


Original article

The boreal soil microbiome of different urban green spaces – Do city residents meet different microbes?

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ABSTRACT

Soil microbes perform a wide range of functions, including nutrient cycling. Soils in urban green spaces also provide a nearby source of natural microbiota for human residents, potentially promoting the development of skin microbiomes and having beneficial impact on health. The soil of urban green spaces, which have distinctive vegetation characteristics, was analysed in two boreal cities to investigate whether residents are exposed to different microbial communities.

The analysis included built, open and forested green spaces. Built green spaces, characterized by flower beds and lawns, and open green spaces, such as meadows, had a similar soil microbiome, although this was an unexpected finding. The bacterial and fungal communities of urban forests differed from those of built and open green spaces. In contrast to the bacterial diversity of urban forests, which increased in the soil of built and open spaces, fungal diversity did not differ. According to metagenomic analyses all the green spaces were potentially denitrifying environments and revealed that all three urban spaces harboured genes that could possibly lead to the formation of more nitrous oxide (N₂O) in urban forest soils. This finding highlights the importance of incorporating greenhouse gas flux measurements and functional microbial analyses in future research on the impact of urbanization on soil microbiology.

From the perspective of urban planning, our results indicate that the soils of built green spaces are microbiologically diverse, offering the potential to design different types of plantations that attract residents to urban parks of their choice. Furthermore, we recommend making efforts to preserve existing urban forests to provide opportunities for local populations to interact with alternative environmental microbiomes.

1. Introduction

Urban green spaces with their vegetation and soil provide essential ecosystem services, such as mitigating air pollution, heat, and noise, infiltrating rainwater, and offering aesthetic, spiritual, educational and recreational values while acting as refuges for biodiversity (Paudel and States, 2023). Preserving green spaces also enhances carbon (C) sequestration (Raymond et al., 2023), and there is growing evidence of the beneficial effects of green spaces on human health and well-being (e.g., Bowler et al., 2010; Fong et al., 2018; James et al., 2015).

Indeed, recent studies suggest that nature and its biodiversity benefit the human immune system by regulating the environmental microbiome (e.g., Hanski et al., 2012; Rook, 2013; Haahtela, 2019; Roslund et al., 2021). For example, the biodiversity hypothesis by Hanski et al. (2012)

postulate that exposure to more biodiverse environments reduces inflammatory diseases due the richer microbiota, influencing the human microbiome. Therefore, living in an urban environment can reduce interactions with microbes living in natural ecosystems (Parajuli et al., 2018) and the density of urban green spaces is reflected in the human microbiome (Zhang et al., 2023).

Soil microbiomes in public urban green spaces are important sources of exposure to diverse microbiomes for a significant proportion of the world's population (Banerjee and van der Heijden, 2023). The soil microbiome reflects the microbiome from the air and plant litter, and the above-ground vegetation can therefore play an important role in shaping the microbial community composition of soil. Soil characteristics may be also important mediators of the microbiome effects as high cation exchange capacity (CEC) has been shown to be associated with

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reduced risk of infectious and parasitic diseases (Liddicoat et al., 2018).

The soil microbiome provides also other essential ecosystem services, such as nutrient recycling through decomposition and the nitrogen (N) cycle. Microorganisms fix, bind, and release gaseous N to and from organic compounds mineralizing it back to gaseous N. Therefore, the presence and abundance of N-cycle genes indicate soil microbiome's potential function. Delgado-Baquerizo et al. (2021) found higher abundance of soil bacterial genes related to the N-cycle (denitrification, ammonification, nitrite reductase) in urban green spaces across 56 cities compared to nearby natural environments (seminatural forests, grasslands and shrublands). However, Zheng et al. (2024) observed neutral responses when comparing urban habitats in three cities representing the boreal, temperate, and tropical climatic zone. Li et al. (2024) reported increased methane (CH₄) flux along an urbanization gradient towards densely populated areas. Since CH₄ flux is controlled by archaea and bacteria we also analyzed the respective *mcrA* and *pmoA* gene levels as representative indicators of the microbial C cycle.

