



Uniqueness of tree stand composition and soil microbial communities are related across urban spruce-dominated forests

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Abstract

Context Urban forest soils represent significant reservoirs of biodiversity in cities. Retaining this diversity under urban land-use change requires understanding on how species richness, community assembly and uniqueness of species assemblages are related to local forest characteristics and surrounding landscape structure.

Objectives Our aim was to assess the significance and relative importance of logging history, tree species composition and urbanization in shaping soil microbial communities across urban spruce-dominated forest landscapes. We investigated responses of microbial diversity from three complementary viewpoints: local diversity, community assembly patterns and community uniqueness.

Methods We collected soil bacterial and fungal metabarcoding data from 73 spruce-dominated forest sites distributed in three urban centers across southern Finland. We related these data to measurements of logging intensity, tree species composition and degree of urbanization.

Results Logging intensity, tree species composition and urbanization affected site-scale microbial diversity, but the effects varied between microbial groups. Only logging intensity had a significant imprint on microbial assembly, and this effect was restricted to bacteria. Relative uniqueness of microbial assemblages at the landscape-scale was coupled with the uniqueness of tree species composition in all microbial groups, and further affected by tree diversity in saprotrophic fungi and urbanization in ectomycorrhizal fungi.

Conclusions In the context of urban spruce-dominated forests, locally diverse tree stands are not necessarily the same as those that contribute the most to landscape-scale diversity. Identifying and preserving contrasting tree stand structures, which support distinctive soil microbial assemblages, may be the winning strategy in maintaining a wide range of soil microbial diversity.

Keywords Urban forestry · Fungi · Bacteria · Metabarcoding · Biodiversity

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Introduction

Preservation of Earth's biodiversity cannot rely solely on remote natural reserves. Instead, it needs to be addressed across all land-use, including cities (Oke et al. 2021). Urban forests, representing true forest vegetation retained within and around cities,

play a key role in providing habitats for urban wildlife (Alvey 2006). Urban forests are often structurally similar with, and grade into, indigenous forest habitats in the ex-urban landscapes (Hedblom and Söderström 2008; Korhonen et al. 2020; Pregitzer et al. 2021). Hence, city forests with rare habitat types or exceptionally good habitat quality (Ives et al. 2016; Korhonen et al. 2020, 2021) can contribute to the preservation of threatened biodiversity as parts of larger regional habitat networks.

Species diversity across a habitat patch network can be divided into components consisting of local species richness (alpha-diversity), variation in species communities between sites (beta-diversity), and total cumulative species diversity across the network (gamma-diversity) (Whittaker 1960). Beta-diversity can be further divided into components representing species replacement and species richness difference between sites (Legendre 2014). From the viewpoint of biodiversity conservation, an optimal set of habitat patches is one that captures and maintains gamma-diversity effectively (Socolar et al. 2016). However, true gamma-diversity in a given area is often unknown, and biodiversity conservation planning must rely on available knowledge on alpha and beta-diversity and their drivers. In systems where beta-diversity is dominated by species replacement, patterns of species richness (alpha-diversity) have limited utility in effective biodiversity management planning. Instead, more focus should be put on identifying suites of sites with contrasting species assemblages and characteristics of the environment that predict biotic uniqueness (Heino et al. 2017). In cities, this question becomes relevant when reconciling between urban development, green area management and biodiversity, e.g., when designating areas to infill development or retention as natural areas, or when deciding on management practices applied in green areas.

Although soils have been estimated to harbor around 59% of all species on Earth, including 90% of fungi (Anthony et al. 2023), soil biodiversity hasn't received proportional attention in biodiversity conservation efforts (Parker 2010). Previous studies in urban forests have highlighted the importance of surrounding landscape matrix (forest fragmentation and urban development) as a significant determinant of forest soil biodiversity (Malmivaara-Lämsä et al. 2008; Scholier et al. 2023; Tatsumi et al. 2023), but less is

known about the role of forest management and tree species composition in the urban context.

Earlier studies on management effects on boreal forest soil biota have focused primarily on forestry operations applied in commercial wood production (e.g., Lazaruk et al. 2005; Hartmann et al. 2012; Parladé et al. 2019; Rähn et al. 2023). However, urban forestry generally operates at smaller scales and with lower intensity of logging, mainly aiming to maintain tree regeneration and public safety (Gundersen et al. 2005). Therefore, management effects from soil microbiome studies in production forests can be difficult to extrapolate into urban forests. Furthermore, urban forests tend to be more variable in tree species composition than respective forest types in managed production forest landscapes (Korhonen et al. 2020), which can be reflected in soil microbial assemblages through litter and symbiotic host related effects (Prescott and Grayston 2013).

In this study, we set out to investigate how soil microbial communities in urban forest soils relate to three sources of variation: (1) forest logging intensity, (2) tree species composition, and (3) degree of urbanization. More specifically, we asked:

- What are the effects and relative contributions of logging intensity, tree species composition and urbanization in explaining local diversity and community composition of bacteria and fungi in the forest soil?
- Can we predict individual sites' contributions to landscape-level soil microbial diversity based on forest characteristics such as past logging intensity, tree diversity, compositional uniqueness of tree species composition, or the degree of urbanization?

