusion**

278

279 Our results demonstrate that, in general, ECM fungi respond to land-use type (urban park vs.
280 non-urban forest stands) and to plant functional types within parks and forests. Although
281 ECM fungal richness was marginally greater in control forests than in urban parks, urban
282 parks still hosted rich and diverse ECM fungal communities. ECM fungal community
283 composition differed between the two habitats, but it was the common taxa that varied in
284 abundance without clear taxon replacements, indicating that key ECM fungi remained mainly
285 the same. In parks, ECM fungal community composition differed between conifer and
286 broadleaf trees. Park age also proved to shape ECM fungal community composition, but this
287 was evident under broadleaf trees only. Interestingly, plant functional group effects tended to
288 be amplified in older parks where ECM fungi have had a longer time to interact with tree
289 roots. We conclude that despite the lack of natural pedogenesis and arrested vegetation
290 succession as well as anthropogenic disturbance that includes raking leaves, mowing and
291 trampling, urban parks host a surprisingly diverse set of ECM fungi. Whether these urban
292 ECM fungal communities functionally approximate those in natural forest stands requires
293 further research.

294

295 **Materials and methods**

296

297 Study area and sampling design

298

299 The study sites have been described in detail previously (17, 18). Briefly, we selected 41
300 urban parks in the cities of Helsinki and Lahti, southern Finland, and 5 additional control
301 forests in the proximity of Lahti. The urban parks represent different ages: more than 100
302 years old (the oldest parks were established over two centuries ago), 50 ± 10 years old and 10
303 years old, referred to as old, intermediate and young parks, respectively. We considered two

304 plant functional types in these parks: conifer (43.3% of the conifer tree species represents
305 *Picea* spp.; 20% represents *Abies* sp.; 13.3% represents *Pseudotsuga menziesii*; 13.3%
306 represents *Pinus sylvestris* 13.3%; 10% represents *Larix* sp.) and broadleaf (*Tilia* x *vulgaris*
307 100%) trees. With a few exceptions, conifer and broadleaf trees existed commonly together
308 within a park. Distance between the two tree types was always greater than the height of the
309 tallest tree. The age of plants within each park age class corresponded with park age, except
310 for the young parks where trees are commonly planted as ca. 10 year old saplings at the time
311 of park construction. The ideal experimental design would have included 15 parks per city,
312 represented by five old, five intermediate and five young parks, with both plant functional
313 types present. However, since some parks did not include both plant functional types, we also
314 selected parks with only one plant functional type. This resulted in a total of 41 urban parks
315 and 58 urban sampling locations with 7-11 replicates per park age and plant functional type.
316 Park sizes varied considerably, ranging from ca. 0.1 ha to several hectares, but with no
317 systematic grouping of size with park age and plant functional type.
318
319 Soil sampling and edaphic conditions
320
321 ECM fungi colonize roots, but they grow from roots into the soil to deliver soil nutrients to
322 the roots. Because of this, soil is a good proxy in studying ECM fungal communities and
323 assigning the detected taxa to ecological roles (47-49). We sampled soils in May 2015 at the
324 edge of the canopy projection so that distance to the nearest tree trunk ranged from 1 m
325 (young parks; samples always collected outside the planting pit) to several meters (old parks).
326 At each sampling point, we subsampled 3 soil cores (top 10 cm) using a steel push corer (10
327 cm deep, 2.54 cm diameter), pooled the three subsamples into one composite for a total of 68
328 samples across the experiment (58 urban park samples and 10 control forest samples). The
329 corer was sterilized using 70% ethanol between samples. Samples were stored in Minigrip

330 bags on ice in the field and frozen at −20 °C in the laboratory. Before DNA extraction, the
331 samples were thawed at room temperature and sieved through a 2 mm mesh to remove any
332 remaining large particles. The edaphic conditions (0-10 cm deep) of all samples were
333 analyzed in our previous studies (18, 50). Five variables, carbon content (C), nitrogen content
334 (N), organic matter (OM), percentage sand (PS) and pH were used in our statistical analyses.
335

336 DNA extraction, PCR and Illumina MiSeq sequencing
337

338 Total DNA was extracted from ~10 g (8.2–10.1 g fresh weight) soil samples using the
339 PowerMax® Soil DNA Isolation Kit (MoBio, Carlsbad, California) following the
340 manufacturer's instructions, and stored at −20 °C until PCR amplification. The hypervariable
341 Internal Transcribed Spacer (ITS2) region of the fungal rRNA gene was amplified with
342 primers fITS7 5'-GTGARTCATCGAATCTTTG-3' incorporating 5'-
343 GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT-3' overhang and ITS4 5'-
344 TCCTCCGCTTATTGATATGC-3' incorporating 5'-
345 ATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT-3' overhang. In the secondary
346 PCR, the full-length P5 and indexed P7 Illumina MiSeq adapters were used. The PCR
347 reactions were performed as in (51). The samples were analyzed using the Fragment Analyzer
348 (Advanced Analytical, USA) and amplicons sequenced with Illumina MiSeq (v.3 2x300bp
349 paired-end) at the Institute of Biotechnology, University of Helsinki. The paired fastq files are
350 available in the Sequence Read Archive at NCBI (www.ncbi.nlm.nih.gov) under accession
351 number SRX1584451.
352