Although the soil microbiota plays an important role in the production of ecosystem services of urban areas, we lack understanding of how soil microbial communities vary across urban green spaces. Mills et al. (2022) found that rare soil bacterial genera can distinguish between urban green spaces of high and low complexity, but they also called for further research. Contributing to this knowledge gap may help us to optimize the capacity of green spaces to provide much needed ecosystem services in urban planning. As vegetation complexity is potentially important for urban green space design, this knowledge can also be used to promote microbial biodiversity.

This study aims to describe the soil microbiome, analysed by metagenomics (bacteria) and amplicon sequencing (fungi), in relation to the aboveground plant diversity in three types of urban green spaces: built green spaces with sown lawns, planted trees and ornamental flowerbeds; open green spaces such as meadows and pastures; and urban forests. The study was conducted in two boreal cities where residents frequently interact with green spaces. A total of 151 urban green space soil samples were included in the sampling campaigns. The overarching hypothesis is that the complexity of urban green space plant communities' controls soil microbes. Specific hypotheses were: H1 - Soil bacterial and fungal richness and diversity in human-built green spaces are lower than in open and forested urban green spaces. H2 - The soil microbiome (bacterial and fungal community composition) differs between the three types of urban green spaces. H3 - Changes in the microbial community are accompanied by alterations in the C and N cycle, analyzed by gene counts involved in the greenhouse gas (GHG) CH₄ and N₂O fluxes.

We expect these hypotheses to be valid because plant legacies modify soil microbial communities (Connell et al., 2021). This should shape the soil microbes of different urban green spaces into their own communities, especially in human-built green spaces due to the prevalence of low plant diversity lawns.

2. Material and methods

2.1. Study area

The study was performed in two cities, Oulu (65°05'11.12''N, 25°28'18.84''E) and Helsinki (60°10'32.16''N, 24°56'3.12''E), Finland (Fig. 1). Oulu has 0.21 million residents, and the municipality covers ca. 3000 km² of land in total. The city is situated in the northern boreal zone and has a yearly mean day temperature of 3.3 °C and 525 mm of yearly precipitation. Helsinki's land area covers ca. 215 km² and has 0.67 million residents. The city is situated in Southern Finland in the hemi boreal zone, with mean day temperature of 6.5 °C and yearly mean precipitation of 651 mm. The selected cities represent two different examples of urbanization level. Helsinki is more densely built, as there are notably more inhabitants relative to the land area compared to Oulu. Furthermore, as the capital, Helsinki is the centre of the wider Helsinki metropolitan area and is thus surrounded by other large Finnish cities,

whereas in Oulu, the city's built-up area is relatively small compared to the municipality's land area and thus surrounded by more sparsely populated and rural area. In Oulu, most of the inhabitants as well as the public green spaces managed by the city, are in the city's built-up area and thus, our study sites were concentrated also therein (Fig. 1).

2.2. Green space types

Spatial data of urban green spaces were provided by the cities of Oulu (Source: Oulu city green area data, not open data) and Helsinki (Register of public areas in the City of Helsinki, 2022). The data includes information on location, green space classification and their maintenance. The classification includes three main types: Built green spaces (B), open green spaces (O), and urban forests (F) (Table 1, Figs. 1 and 2).

Built green spaces (B) include three different subtypes, which are typically characterized by sown lawns dominated by fast-growing grasses and herbs. They can also include a varying number of planted shrubs, trees, and flowerbeds. They are maintained regularly, and the maintenance level is highest in subtype B1 and lowest in B3 (Table 1). Open green spaces (O) include five subtypes that consist of different types of meadows, fields, pastures, and other habitats that are maintained open (Table 1). Oulu has only representative of subtype O3: landscape meadow and grazing area (Table 1). Helsinki includes all five O subtypes. Urban Forest (F) include five subtypes that are characterized by forest vegetation, and are managed for different purposes including recreation, commercial forestry, shelter forest protecting against disruptive activities, and for preserving or re-introducing landscape and biodiversity protection (Table 1). Representatives of subtype F4, commercial forests, are only found in Oulu (Table 1).