To answer these questions, we sampled 73 urban spruce-dominated forest plots distributed in three urban regions in southern Finland, representing typical variability in management intensity and forest structure. We analyzed microbial diversity and taxonomic composition in soil samples collected at each site with DNA metabarcoding. We then tested the effects of the three focal environmental covariates on microbial community data at different levels or organization: (1) local diversity and other site-level community characteristics, (2) community assembly, i.e., variation in community composition across sites, and

(3) community uniqueness, i.e., magnitude of site-specific contributions to microbial taxonomic turnover across sites.

Methods

Study area and sample plots

We collected data from 73 forest plots distributed in three urban centers in southern Finland (Fig. 1): Helsinki region (45 sites in cities of Helsinki, Espoo and Vantaa; 1 581 residents per km²), Lahti (8 sites, 261 residents per km²), and Tampere (20 sites, 473 residents per km²) (StatFin 2022). We targeted Norway spruce (*Picea abies* L. H. Karst.) dominated city-owned forests situated within inner urban, outer urban or peri-urban zones around the urban centers (Helminen et al. 2020) with bilberry (*Vaccinium myrtillus* L.) as the dominant field layer shrub species. To achieve a sample of sites representing typical management intensities in urban

forests, we consulted cities’ forest databases to locate forest stands with prior records of partial or selective cuttings, as well as sites that were known to be unmanaged. In addition, we included sites with no prior information available regarding management history. Logging intensity was quantified later during field sampling based on quantities of cut stumps. All sites were on mineral soils 10–160 m above sea level, with mean annual temperature 4.9–6.3 °C and rainfall 600–650 mm. Canopy tree layer consisted of mature (≥ 60 year old) spruce alone or mixed with other tree species. Basal area of living trees varied between 17.5 and 48.3 m²/ha (mean 32.7), and the share of Norway spruce was on average 77%.

Sample plots consisted of four interconnected 20×20 m squares that were placed inside forest stands (Appendix A). We avoided large continuous canopy openings that were larger than a few trees wide as well as heavily trampled areas with worn understorey vegetation.

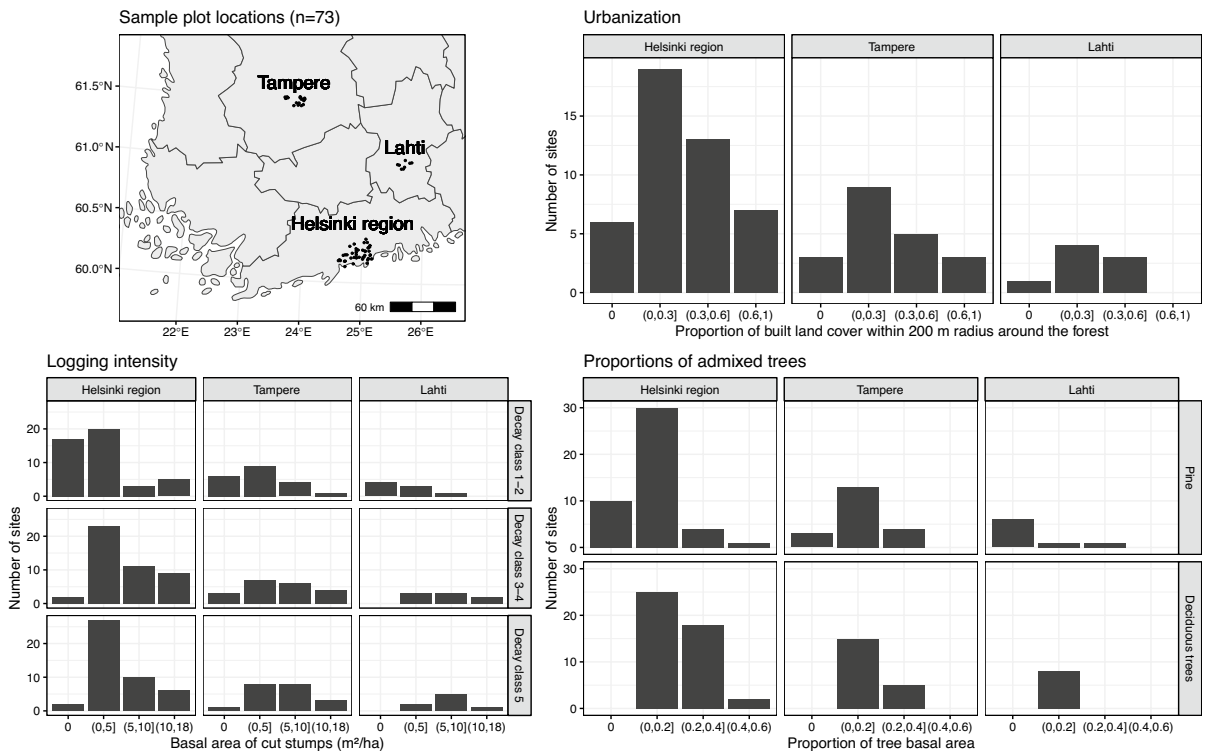


Fig. 1 Distribution of study plots in space and across gradients of urbanization, logging intensity (based on amounts and decay class distribution of cut stumps) and tree species composition

Forest stand characteristics and urbanization

We measured all living trees ≥ 5 cm at breast height (1.3 m), all cut tree stumps with diameter ≥ 10 cm at cut surface, and their decay class (1 to 5, from least to most decayed; Renvall 1995) within the study plots.