353 Bioinformatics
354

355 We extracted the ECM fungus dataset from a broader environmental sequence dataset
356 described in our previous study (17). In the current contribution, we explicitly focused on
357 mycorrhizal communities, because our previous effort on general fungal communities poorly
358 permitted us to address changes in ECM fungal community composition and diversity.
359 Briefly, we processed the paired end sequence data (.fastq) using mothur version 1.36.1(52).
360 The fungal .fastq files were contiged and any sequences with ambiguous bases, with more
361 than one mismatch to the primers, homopolymers longer than 8 bp and any without a
362 minimum overlap of 50 bp were removed. The sequences were screened for chimeras using
363 UCHIME (53) and putative chimeras removed. To permit pairwise alignment of fungal ITS
364 sequences to calculate a pairwise distance matrix, we omitted sequences that were shorter
365 than 300bp, and truncated the remaining sequences to the first 300bp. These fungal sequences
366 were assigned to taxa using the Naïve Bayesian Classifier and the UNITE-curated
367 International Nucleotide Sequence Database reference database (54). Any sequences not
368 assigned to Kingdom Fungi were removed. A pairwise distance matrix was derived from
369 pairwise alignments and sequences clustered to OTUs at a 97% threshold using nearest
370 neighbour joining. All low abundance OTUs were removed (≤ 10 sequences across all
371 experimental units) as they may be PCR or sequencing artifacts (55-57). We assigned OTUs
372 into trophic modes using the FUNGuild database (58) and selected ECM OTUs at the cut
373 value of "highly probability". This resulted in a total of 216 916 sequences representing 357
374 ECM OTUs. We estimated richness and diversity indices for ECM fungal communities in
375 mothur. Observed OTU richness (S_{obs}), the complement of Simpson's diversity (1/D: $1/\sum p_i^2$),
376 and Simpson's evenness (E_D: $1/\sum p_i^2/S$), with p_i representing the abundance of each OTU
377 within a sample, were iteratively calculated and subsampled at 517 sequences per sample.
378
379 Statistical analyses
380

All statistical analyses were performed in R (version 3.2.1, R Development Core Team, 2015) using various packages.

ECM fungal community data were analyzed using two different strategies. First, we evaluated differences between urban parks and the control forests (land-use type), using a dataset including the 10 controls (five control forests with conifer and broadleaf species in the vicinity of the city of Lahti) and old parks (five parks with conifer and broadleaf species within the city of Lahti), for a total of 20 experimental units. We compared controls to old parks because they have trees of virtually the same age class, which enabling comparison between habitat types and excluding tree age. In this analysis, we specifically explored differences in ECM fungal communities in control forests and comparable park treatments. Differences in ECM fungal diversity indices (Ln-transformed where necessary) and counts (sequence abundance) of the dominant genera (the 10 most abundant genera) between land-use types were evaluated using generalized linear mixed models (GLMM) with the *lmer* and *glmer* functions in the lme4 package in R. Diversity index data were modeled following a Gaussian distribution, while count data (the dominant genera) were modeled following a Poisson error distribution, with an individual-level random effect included to account for possible overdispersion (59). Predictor variables included plant functional type as a factor, land-use type as a factor and their interaction, as well as C and N content of the soil, OM, percentage sand (PS) and soil pH. Since our samples were from two different vegetation treatments that may locate in the same park, park location was added as a random term. We performed model selection by removing non-significant terms, starting with the term with the highest p-value. C, N, OM, PS and pH were initially subject to model simplification until only terms with p-values < 0.1 were left. If the land-use type x plant functional group interaction remained non-significant (p-values > 0.1) after this procedure, it was also removed. However, to remain true to our experimental design, the main effects (land-use type and plant functional

type) were always retained in the model irrespective of their significance. Second, we evaluated the effects of plant functional type and park age on ECM fungi. Here, we analyzed a dataset including all park age categories and plant functional types, but omitted the control stands. Similarly to the above, the response of individual ECM taxa (the 10 most abundant genera only) (count data) and diversity indices (Ln-transformed when necessary) to park age and plant functional type were tested using GLMM. In these analyses, land-use was replaced with park age; otherwise the analyses were identical to those described for the first strategy.

For each of the two discrete analyses, we also utilized non-metric multidimensional scaling (NMDS, vegan package in R) to visualize community wide responses to the factors included, based on Bray-Curtis dissimilarity. Soil carbon and nitrogen content, OM, PS and soil pH were correlated with the community structure using permutation tests as the vector fitting procedure (the *envfit* function in vegan). We did the same ordination analyses on the park ECM fungal communities under conifer and broadleaf trees separately. These analyses were motivated by the distinctions between the two plant functional types, thus permitting a more detailed focus on the effects of park age and multi tree species within the conifer group. In addition, because the same conifer tree species were not consistently present in our parks, we included 5 tree species in this plant functional group and tested the tree species effect on the ECM fungal communities. Although the five conifer tree species differed in their ECM fungal communities ($r^2 = 0.110$, $p = 0.029$, Fig. 5) when comparing "inter-tree type variation" with variation between the two plant functional types, the tree species effect within the conifer group was minor.

Acknowledgements

We thank Tuukka Ryynänen and John Allen for help in the field. Satu Tegel, Tuuli Ylikotila and Pekka Engblom provided data for the parks in Helsinki, and Markku Saari in Lahti. Lars Paulin provided DNA sequencing services. The research was funded by an Academy of Finland grant (Grant number 268548).