2.3. Study sites, study plot design, soil sampling, and vegetation analysis

In 2022, 151 study sites were selected for aboveground vegetation and belowground soil microbiome diversity analysis. The following selection criteria were used: i) The site is within 1.5 km of the residential area of Northern Birth Cohort 1966 and 1986 members, whose clinical records are known due to regular monitoring. This enables future research on environmental diversity in relation to human health. ii) The site is known to be popular for recreational use. iii) Replications for each type of green space exist. The number of replications of each green space type was influenced by its prevalence and total area in the city. iv) The selected sites form a gradient from densely to more sparsely built urban areas containing larger green spaces.

In each study site, we established a study plot for the field inventory (Supplementary Fig. S1). Each study plot consisted of two lines that were perpendicular to each other. The lines were 10 or 20 m depending on the size of the green area. The first line started from a randomly pre-assigned coordinate within the green space, facing north. If the line could not be oriented in a true north-south direction, it was directed as closely to that as possible. Similarly, the second line ran primarily from west to east and was always perpendicular to the first line. Soil samples were collected from a total of seventeen sample plots evenly spaced along these two lines. Green vegetation and litter where plant particles were structurally integrated were removed before the soil sample was taken. A soil auger with a diameter of 30 mm was used to collect a soil sample from the top layer, reaching a depth of 3 cm. Sampling the uppermost 3 cm of soil was chosen to provide a more representative microbiota in terms of potential human exposure, compared to traditional soil sampling at a depth of 10 cm, for example. In forest sites with podzolic soil development, where the organic F/H layer was shallower than 3 cm before the appearance of mineral soil, the sampling was adjusted to this depth. Finally, all 17 soil samples were combined into one composite soil sample per study plot. Between study plots, the soil auger was cleaned with disposable alcohol wipes, and the first soil sample taken in each new study plot was collected outside the plot and discarded. Disposable gloves were used during sampling to prevent cross contamination. The