To quantify management intensity in forest sites, we reduced the logging history data into two main gradients by running a principal component analysis on square root transformed basal areas of cut stumps and extracting site scores for the two first principal components (Appendix B). The first component axis (Logging_recent) reflected the intensity of relatively recent logging (<decadal timescale), while the second axis (Logging_old) mostly associated with the oldest signs of logging (>decadal timescale).

To quantify forest tree diversity at each site, we calculated Shannon diversity of tree species (TreeDiv) based on proportional tree basal areas, and the number of ectomycorrhizal tree species (ECM tree richness).

Variability in tree species composition was summarized in two principal component axes calculated from square root transformed basal areas of tree species (Appendix B). The first component axis (Trees_Pinus) was mostly associated with the abundance of pines (*Pinus sylvestris*), while the second axis (Trees_Betula) was mostly associated with the abundance of birches (*Betula pendula* and *B. pubescens*).

The relative uniqueness of tree species composition was quantified by calculating an index of local contribution to beta-diversity (Legendre and De Cáceres 2013). The index (LCBD-Trees) was calculated from a distance matrix (Sørensen dissimilarity) derived from tree composition data (square root transformed basal areas of tree species) using the *beta.div.comp* and *LCBD.comp* functions in the *adespatial* package (v0.3-21; Dray et al. 2023).

We assessed the degree of urbanization around sample plots by calculating the proportion of built land surface within a 200 m radius around sample plot center based on Corine Land Cover 2018 GIS-dataset. Land classes considered as built land included Urban fabric; Industrial, commercial and transport units; Mine, dump and construction sites; and Artificial, non-agricultural vegetated areas.

Soil sampling

Soil sampling was done between May 19th and July 19th 2022. Samples were collected from 16 regularly spaced grid points across each study plot (Appendix A). The minimum distance between two sampling points was 10 m. If the grid point was on a rock, bare mineral soil or path, we moved the sampling point to the nearest suitable spot in the vicinity. Material was collected from each sampling point into one pooled sample per site.

At each point, we collected ca. 0.5 dl of material from the humus layer between the undecomposed litter layer and mineral soil. After removing the undecomposed surface litter and roots, material was extracted with a DNA-sterilized hand shovel down to the boundary between humus layer and mineral soil to a maximum depth of 15 cm. Material was collected into a PE zip lock bag and stored on ice during transport. Within 12 h from collecting, the samples were sieved through 2 mm mesh in the laboratory and stored in -20 °C until further processing.

Soil chemical analyses

We measured the carbon (C) and nitrogen (N) content of the soil samples from combustion products with an elemental analyzer (Leco TruMac, St. Joseph, US-MI). Soil acidity was quantified by measuring pH from an aqueous solution with 10 ml of soil sample mixed with 50 ml of distilled water.

Ergosterol was measured from soil samples as a biomarker of living fungal biomass following the protocol of Adamczyk et al. (2019). Analyses were done with high-performance liquid chromatography (Waters Arc HPLC, Milford, US-MA). We calculated estimates of ectomycorrhizal and saprotrophic fungal biomasses based on ergosterol content and relative read abundances corresponding to each functional group (i.e., relative read abundance * ergosterol content; Tedersoo et al. 2016).

DNA extraction and sequencing

DNA was extracted from two ca. 200 mg portions of material from each sample. Extractions were done with DNeasy PowerSoil Pro (Qiagen, Hilden, DE)

according to the manufacturer's instructions to a final volume of 100 μl . We combined replicate extractions from the same soil sample before DNA analyses.

For bacterial metabarcoding, V4 region of the 16S rRNA gene was amplified with primers 515F (GTG CCAGCMGCCGCGGTAA) and 806R (GACTA CHVGGGTWTCTAAT). For fungal metabarcoding, the Internal Transcribed Spacer 2 of the nrRNA coding region was amplified with primers ITS3-2024F (GCATCGATGAAGAACGCAGC) and ITS4-2409R (TCCTCCGCTTATTGATATGC). Indexed amplicons were sequenced with Illumina NovaSeq 6000 (paired-end 250 bp) at Novogene Co (Cambridge, GB).

