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

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Running Head: Ectomycorrhizal fungal communities in urban parks

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

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

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

Effects of plant functional group and park age on ECM fungi in urban parks

In parks, ECM OTU richness and evenness had significant plant functional group x park age interactions (Table S2). In young parks, both ECM fungal richness and diversity were indistinguishable between the two plant functional groups. However, ca. 50 years after park establishment, soils under broadleaf trees tended to host more diverse ECM fungal communities than under conifer trees (Fig. 1 a, b). Diversity and evenness of the ECM fungal communities in parks were negatively correlated with soil pH. In our case, all soils were acidic with maximum pH ~ 6.9 (18). As a result, the diversity and evenness declined as pH approached neutral. All diversity indices correlated negatively with soil C (Table S2).

ECM fungal community composition differed clearly between conifer and broadleaf trees in old parks in Lahti. As a result, we conducted analyses separately for the two tree functional groups in parks: ECM fungal communities under broadleaf trees responded to park age ($r^2 = 0.110$, $p = 0.029$, Fig. 2b), but this was not the case under conifer trees ($r^2 = 0.041$, $p = 0.697$).

To study the effects of plant functional type and park age on common ECM fungi in parks, we analyzed the ten most abundant ECM fungal genera using GLMM. The abundances of *Inocybe*, *Wilcoxina* and *Cenococcum* responded similarly and the differences among the two plant functional types became more pronounced in intermediate and old parks than in young parks (Fig. 4). In intermediate and old parks, *Wilcoxina* was more abundant under conifer trees than under broadleaf trees, whereas *Cenococcum* and *Tuber* showed the opposite trend. *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).
Presumably ECM diversity would increase as plants grow older, because trees in young parks lack an extensive root system for ECM colonization (43). Further, young soils may lack an extensive ECM propagule bank because landfill top soils are common in park construction and because of insufficient propagule dispersal to recently established habitats (44). The relatively stable ECM diversity that we observed across park ages may result from the minimal competition in the urban environments allowing many ECM fungi to rapidly colonize roots of young trees. It appears that young trees are equally suitable hosts for these ECM fungal spores to colonize their roots. Although parks soil characteristics change by age (18), the modifications do not influence the colonization of ECM fungi, suggesting that ECM spores are ubiquitously present in the urban environment. However, to our knowledge, studies that explicitly explore the effects of host age on ECM fungal communities in urban soils are non-existent.

Despite the lack of an overall park age effect on ECM fungal community diversity, community composition responded to park age in the broadleaf tree rhizospheres. Similar responses were absent with the conifer hosts. The lack of this response under conifer trees is surprising, given that soil chemistry changes (lower pH, higher OM, C and N) were particularly pronounced underneath conifers in our parks (18). Previous studies on ECM succession in natural forests suggest some context dependency of community responses to stand age. ECM fungal composition and diversity were insensitive to the age of oak (*Quercus ilex*) stands (45), whereas Kyaschenko et al. reported changes in the ECM fungus species composition along an age gradient of managed *Pinus sylvestris* stands (46). Taken together, these suggest that ECM fungal communities are primarily shaped by host-fungus interactions rather than by abiotic habitat conditions such as soil chemistry.

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

Materials and methods

Study area and sampling design

The study sites have been described in detail previously (17, 18). Briefly, we selected 41 urban parks in the cities of Helsinki and Lahti, southern Finland, and 5 additional control forests in the proximity of Lahti. The urban parks represent different ages: more than 100 years old (the oldest parks were established over two centuries ago), 50 ± 10 years old and 10 years old, referred to as old, intermediate and young parks, respectively. We considered two
plant functional types in these parks: conifer (43.3% of the conifer tree species represents
*Picea* spp.; 20% represents *Abies* sp.; 13.3% represents *Pseudotsuga menziesii*; 13.3%
represents *Pinus sylvestris* 13.3%; 10% represents *Larix* sp.) and broadleaf (*Tilia x vulgaris*
100%) trees. With a few exceptions, conifer and broadleaf trees existed commonly together
within a park. Distance between the two tree types was always greater than the height of the
tallest tree. The age of plants within each park age class corresponded with park age, except
for the young parks where trees are commonly planted as ca. 10 year old saplings at the time
of park construction. The ideal experimental design would have included 15 parks per city,
represented by five old, five intermediate and five young parks, with both plant functional
types present. However, since some parks did not include both plant functional types, we also
selected parks with only one plant functional type. This resulted in a total of 41 urban parks
and 58 urban sampling locations with 7-11 replicates per park age and plant functional type.
Park sizes varied considerably, ranging from ca. 0.1 ha to several hectares, but with no
systematic grouping of size with park age and plant functional type.

Soil sampling and edaphic conditions

ECM fungi colonize roots, but they grow from roots into the soil to deliver soil nutrients to
the roots. Because of this, soil is a good proxy in studying ECM fungal communities and
assigning the detected taxa to ecological roles (47-49). We sampled soils in May 2015 at the
dge of the canopy projection so that distance to the nearest tree trunk ranged from 1 m
(young parks; samples always collected outside the planting pit) to several meters (old parks).
At each sampling point, we subsampled 3 soil cores (top 10 cm) using a steel push corer (10
cm deep, 2.54 cm diameter), pooled the three subsamples into one composite for a total of 68
samples across the experiment (58 urban park samples and 10 control forest samples). The
corer was sterilized using 70% ethanol between samples. Samples were stored in Minigrip
bags on ice in the field and frozen at −20 °C in the laboratory. Before DNA extraction, the samples were thawed at room temperature and sieved through a 2 mm mesh to remove any remaining large particles. The edaphic conditions (0-10 cm deep) of all samples were analyzed in our previous studies (18, 50). Five variables, carbon content (C), nitrogen content (N), organic matter (OM), percentage sand (PS) and pH were used in our statistical analyses.

DNA extraction, PCR and Illumina MiSeq sequencing

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

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

Statistical analyses

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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.

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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.
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