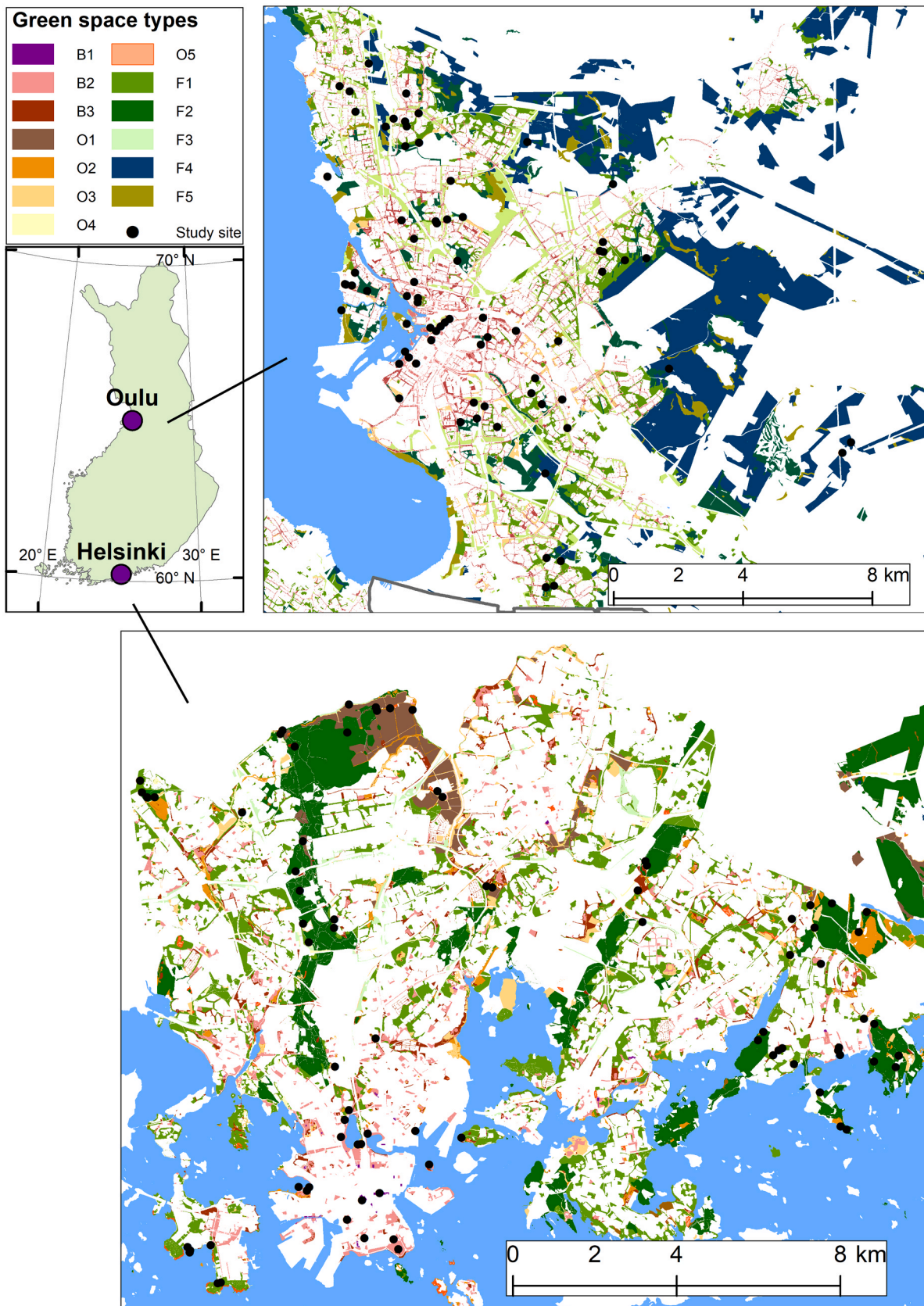


Fig. 1. Maps showing the location of the studied Finnish cities, Oulu and Helsinki, and the distribution of urban green spaces in those cities. Sources: Oulu city green area data and Register of public areas in the city of Helsinki.

Table 1
The urban green spaces, their subtypes, descriptions, and the number of study sites in Oulu and Helsinki.

Green space type	Green space description	Oulu	Helsinki	Total
Built green spaces (B)				
B1 Representative green space	Yards of important public buildings, central urban parks, and squares.	2	5	7
B2 Recreational green space	Urban parks and squares, playgrounds, traffic green areas in the downtown area, courtyards and green spaces for sports and activities.	8	9	17
B3 Recreational and shelter green space	Includes generally large parks, protective zones, or more environmentally managed shelter parts of the property's yard areas, sports greenery and street green areas situated outside the city center.	10	9	19
Open green spaces (O)				
O1 Landscape field	Modified and sown areas of land where landscape plants are cultivated.	-	6	6
O2 Recreational meadow	Open or semi-open meadows usually available for recreational use throughout their area.	-	7	7
O3 Landscape meadow and grazing area	Open or semi-open meadows where recreational use has been directed to e.g. trails. The pasture areas are meadows that are managed by grazing.	8	6	14
O4 Open areas	Areas that are maintained open for scenic purposes or e.g., a technical network installation (electric lines).	-	6	6
O5 Valued meadow	Meadows of significance for cultural tradition, landscape, or biodiversity.	-	7	7
Urban Forests (F)				
F1 Near-by forest	Forests that are located close to settlement and are subject to abundant recreational use and trampling.	10	10	20
F2 Recreational forest	Forests consist of wider forest areas that are intended to outdoor recreation and hiking/camping and can contain maintained trails and other recreational facilities.	10	7	17
F3 Shelter forest	Forests located between settlement or other built environment and disruptive activities such as bigger roads. Protects against small particles, dust, and noise damage.	10	5	15
F4 Commercial forest	Commercial forests located further away from the settlement or outside the built-up area. Management and use of forest is carried out in accordance with the principles of sustainable forestry.	7	-	7
F5 Valued Forest	Forests that are valuable for preserving cultural heritage, landscape, or biodiversity.	6	3	9
Total		71	80	151

soil samples were frozen at -20°C at the end of each field collection day and then divided into two parts before analysis: one for DNA extraction and the other for soil physico-chemical analysis.