Bioinformatics

Sequence reads were demultiplexed, and index and primer sequences were removed from paired-end reads. We applied *vsearch* (v2.18.0; Rognes et al. 2016) for further sequence processing. Individual R1 and R2 reads were first quality filtered, and then assembled. Assembled reads were chimera filtered de novo. Remaining reads were clustered into OTUs with 98% similarity threshold. OTUs were taxonomically assigned with 80% confidence cutoff using Naïve Bayesian Classifier trained with bacterial SILVA (v138; Quast et al. 2012; Yilmaz et al. 2014) and fungal UNITE (v9, dynamic, all eucaryotes; Abarenkov et al. 2022) databases in *mothur* v.1.36.1 (Schloss et al. 2009). Fungal OTUs were further assigned to functional groups according to Fungal-Traits database (Pölme et al. 2020) based on genus-level taxonomical assignments. OTU tables were finally filtered by discarding bacterial OTUs that had less than 1‰ relative abundance and fungal OTUs that had less 0.1‰ relative abundance in all samples and OTUs that had maximum read counts in negative controls. Taxonomic and functional composition of the bacterial and fungal communities are summarized in Appendix C.

Community data preparation

Data preparation and further analyses of community data were done in R (v4.2.2; R Core Team 2022). We calculated diversity indices and distance matrices for all bacteria, ectomycorrhizal (ECM) fungi (46% of fungal reads) and saprotrophic (SAP) fungi (30% of fungal

reads). We considered SAP fungi to include soil, litter, wood and unspecified saprotrophs. We presumed that the occurrence of dung, nectar/tap saprotrophs and sooty molds (<0.2% of all fungal reads) in forest soil samples was mostly incidental, and thus, we omitted them from the SAP group.

We estimated Shannon diversity with sample read depth of 32k for bacteria, 66k for ECM fungi, and 58k for SAP fungi using the *estimateD* function in the *iNEXT* package (v3.0.9; Chao et al. 2014; Hsieh et al. 2022). In each case, the applied read depth corresponded to estimated sample coverage of >99%.

We calculated Bray–Curtis distance matrices with rarefaction by randomly subsampling OTU datasets with 100 iterations to the read depths listed above using the *avgdist* function in the *vegan* package (v2.6-4; Oksanen et al. 2022). OTU read counts were square root transformed before distance calculations to improve ecological signal detection.

To quantify relative uniqueness of bacterial, ECM fungal and SAP fungal communities, we calculated local contributions to beta-diversity indices representing the replacement component of beta-diversity ($\text{LCBD}_{\text{repl}}$) using the *beta.div.comp* and *LCBD.comp* functions in the *adespatial* package. $\text{LCBD}_{\text{repl}}$ is an index that measures how exceptional each site is, when compared with the other sites, in terms of taxon replacement (Legendre 2014). We calculated $\text{LCBD}_{\text{repl}}$ for bacteria based on the quantitative indices of replacement (Sørensen, Podani family) from the full OTU data. For ECM and SAP fungi, we calculated the $\text{LCBD}_{\text{repl}}$ indices based on presence-absence (Jaccard, Podani family) from a reduced dataset focusing on taxa identified to species-level. This was done to avoid conflating fungal taxa with (potentially artefactual) intraspecific sequence variants and other sequence reads with uncertain taxonomic affinity. First, we pooled together OTUs that were assigned to the same species. Then, we assigned the species-level OTUs as present if their relative abundance in relation to the total read count per sample was >0.1‰. Relationships between the $\text{LCBD}_{\text{repl}}$ indices, Shannon diversity and numbers of rare species are reported in Appendix D.

Statistical analyses

Analyses of local microbial diversity, community characteristics and relative uniqueness

We used linear mixed models (LMMs) estimated by the *lme* function in the *nlme* package (v3.1–164; Pinheiro et al. 2023) to test the effects of environmental predictors on univariate responses. Firstly, we analyzed local microbial diversity (Shannon diversity of bacteria, ECM fungi and SAP fungi) and other community characteristics including Proteobacteria:Acidobacteriota ratio (see Zhou et al. 2018) and ECM and SAP fungal biomasses (Table 1). Secondly, we analyzed measures of relative community uniqueness for each microbial group (LCBD_{repl}-Bacteria, LCBD_{repl}-ECM and LCBD_{repl}-SAP, Table 1).

For the first set of response variables, we included explanatory variables describing logging intensity (Logging_recent and Logging_old), tree stand composition (Trees_Pinus and Trees_Betula) and urbanization (BuiltLand_200m). To control for variation driven by soil properties, we also included variables describing soil chemistry (CN_ratio and pH). ECM tree richness was included as an additional explanatory variable for ECM fungal diversity. For the second set of response variables (i.e., community uniqueness), we included logging intensity (Logging_recent and Logging_old), tree species diversity (TreeDiv), uniqueness of tree species composition (LCBD-Trees) and urbanization (BuiltLand_200m). Before model estimation, the explanatory variables were centered and scaled to mean=0 and sd=1. Region was included as a random factor in all LMMs to account for the geographical clustering of sites at regional level. We checked that all LMMs satisfied assumptions of homogeneity of variances and normality of residuals by inspecting diagnostic plots (Pinheiro and Bates 2000). To avoid problems with multicollinearity, we checked that variance inflation factors for all explanatory variables were < 2 in all LMMs.