Reference

1. Gómez-Baggethun E, Barton DN. 2013. Classifying and valuing ecosystem services for urban planning. Ecol Econ 86:235-245.
2. Grote R, Samson R, Alonso R, Amorim JH, Cariñanos P, Churkina G, Fares S, Thiec DL, Niinemets Ü, Mikkelsen TN. 2016. Functional traits of urban trees: air pollution mitigation potential. Front Ecol Environ 14:543-550.
3. Smith S, Read D. 2008. Mineral nutrition, toxic element accumulation and water relations of arbuscular mycorrhizal plants. Mycorrhizal symbiosis, 3rd edn Academic, London:145-148.
4. Jonsson LM, Nilsson MC, Wardle DA, Zackrisson O. 2001. Context dependent effects of ectomycorrhizal species richness on tree seedling productivity. Oikos 93:353-364.
5. Velmala SM, Rajala T, Heinonsalo J, Taylor AF, Pennanen T. 2014. Profiling functions of ectomycorrhizal diversity and root structuring in seedlings of Norway spruce (Picea abies) with fast-and slow-growing phenotypes. New Phytol 201:610-622.
6. Luo Z-B, Wu C, Zhang C, Li H, Lipka U, Polle A. 2014. The role of ectomycorrhizas in heavy metal stress tolerance of host plants. Environ Exp Bot 108:47-62.
7. Dighton J, Tuininga AR, Gray DM, Huskins RE, Belton T. 2004. Impacts of atmospheric deposition on New Jersey pine barrens forest soils and communities of ectomycorrhizae. For Ecol Manage 201:131-144.

8. Bainard LD, Klironomos JN, Gordon AM. 2011. The mycorrhizal status and colonization of 26 tree species growing in urban and rural environments. Mycorrhiza 21:91-96.

9. Jumpponen A, Jones KL, David Mattox J, Yaege C. 2010. Massively parallel 454-sequencing of fungal communities in Quercus spp. ectomycorrhizas indicates seasonal dynamics in urban and rural sites. Molecular Ecology 19:41-53.

10. Visser S. 1995. Ectomycorrhizal fungal succession in jack pine stands following wildfire. New Phytol 129:389-401.

11. Kernaghan G, Widden P, Bergeron Y, Légaré S, Paré D. 2003. Biotic and abiotic factors affecting ectomycorrhizal diversity in boreal mixed-woods. Oikos 102:497-504.

12. Twieg BD, Durall DM, Simard SW. 2007. Ectomycorrhizal fungal succession in mixed temperate forests. New Phytol 176:437-447.

13. Tedersoo L, Sadam A, Zambrano M, Valencia R, Bahram M. 2010. Low diversity and high host preference of ectomycorrhizal fungi in Western Amazonia, a neotropical biodiversity hotspot. The ISME journal 4:465-471.

14. Prescott CE, Grayston SJ. 2013. Tree species influence on microbial communities in litter and soil: current knowledge and research needs. Forest Ecology and Management 309:19-27.

15. Baxter JW, Pickett ST, Carreiro MM, Dighton J. 1999. Ectomycorrhizal diversity and community structure in oak forest stands exposed to contrasting anthropogenic impacts. Canadian Journal of Botany 77:771-782.

16. Timonen S, Kauppinen P. 2008. Mycorrhizal colonisation patterns of Tilia trees in street, nursery and forest habitats in southern Finland. Urban Forestry & Urban Greening 7:265-276.

17. Hui N, Jumpponen A, Francini G, Kotze DJ, Liu X, Romantschuk M, Strömmer R, Setälä H. 2017. Soil microbial communities are shaped by vegetation type and park age in cities under cold climate. Environ Microbiol 19:1281-1295.

18. Setälä HM, Francini G, Allen JA, Hui N, Jumpponen A, Kotze DJ. 2016. Vegetation type and age drive changes in soil properties, nitrogen and carbon sequestration in urban parks under cold climate. Frontiers in Ecology and Evolution 4:93.

19. Sato H, Morimoto S, Hattori T. 2012. A thirty-year survey reveals that ecosystem function of fungi predicts phenology of mushroom fruiting. PLoS One 7:e49777.

20. Last FT, Dighton J, Mason PA. 1987. Successions of sheathing mycorrhizal fungi. Trends Ecol Evol 2:157-161.

21. Hobbie EA, Macko SA, Shugart HH. 1999. Insights into nitrogen and carbon dynamics of ectomycorrhizal and saprotrophic fungi from isotopic evidence. Oecologia 118:353-360.

22. Schmidt DJE, Pouyat R, Szlavecz K, Setälä H, Kotze DJ, Yesilonis I, Cilliers S, Hornung E, Dombos M, Yarwood SA. 2017. Urbanization erodes ectomycorrhizal fungal diversity and may cause microbial communities to converge. Nature Ecology & Evolution 1:0123.

23. Amossé J, Dózsa-Farkas K, Boros G, Rochat G, Sandoz G, Fournier B, Mitchell EAD, Le Bayon R-C. 2016. Patterns of earthworm, enchytraeid and nematode diversity and community structure in urban soils of different ages. Eur J Soil Biol 73:46-58.

24. Baxter JW, Dighton J. 2005. Diversity-functioning relationships in ectomycorrhizal fungal communities. MYCOLOGY SERIES 23:383.

25. Ochimaru T, Fukuda K. 2007. Changes in fungal communities in evergreen broad-leaved forests across a gradient of urban to rural areas in JapanThis article is one of a selection of papers published in the Special Forum on Towards Sustainable Forestry

— The Living Soil: Soil Biodiversity and Ecosystem Function. Can J For Res 37:247-258.