The vegetation structure was surveyed within five $0.5\text{ m} \times 0.5\text{ m}$ squares placed along the lines, with two squares located at both ends of the first line and one in the middle, at the intersection of the two lines

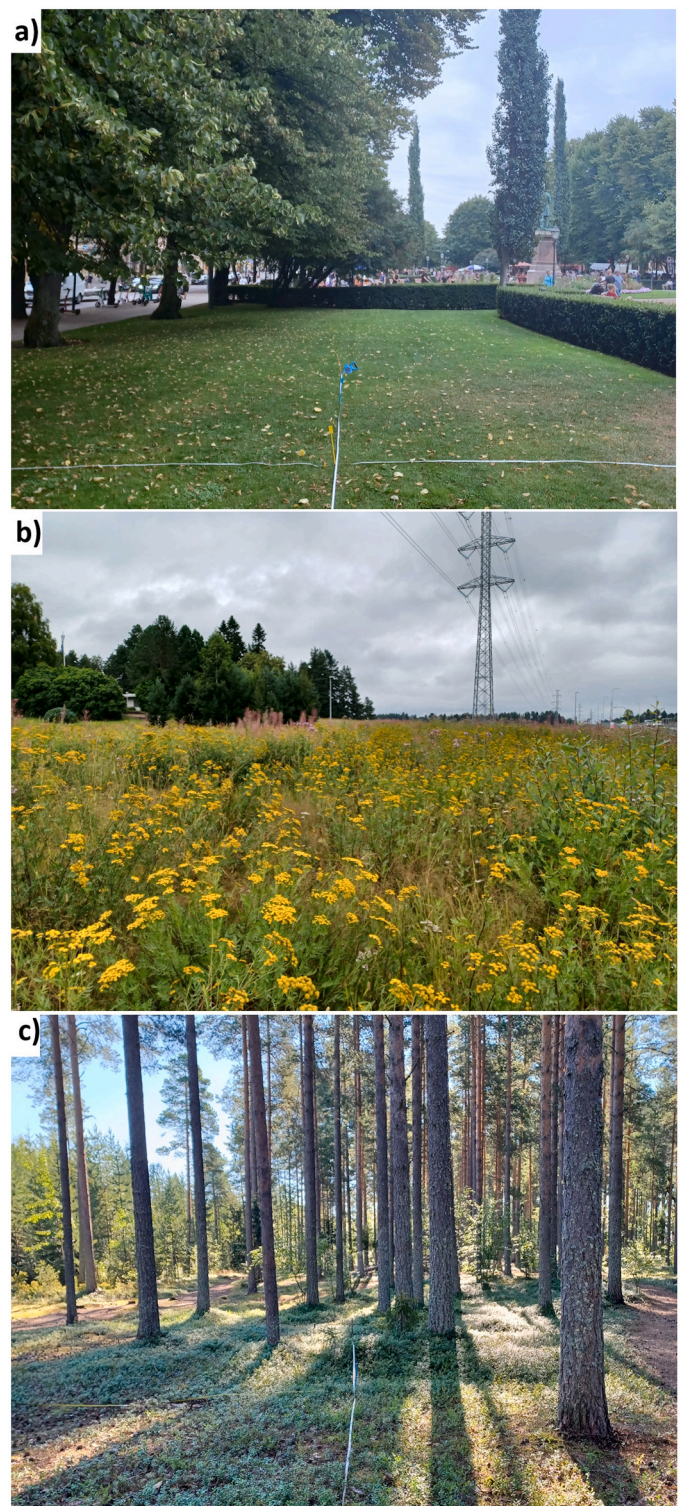


Fig. 2. The studied green space types: a) built green areas, b) open green areas, and c) urban forests.