Analyses of microbial community assembly

We applied generalized dissimilarity modeling (GDM) based on Bray–Curtis distance matrices (R package *gdm* v1.5.0–9.1; Fitzpatrick et al. 2022) to test the significance of environmental predictors in

explaining bacterial and fungal OTU community composition and to assess their relative importance. We included variables describing logging intensity (Logging_recent and Logging_old), tree stand composition (Trees_Pinus and Trees_Betula), urbanization (BuiltLand_200m), soil chemistry (CN_ratio and pH) and the spatial arrangement of sites (geographic coordinates) as predictors in the models. We assessed predictor importance and statistical significance based on permutational analysis (nPerm = 400).

To further illustrate how microbial diversity was distributed across spatial scales, we applied additive partitioning of the total Shannon diversity of microbial communities into components representing mean diversity in individual sites and variability between sites within and between regions. Partitioning was done with the *adipart* function in the *vegan* package.

Results

Predictors of local microbial diversity and community characteristics

Forest stands with higher amounts of old logging (Logging_old) had higher local diversity of bacteria and SAP fungi (Table 2). Furthermore, higher amount of old logging was associated with lower Proteobacteria:Acidobacteriota ratio and a marginally significant shift to a higher biomass of ECM fungi and lower biomass of SAP fungi. Intensity of recent logging (Logging_recent) had no significant associations with any of the investigated microbial responses.

Tree species composition influenced microbial diversity and community characteristics along a direction of variation that was mostly associated with the abundance of birches (Trees_Betula). Diversities of bacteria and SAP fungi decreased with increasing abundance of birches, while the biomass of SAP fungi increased. Variation related to the abundance of pines (Trees_Pinus) had no significant associations with any of the microbial responses. Forest stands with higher number of ECM tree species had marginally higher diversity of ECM fungi (Table 2).

Forest stand situated in more urbanized areas (higher BuiltLand_200m) had marginally higher diversity of bacteria but significantly lower

Table 1 Variables included in the analyses and their explanations

Variable group	Variable name	Mean ± SD	Explanation
Microbial community characteristics	Bacterial diversity	166.21 ± 21.10	Rarefied estimate of bacterial Shannon diversity
	ECM diversity	34.50 ± 12.56	Rarefied estimate of ectomycorrhizal fungal Shannon diversity
	SAP diversity	71.89 ± 21.86	Rarefied estimate of saprotrophic fungal Shannon diversity
	Proteobacteria: Acidobacteriota ratio	1.69 ± 0.45	Ratio of relative read abundance of bacterial phyla <i>Proteobacteria</i> (i.e., <i>Pseudomonadota</i>) and <i>Acidobacteriota</i>
	ECM biomass	37.55 ± 14.56	Relative read abundance of ectomycorrhizal fungi * ergosterol (µg/g organic content)
	SAP biomass	24.80 ± 11.25	Relative read abundance of saprotrophic fungi * ergosterol (µg/g organic content)
	LCBD _{repl} -bacteria	0.014 ± 0.005	Relative community uniqueness, i.e., local contribution to the replacement component of beta-diversity. The index was calculated separately for bacteria, ECM fungi and SAP fungi
	LCBD _{repl} -ECM	0.014 ± 0.005	
	LCBD _{repl} -SAP	0.014 ± 0.005	
		ECM biomass	37.55 ± 14.56
	SAP biomass	24.80 ± 11.25	Relative read abundance of saprotrophic fungi *ergosterol (µg/g organic content)
Logging intensity	Logging_recent	0.00 ± 0.49	Principal component primarily corresponding to recent logging intensity (least decayed cut stumps)
	Logging_old	0.00 ± 0.49	Principal component primarily corresponding to old logging intensity (most decayed cut stumps)
Composition of the tree stand	Trees_ <i>Pinus</i>	0.00 ± 0.46	Principal component primarily corresponding to the abundance on pine (<i>Pinus</i>) in the tree stand
	Trees_ <i>Betula</i>	0.00 ± 0.46	Principal component primarily corresponding to the abundance on birch (<i>Betula</i>) in the tree stand
	ECM tree richness	3.73 ± 1.06	Number of ectomycorrhizal tree genera present in the sample plot (<i>Abies</i> , <i>Alnus</i> , <i>Betula</i> , <i>Corylus</i> , <i>Picea</i> , <i>Pinus</i> , <i>Populus</i> , <i>Quercus</i> , <i>Salix</i> , <i>Tilia</i>)
	TreeDiv	0.68 ± 0.28	Shannon diversity of tree species calculated from relative abundances in terms of basal area
	LCBD-trees	0.014 ± 0.004	Relative uniqueness of a site in terms of tree species composition
Urbanization	BuiltLand_200m	0.29 ± 0.24	Proportion of built land surface within 200 m radius around sample plot
Soil chemistry	CN_ratio	24.71 ± 2.53	Mass ratio of C and N in soil sample
	pH	4.26 ± 0.35	Soil acidity measured as hydrogen potential
Geographic location	Region		Location of the sample plot: Helsinki region, Lahti or Tampere

diversity of SAP fungi and lower ECM fungal biomass (Table 2).