26. Hartmann M, Niklaus PA, Zimmermann S, Schmutz S, Kremer J, Abarenkov K, Lüscher P, Widmer F, Frey B. 2014. Resistance and resilience of the forest soil microbiome to logging-associated compaction. The ISME journal 8:226-244.

27. Mäkipää R, Rajala T, Schigel D, Rinne KT, Pennanen T, Abrego N, Ovaskainen O. 2017. Interactions between soil-and dead wood-inhabiting fungal communities during the decay of Norway spruce logs. The ISME Journal 11:1964–1974.

28. Wang X-H, Benucci GMN, Xie X-D, Bonito G, Leisola M, Liu P-G, Shamekh S. 2013. Morphological, mycorrhizal and molecular characterization of Finnish truffles belonging to the Tuber anniae species-complex. Fungal Ecol 6:269-280.

29. Pouyat RV, Yesilonis I, Russell-Anelli J, Neerchal N. 2007. Soil chemical and physical properties that differentiate urban land-use and cover types. Soil Sci Soc Am J 71:1010-1019.

30. Tedersoo L, Mett M, Ishida TA, Bahram M. 2013. Phylogenetic relationships among host plants explain differences in fungal species richness and community composition in ectomycorrhizal symbiosis. New Phytol 199:822-831.

31. Korkama T, Pakkanen A, Pennanen T. 2006. Ectomycorrhizal community structure varies among Norway spruce (Picea abies) clones. New Phytol 171:815-824.

32. Hartmann A, Schmid M, Van Tuinen D, Berg G. 2009. Plant-driven selection of microbes. Plant Soil 321:235-257.

33. Urbanová M, Šnajdr J, Baldrian P. 2015. Composition of fungal and bacterial communities in forest litter and soil is largely determined by dominant trees. Soil Biol Biochem 84:53-64.

34. Tedersoo L, Bahram M, Toots M, Diedhiou AG, Henkel TW, Kjøller R, Morris MH, Nara K, Nouhra E, Peay KG. 2012. Towards global patterns in the diversity and community structure of ectomycorrhizal fungi. Mol Ecol 21:4160-4170.

35. Cox F, Barsoum N, Lilleskov EA, Bidartondo MI. 2010. Nitrogen availability is a primary determinant of conifer mycorrhizas across complex environmental gradients. Ecology letters 13:1103-1113.

36. Högberg MN, Högberg P, Myrold DD. 2007. Is microbial community composition in boreal forest soils determined by pH, C-to-N ratio, the trees, or all three? Oecologia 150:590-601.

37. Avis PG. 2012. Ectomycorrhizal iconoclasts: the ITS rDNA diversity and nitrophilic tendencies of fetid Russula. Mycologia 104:998-1007.

38. Lilleskov EA, Wargo PM, Vogt KA, Vogt DJ. 2008. Mycorrhizal fungal community relationship to root nitrogen concentration over a regional atmospheric nitrogen deposition gradient in the northeastern USA. Canadian Journal of Forest Research 38:1260-1266.

39. Lilleskov EA, Hobbie EA, Fahey TJ. 2002. Ectomycorrhizal fungal taxa differing in response to nitrogen deposition also differ in pure culture organic nitrogen use and natural abundance of nitrogen isotopes. New Phytologist 154:219-231.

40. Pouyat RV, Russell-Anelli J, Yesilonis ID, Groffman PM. 2003. Soil carbon in urban forest ecosystems. CRC Press: Boca Raton, FL, USA.

41. Aučina A, Rudawska M, Leski T, Ryliškis D, Pietras M, Riepšas E. 2011. Ectomycorrhizal fungal communities on seedlings and conspecific trees of Pinus mugo grown on the coastal dunes of the Curonian Spit in Lithuania. Mycorrhiza 21:237-245.

42. Hui N, Jumpponen A, Niskanen T, Liimatainen K, Jones KL, Koivula T, Romantschuk M, Strömmer R. 2011. EcM fungal community structure, but not

558 diversity, altered in a Pb-contaminated shooting range in a boreal coniferous forest site
559 in Southern Finland. FEMS microbiology ecology 76:121-132.
560 43. Kranabetter JM, Friesen J, Gamiet S, Kroeger P. 2005. Ectomycorrhizal mushroom
561 distribution by stand age in western hemlock – lodgepole pine forests of northwestern
562 British Columbia. Can J For Res 35:1527-1539.
563 44. Huang J, Nara K, Zong K, Lian C. 2015. Soil propagule banks of ectomycorrhizal
564 fungi along forest development stages after mining. Microb Ecol 69:768.
565 45. Richard F, Millot S, Gardes M, Selosse MA. 2005. Diversity and specificity of
566 ectomycorrhizal fungi retrieved from an old-growth Mediterranean forest dominated
567 by Quercus ilex. New Phytol 166:1011-1023.
568 46. Kyaschenko J, Clemmensen KE, Hagenbo A, Karltun E, Lindahl BD. 2017. Shift in
569 fungal communities and associated enzyme activities along an age gradient of
570 managed Pinus sylvestris stands. The ISME journal 11:863–874.
571 47. Coince A, Caël O, Bach C, Lengellé J, Cruaud C, Gavory F, Morin E, Murat C,
572 Marçais B, Buée M. 2013. Below-ground fine-scale distribution and soil versus fine
573 root detection of fungal and soil oomycete communities in a French beech forest.
574 Fungal Ecol 6:223-235.
575 48. Lothamer K, Brown SP, Mattox J, Jumpponen A. 2014. Comparison of root-
576 associated communities of native and non-native ectomycorrhizal hosts in an urban
577 landscape. Mycorrhiza 24:267-280.
578 49. Baldrian P. 2017. Forest microbiome: diversity, complexity and dynamics. FEMS
579 Microbiol Rev 41:109-130.
580 50. Setälä H, Francini G, Allen J, Jumpponen A, Hui N, Kotze D. 2017. Urban parks
581 provide ecosystem services by retaining metals and nutrients in soils. Environmental
582 Pollution 231:451-461.