(Supplementary Fig. S1). In addition, two squares were located on the second line, placed midway between the end and intersection point of the line. From these squares the cover of field layer vegetation, ground layer vegetation, and plant functional types, as well as the species number were surveyed. Tree seedlings and shrubs under 0.5 m in height were included in the field layer vegetation, together with dwarf shrubs, herbs and graminoids. The ground layer vegetation consisted of bryophytes and lichens. Some of the observations which could not be

determined at species level, for example *Poa* spp., were included at genus level. In addition, in each sample site we surveyed the number of trees and shrubs and estimated the canopy cover. The tree and shrub species were surveyed from the study site extending 5 m to its surroundings.

2.4. Soil physico-chemical analysis

Before analysis the soil was air dried and milled to pass a 2 mm sieve. A detailed description of the performed analysis to determine soil dry matter weight, pH, C, N, organic matter (OM), cation exchange capacity (CEC) and the soil particle size distribution (sand, silt, clay), which was performed to 111 samples where the OM content was below 40 %, is given in Rätty et al. (2021).

2.5. DNA extraction and sequencing

DNA was extracted from approximately 250 mg un-sieved soil with the DNeasy PowerSoil Pro Kit (Qiaqen, Germany) according to the protocol of the manufacturer. Metagenome and fungal amplicon library preparations and sequencing were conducted by Novogene (Cambridge, UK). In amplicon sequencing, the following primer set targeting fungal ITS2 region for 380 bp product was used: ITS3–2024F forward (GCATCGATGAAGAAGCAGC) and ITS4–2409R reverse primers (TCCTCCGCTTATTGATATGC) (White et al., 1990; Gardes and Bruns, 1993). PCR reactions were conducted with 15 µL of Phusion® High-Fidelity PCR Master Mix (New England Biolabs); 2 µM of forward and reverse primers, and about 10 ng template DNA. Thermal cycling consisted of initial denaturation at 98°C for 1 min, followed by 30 cycles of denaturation at 98°C for 10 s, annealing at 50°C for 30 s, and elongation at 72°C for 30 s. The final step included 72°C for 5 min. Amplicon sequencing libraries were generated using TruSeq® DNA PCR-Free Sample Preparation Kit (Illumina Inc., San Diego, California, USA) following manufacturer's recommendations and index codes were added. The library quality was assessed on the Qubit® 2.0 Fluorometer (Thermo Scientific) and Agilent Bioanalyzer 2100 system. The fungal ITS2 region was sequenced on an Illumina NovaSeq platform (Illumina Inc., San Diego, California, USA) with 250 bp paired-end reads, yielding 50,000 tags per sample. Metagenomic libraries were constructed with Nextera XT DNA Library Preparation Kit (Illumina, San Diego, CA, USA) and paired-end sequenced with Illumina NovaSeq S4 lane.

2.6. Bioinformatics for metagenomic data

The obtained raw paired end reads (mean: 10.6 Gb, minimum: 5.1 Gb, maximum: 16.2 Gb) from the 160 metagenomes were filtered with Cutadapt (Martin, 2011) for potential adapter contamination and low-quality reads ($q < 20$) as well as too short reads (50 b). The filtered reads were annotated for taxonomy using Metaxa2 (Bengtsson-Palme et al., 2015) and the resulting SSU rRNA reads re-annotated against SILVA 138 database (Quast et al. 2013) with Wang's naive Bayesian classifier (Wang et al., 2007) in Mothur (Schloss et al., 2009). For community composition analyses, bacterial 16S rRNA gene based taxa were collected from the metagenomes and the data were divided by their city origin. Taxa that were observed fewer than 10 times were removed from the datasets, consisting of 664 048 reads and 930 bacterial taxa in Oulu, and 810 059 reads clustering into 954 bacterial taxa in Helsinki. Functional genes were annotated with Diamond (–max-target-seqs 1, –max-hsps 1) against a custom database of 50 metabolic marker protein databases (Leung and Greening, 2020) covering the major metabolic pathways for respiration, energy conservation carbon fixation, N cycle and phototrophy.