Predictors of microbial community assembly

Additive partitioning of Shannon diversity indicated that the dominant bacterial and fungal OTU

composition was relatively homogeneous across sites, and that regional differences accounted only for a small fraction of the total variability between sites (Fig. 2a). GDMs explained 38.4% of the variation in OTU assembly for bacteria, 13.8% for ECM fungi and 29.0% for SAP fungi. The intensity of old logging was significantly associated with bacterial assembly,

Table 2 Effects of environmental predictors on bacterial and fungal diversities, Proteobacteria:Acidobacteriota ratio and fungal biomasses based on linear mixed models

Variable	Bacterial diversity		ECM fungal diversity		SAP fungal diversity	
	Coeff. \pm SE	p	Coeff. \pm SE	p	Coeff. \pm SE	p
Intercept	161.10 \pm 7.77	<0.001	34.52 \pm 1.40	<0.001	72.48 \pm 2.88	<0.001
Logging_recent	1.58 \pm 1.85	0.395	- 2.44 \pm 1.50	0.109	- 0.93 \pm 2.45	0.705
Logging_old	4.37 \pm 1.78	0.017	- 0.59 \pm 1.44	0.685	5.67 \pm 2.34	0.019
Trees_Pinus	1.30 \pm 1.82	0.478	- 1.72 \pm 1.45	0.241	- 0.06 \pm 2.35	0.981
Trees_Betula	- 4.82 \pm 2.02	0.020	0.64 \pm 1.58	0.686	- 6.64 \pm 2.40	0.007
BuiltLand_200m	3.62 \pm 1.87	0.057	- 0.59 \pm 1.52	0.702	- 5.17 \pm 2.47	0.041
CN_ratio	- 7.51 \pm 2.14	0.001	0.30 \pm 1.69	0.861	0.56 \pm 2.77	0.842
pH	5.17 \pm 2.14	0.018	3.41 \pm 1.68	0.047	4.36 \pm 2.75	0.117
ECM tree richness	NA	NA	2.73 \pm 1.61	0.095	NA	NA
	Proteobacteria:Acidobacteriota ratio		ECM fungal biomass		SAP fungal biomass	
	Coeff. \pm SE	p	Coeff. \pm SE	p	Coeff. \pm SE	p
Intercept	1.73 \pm 0.09	<0.001	37.48 \pm 2.00	<0.001	24.11 \pm 1.94	<0.001
Logging_recent	- 0.06 \pm 0.04	0.181	- 0.33 \pm 1.61	0.837	- 0.07 \pm 1.33	0.961
Logging_old	- 0.10 \pm 0.04	0.015	2.71 \pm 1.54	0.083	- 2.34 \pm 1.28	0.072
Trees_Pinus	0.04 \pm 0.04	0.327	- 0.26 \pm 1.55	0.866	0.63 \pm 1.29	0.624
Trees_Betula	0.02 \pm 0.04	0.680	0.20 \pm 1.59	0.900	2.77 \pm 1.34	0.043
BuiltLand_200m	0.00 \pm 0.04	0.970	- 4.00 \pm 1.62	0.016	1.82 \pm 1.35	0.180
CN_ratio	0.00 \pm 0.05	0.993	2.02 \pm 1.82	0.273	0.56 \pm 1.52	0.713
pH	0.26 \pm 0.05	<0.001	- 4.60 \pm 1.81	0.013	- 0.33 \pm 1.51	0.828

Coefficient values with standard error of means (\pm SE) and p-values are shown

Effects are shown in bold when $p < 0.05$ and italicized when $0.05 < p < 0.10$

while none of the other forest stand characteristics or urbanization predicted variation in bacterial or fungal assembly significantly (Fig. 2b). All microbial groups showed significant spatial signal, indicating that sites that were geographically closer to one another tended to have more similar communities. However, variation in soil chemical properties accounted for the largest proportion of the variance that was explained.

Predictors of microbial community uniqueness

Taxon turnover accounted for 67.3% of the total beta-diversity for bacteria (calculated from the full OTU dataset), 63.0% for ECM fungi and 62.6% for SAP fungi (calculated from the species-level presence-absence dataset). The magnitude of site-specific contributions to microbial taxonomic turnover across sites, i.e., relative uniqueness of microbial communities (LCBD_{rep}), was positively associated

with the uniqueness of tree species composition in all three microbial groups: bacteria, ECM fungi and SAP fungi (Table 3). Thus, forest sites where tree species composition deviated the most from the total average tended to have the most unique soil microbial assemblages. Furthermore, the uniqueness of ECM fungi was negatively associated with urbanization (Table 3), indicating that ECM fungal assemblages in forest patches surrounded by large proportion of built-up area tended to be less unique than communities in less urbanized areas. Finally, the uniqueness of SAP fungi was negatively associated with tree diversity (Table 3), indicating that stands with relatively high dominance of Norway spruce tended to have more unique SAP fungal assemblages. Logging history did not predict community uniqueness in any of the three microbial groups.