583 51. Koskinen K, Hultman J, Paulin L, Auvinen P, Kankaanpää H. 2011. Spatially
584 differing bacterial communities in water columns of the northern Baltic Sea. FEMS
585 Microbiol Ecol 75:99-110.
586 52. Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB, Lesniewski
587 RA, Oakley BB, Parks DH, Robinson CJ. 2009. Introducing mothur: open-source,
588 platform-independent, community-supported software for describing and comparing
589 microbial communities. Appl Environ Microbiol 75:7537-7541.
590 53. Edgar RC, Haas BJ, Clemente JC, Quince C, Knight R. 2011. UCHIME improves
591 sensitivity and speed of chimera detection. Bioinformatics 27:2194-2200.
592 54. Abarenkov K, Henrik Nilsson R, Larsson KH, Alexander IJ, Eberhardt U, Erland S,
593 Høiland K, Kjøller R, Larsson E, Pennanen T. 2010. The UNITE database for
594 molecular identification of fungi–recent updates and future perspectives. New Phytol
595 186:281-285.
596 55. Tedersoo L, Nilsson RH, Abarenkov K, Jairus T, Sadam A, Saar I, Bahram M,
597 Bechem E, Chuyong G, Kõljalg U. 2010. 454 Pyrosequencing and Sanger sequencing
598 of tropical mycorrhizal fungi provide similar results but reveal substantial
599 methodological biases. New Phytol 188:291-301.
600 56. Brown SP, Veach AM, Rigdon-Huss AR, Grond K, Lickteig SK, Lothamer K, Oliver
601 AK, Jumpponen A. 2015. Scraping the bottom of the barrel: are rare high throughput
602 sequences artifacts? Fungal Ecol 13:221-225.
603 57. Oliver AK, Brown SP, Callaham MA, Jumpponen A. 2015. Polymerase matters: non-
604 proofreading enzymes inflate fungal community richness estimates by up to 15%.
605 Fungal Ecol 15:86-89.
606 58. Nguyen NH, Song Z, Bates ST, Branco S, Tedersoo L, Menke J, Schilling JS,
607 Kennedy PG. 2016. FUNGuild: an open annotation tool for parsing fungal community
608 datasets by ecological guild. Fungal Ecol 20:241-248.

59. Harrison XA. 2014. Using observation-level random effects to model overdispersion in count data in ecology and evolution. PeerJ 2:e616.

Figure legends

Fig. 1 Predicted (mean ± SE) ECM fungal OTU richness (a), diversity (b) and evenness (c) (GLMM results) in parks (both Lahti and Helsinki parks, left side panels) across plant functional types (symbols) and park ages (x-axis), and between old Lahti parks and control forests (right side panels) across plant functional types.

Fig. 2 NMDS plots for ECM fungal communities. ECM fungal communities (a) of soils below broadleaf and conifer trees in old Lahti parks and control forests; ECM fungal communities (b) of soils below broadleaf trees in parks. Statistically significant ($p < 0.05$) vectors (soil pH, %N, %C, organic matter and percentage sand) are shown. All NMDS plots showed significant differences ($p < 0.05$) either across land-use type (control forest vs. urban park), plant functional type or park age by *envfit* analyses.

Fig. 3 Predicted count (mean ± SE) of the ten most abundant ECM fungal genera across land-use type (control forest vs. old urban park) and plant functional type (GLMM results).

Fig. 4 Predicted count (mean ± SE) of the ten most abundant ECM fungal genera across plant functional type and park age (GLMM results).

Fig. 5 NMDS plot for ECM fungal communities under conifer trees in parks. The NMDS plot shows significant differences ($p < 0.05$) across conifer tree species by *envfit* analyses.

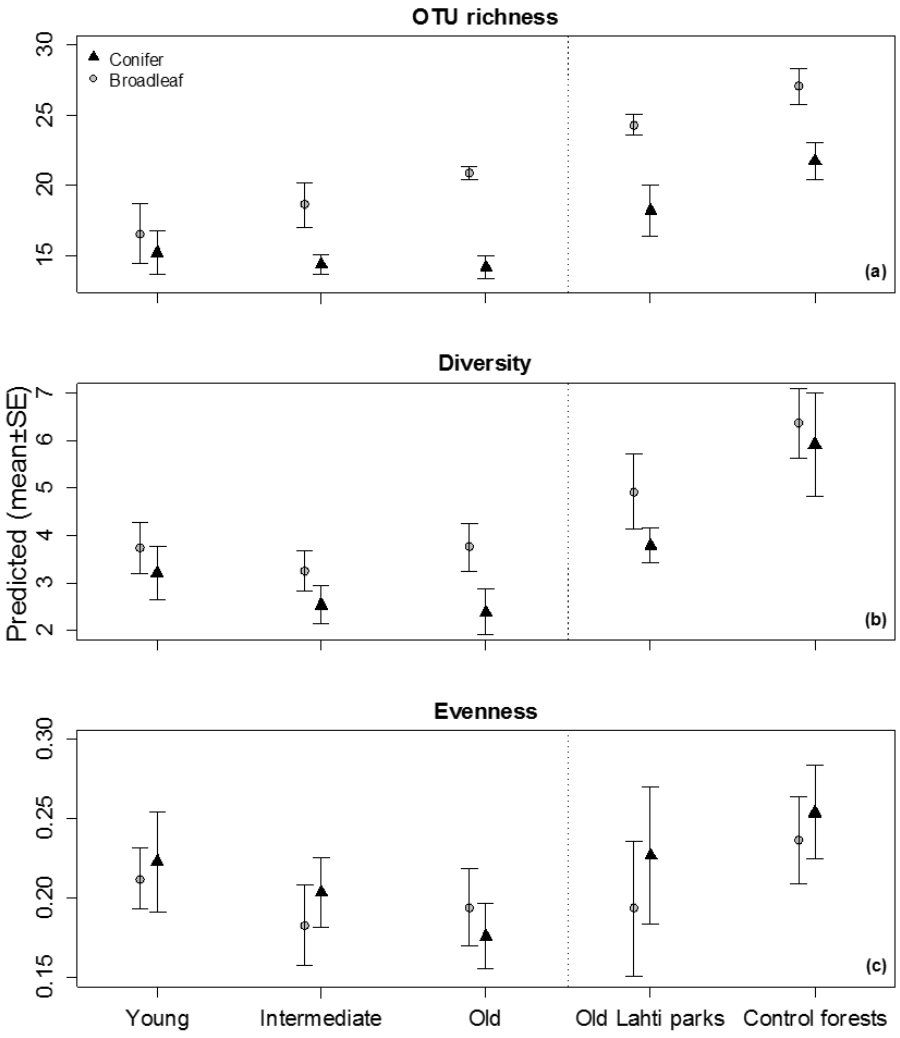

Fig. 1 Predicted (mean ± SE) ECM fungal OTU richness (a), diversity (b) and evenness (c) (GLMM results) in parks (both Lahti and Helsinki parks, left side panels) across plant functional types (symbols) and park ages (x-axis), and between old Lahti parks and control forests (right side panels) across plant functional types.

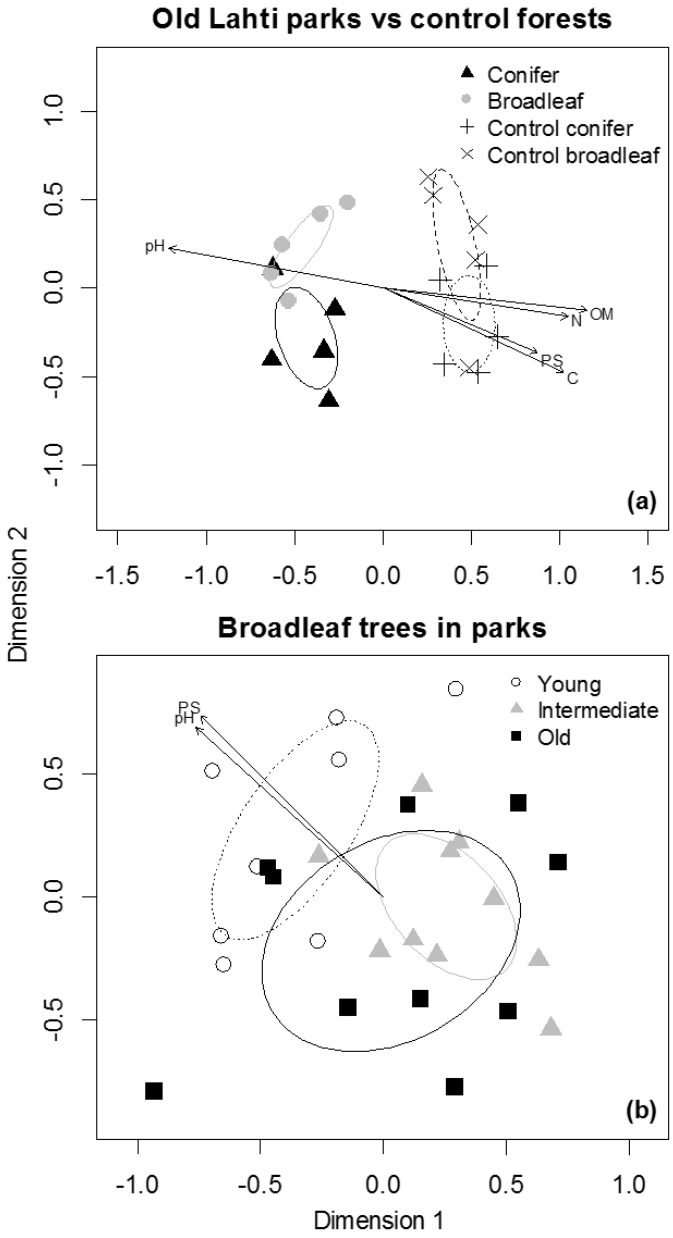

Fig. 2 NMDS plots for ECM fungal communities. ECM fungal communities (a) of soils below broadleaf and conifer trees in old Lahti parks and control forests; ECM fungal communities (b) of soils below broadleaf trees in parks. Statistically significant ($p < 0.05$) vectors (soil pH, %N, %C, organic matter and percentage sand) are shown. All NMDS plots showed significant differences ($p < 0.05$) either across land-use type (control forest vs. urban park), plant functional type or park age by *envfit* analyses.