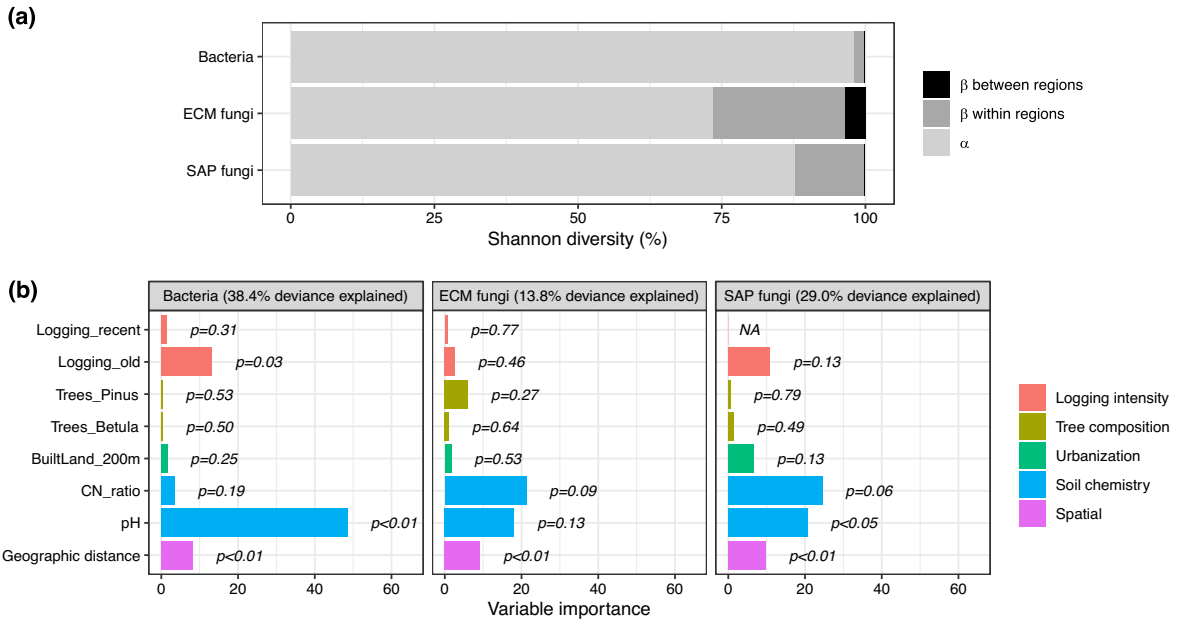


Fig. 2 Distribution of microbial diversity and predictors of community assembly. Additive partitioning of Shannon diversity. **a** represents how much of the total diversity was accounted for by diversity in individual sites (α) and variability (β) within and between regions in each microbial group. Results from the GDMs of microbial OTU assembly. **b** repre-

sent how much of the variation between sites was explained by environmental predictors. Bar widths show variable importance calculated as the percent change in deviance explained between a model fit with and without permuting the variable in question

Table 3 Effects of forest stand characteristics and urbanization on bacterial and fungal community uniqueness (LCBD_{rep}) based on linear mixed models

Variable	Bacteria		ECM fungi		SAP fungi	
	Coeff. \pm SE	p	Coeff. \pm SE	p	Coeff. \pm SE	p
Intercept	$14 \times 10^{-3} \pm 0.9 \times 10^{-3}$	< 0.001	$15 \times 10^{-3} \pm 1.7 \times 10^{-3}$	< 0.001	$14 \times 10^{-3} \pm 0.6 \times 10^{-3}$	< 0.001
Logging_recent	$-2.9 \times 10^{-4} \pm 4.4 \times 10^{-4}$	0.519	$7.1 \times 10^{-4} \pm 5.9 \times 10^{-4}$	0.232	$3.9 \times 10^{-4} \pm 6.3 \times 10^{-4}$	0.542
Logging_old	$2.5 \times 10^{-4} \pm 4.3 \times 10^{-4}$	0.568	$-4.7 \times 10^{-4} \pm 5.8 \times 10^{-4}$	0.416	$-3.3 \times 10^{-4} \pm 6.2 \times 10^{-4}$	0.596
TreeDiv	$0.3 \times 10^{-4} \pm 4.8 \times 10^{-4}$	0.944	$-0.8 \times 10^{-4} \pm 6.6 \times 10^{-4}$	0.903	$-15 \times 10^{-4} \pm 6.5 \times 10^{-4}$	0.026
LCBD-Trees	$37 \times 10^{-4} \pm 4.6 \times 10^{-4}$	< 0.001	$16 \times 10^{-4} \pm 6.2 \times 10^{-4}$	0.014	$13 \times 10^{-4} \pm 6.4 \times 10^{-4}$	0.039
BuiltLand_200m	$-2.0 \times 10^{-4} \pm 4.5 \times 10^{-4}$	0.653	$-12 \times 10^{-4} \pm 6.0 \times 10^{-4}$	0.046	$3.6 \times 10^{-4} \pm 6.4 \times 10^{-4}$	0.582

Coefficient values with standard error of means (\pm SE) and p-values are shown

Effects are shown in bold when $p < 0.05$

Discussion

Urban forest landscapes are continuously shaped by planned and inadvertent human influence through active forest management and land-use development in the surroundings. In this study, we aimed to show how above-ground forest characteristics and

disturbances are reflected in below-ground microbial assemblages as drivers of (1) community diversity and composition, and as determinants of (2) community assembly patterns and (3) community uniqueness across a spruce-dominated forest landscape. Our results show that logging history, tree species composition and urbanization each have predictable

effects on soil microbiomes at one or more levels of diversity. However, our analyses also showed that the above-ground predictors had relatively limited power in explaining community assembly at the OTU-level, highlighting the significance of soil quality data in predicting microbial community composition at fine taxonomic levels.

Effective conservation prioritization aiming to preserve maximum species diversity within a habitat network, such as an urban forest landscape, should be based on understanding about which areas provide highest complementarity to the landscape-scale diversity. As biodiversity data covering whole landscapes is usually unavailable, particularly for soil organisms, such prioritization needs to rely on more readily available proxies, e.g., occurrence patterns of easily observable indicator species or forest structural data to point out potential biodiversity or rarity hotspots. Our results show that above-ground forest features such as the proportion of built-up land surrounding the forest, tree diversity within the forest, and the uniqueness of tree species composition have the potential to predict community uniqueness across a forest network. While the degree of urbanization and tree diversity are relatively easy to measure and compare between just two forest stands, comparisons of relative uniqueness of tree species composition require information about how forest structure varies throughout the focal landscape. However, a potentially generalizable implication of our result is that a network of sites with contrasting tree species compositions is likely to capture more soil microbial diversity than respective network with higher local tree species diversity but lower between-site variability in tree species composition (see Heinrichs et al. 2019). In the following sections, we discuss the implications of our findings more specifically in terms of logging disturbance, tree species composition and urbanization in the context of boreal spruce-dominated forest landscapes.

Logging intensity

Earlier studies in boreal Norway spruce dominated stands have shown that harvesting of 30% of tree basal area results in predictable shifts in soil fungal communities within a timeframe of few years (Kim et al. 2021). Based on our observations of cut stumps in early stages of decay (decay classes 1–2), only a

few sites in this study had been harvested with comparable intensity in recent years. Paucity of responses to recent logging activity suggests that microbial communities were generally well buffered against acute disturbance effects at the prevailing levels of tree harvesting observed across the plot network.

Although no short-term logging related effects were found, soil microbial communities showed responses to decades old logging activities. We presume that these effects may relate to long-term changes in tree layer following logging disturbance. Stands with higher amounts of old logging tended to have higher basal area of living trees and lower cover of vascular plants in the field layer (see Appendix E), indicating that the effect was potentially mediated by increasing influence of recalcitrant tree (needle) litter and/or dominance of competitive tree-root associated fungi in the soil organic layer. This interpretation is consistent with the observed decrease in Proteobacteria:Acidobacteriota ratio, indicating a shift towards more oligotrophic conditions (Fierer et al. 2007), and the change in fungal biomasses to higher ECM and lower SAP fungal abundance,

Tree species mixture

Our results revealed contrasting responses to tree diversity and deciduous tree admixture among microbial groups. While ECM fungal diversity was marginally higher in stands with higher tree species richness (consistent with, e.g., Kernaghan et al. 2003 and Tedersoo et al. 2016), stands with low tree species diversity (i.e., high relative dominance of spruce) contributed the most to the landscape-scale diversity of SAP fungi. This effect was probably driven primarily by birch admixture, which was also associated with lower site-level diversity of SAP fungi. In the context of spruce-dominated forests, it is likely that deciduous tree admixtures mainly benefit generalist species, whereas specialized spruce forest associated species are more restricted to pure spruce stands (see also Kebrle et al. 2021). Thus, allowing a certain share of the forest area to develop as close to pure spruce stands is probably necessary for maintaining soil microbial diversity at the landscape-scale, despite concerns over the resilience of such forest structures with increasing risks of climate driven forest damages (Venäläinen et al. 2020).

Urbanization

Aside from marginally positive effect on site-level diversity of bacteria, the effects of urbanization on microbial diversity were mainly negative. While SAP fungi decreased in diversity at the stand-scale, urbanization had no apparent role in explaining the uniqueness of SAP fungal assemblages across sites. In contrast, urbanization had no significant effect on ECM fungal diversity at the stand scale but promoted ECM fungal assemblages that were less unique. In growing and densifying cities, further fragmentation of remaining forest areas with built-up land cover may therefore be expected to result in increasingly generic ECM fungal communities, possibly due to the loss of disturbance sensitive forest specialist species.

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Data availability Sequence read data files will be made available under BioProject ID PRJNA1012880 in NCBI sequence read archive. OTU tables, and metadata on OTUs, samples and study sites are deposited in the Dryad Digital Repository <https://doi.org/10.5061/dryad.h44j0zps5>.

Declarations

Conflict of interest The authors declare no competing interests.

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