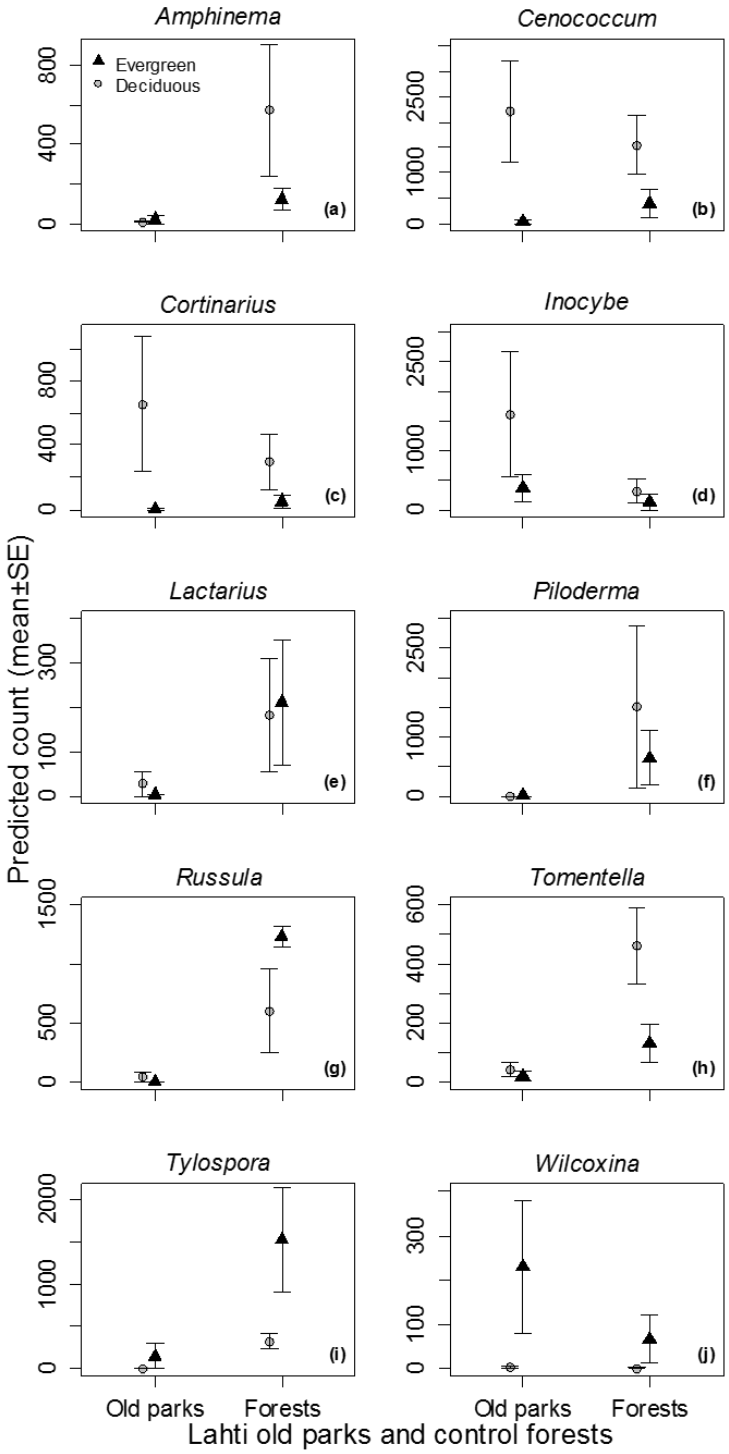

Fig. 3 Predicted count (mean ± SE) of the ten most abundant ECM fungal genera across land-use type (control forest vs. old urban park) and plant functional type (GLMM results).

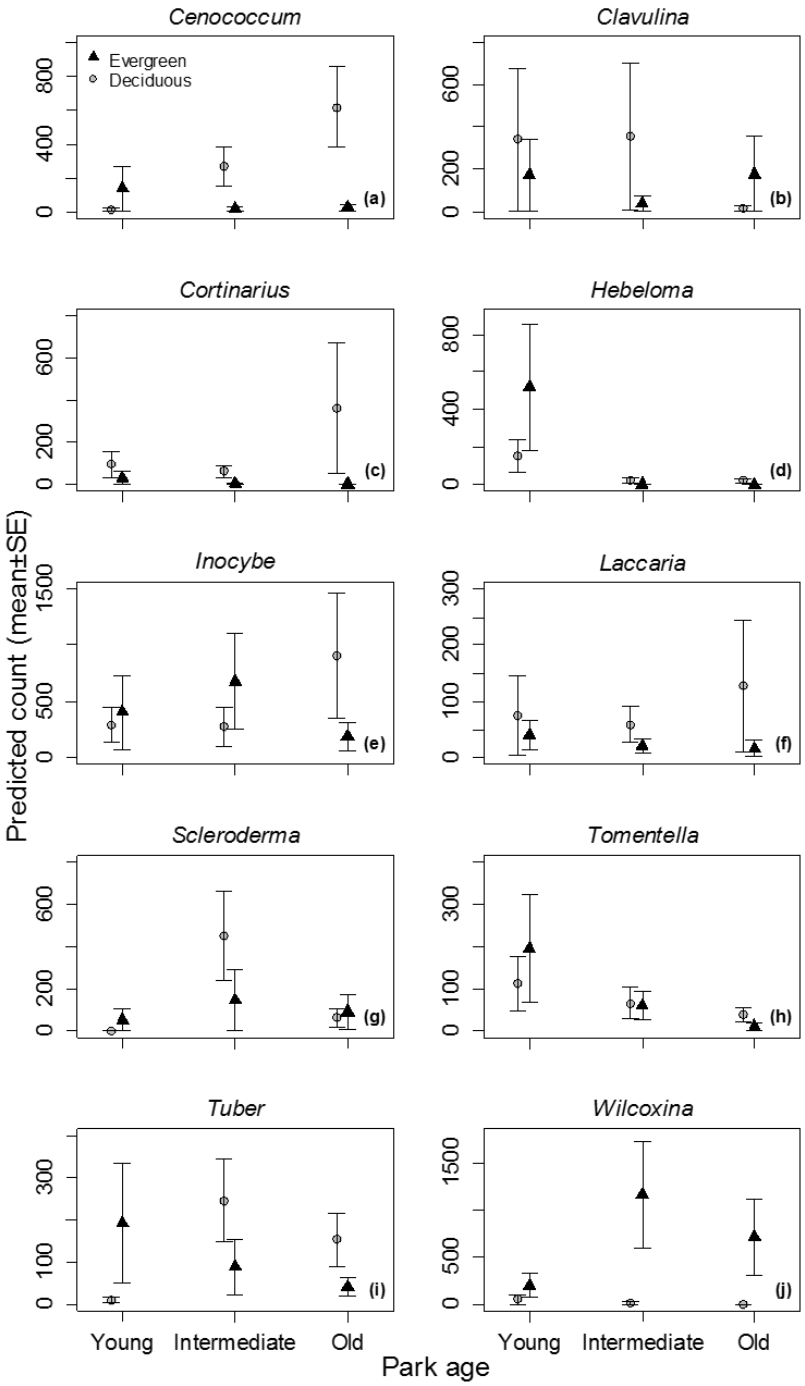

Fig. 4 Predicted count (mean ± SE) of the ten most abundant ECM fungal genera across plant functional type and park age (GLMM results).

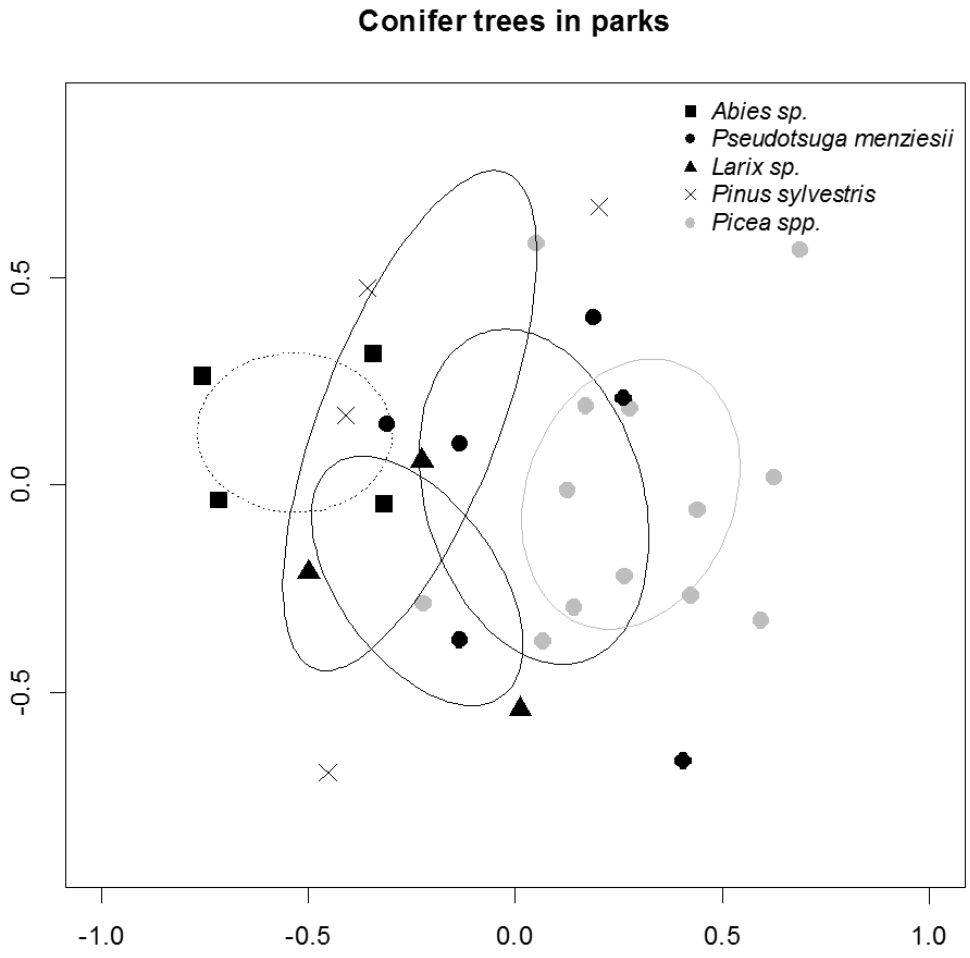

Fig. 5 NMDS plot for ECM fungal communities under conifer trees in parks. The NMDS plot shows significant differences ($p < 0.05$) across conifer tree species by envfit analyses.