2.7. Bioinformatics for fungal ITS amplicon reads

The average amount of raw reads per sample library was 179 671

(standard deviation 22 853). Sequence assembly, quality filtering, removal of artifacts, primer-dimers, and primers from ITS2 sequence reads along with clustering and taxonomical annotations were conducted with PipeCraft 1.0 pipeline (Anslan et al., 2017) as described by Soinne et al. (2020) with slight modifications. Reference ITS2 database *sh_general_release_dynamic_17.8.2023.fasta* from UNITE (Nilsson et al., 2019) was used for identification of fungal operational taxonomic units (OTU). After the first quality filtering steps raw ITS2 data consisted of 21 560 350 reads clustering into 90 413 OTUs. Then, we filtered out fungal OTUs that had e-value higher than e^{-25} and identity less than 75 % with the database match. OTUs that had affiliation other than fungi, as well as singleton OTUs were removed from the data. Furthermore, fungal OTUs were consolidated according to the exact same Species Hypothesis in UNITE (Kõljalg et al., 2013). The final fungal ITS2 data for richness and diversity measures consisted of 21 220 972 reads clustering into 16 840 OTUs with the same Species Hypothesis. The average amount of filtered reads per sample library was 136 032 (standard deviation 23 138). Fungal guilds derived from OTUs representing potentially different ecological roles were detected using FUNGuild (Nguyen et al., 2016). For community composition analyses, fungal data were divided by their city origin and reads that were observed fewer than 10 times were removed from the data sets, consisting of 9 644 496 reads and 7470 OTUs in Oulu, and 10 821 971 reads clustering into 8638 OTUs in Helsinki.

2.8. C and N-cycle gene count ratios

Gene counts for the genes involved in C and N cycling were collected from the custom database alignments and normalized by the number of base pairs sequenced. For the C-cycle we focused on the analysis of *mcrA* and *pmoA* genes. They are responsible for CH₄ production and consumption, respectively, and successfully analyzed by metagenomics (Laiho et al., 2024). For the N-cycle we focused on two calculations: the difference in counts of *nfrA* and *nir* genes [*nfrA*–(*nirS*+*nirK*)] (Saghaei et al., 2023) and the ratio of *nir* and *nos* genes (*nirK*+*nirS*)/*NosZ*, which was used to understand the fate of N. The first calculation abbreviated from here as $\delta nfrA-nir$ indicates immobilization of N as ammonium if values are > 0 or losing of N as gaseous products if values are < 0 . The second calculation abbreviated as *nir/nosZ*-ratio provides an indication in which gaseous form N is lost from the soil – in comparison higher values produce more N₂O.

2.9. Spatial variables

Spatial analyses and map visualization were carried out using ESRI ArcGIS for Desktop software version 10.6.1. and ArcGis Pro 2.1 (ESRI, Redlands, CA). Spatial variables describing the characteristics of the studied green spaces with varying radius were calculated for each study plot (Supplementary Table S1). The size of the studied green space, and the number and the total area of surrounding green spaces were calculated from the green space data provided by the cities. We further calculated several variables indicating the level of urbanization (Supplementary Table S1). The number of length of roads as well as light traffic roads were calculated from the Digiroad, Finnish Transport Infrastructure Agency, 2023). The population density was calculated from Population grid data 1 km x 1 km by Statistics Finland (Statistics Finland, 2022). The area of different types of built urban areas were calculated using the Corine Land Cover 2018 dataset (resolution 20 m x 20 m) provided by the Open Spatial datasets of the Finnish Environment Institute (SYKE) (Finnish Environment Institute, 2023).

2.10. Statistics

Statistical analyses including microbiome analyses were performed using R Statistical Software (v 4.3.1; R Core Team, 2023).

2.10.1. Physico-chemical and vegetation data analysis

The green space type and subtype differences in soil physiochemistry variables and vegetation were tested with a linear model and pairwise comparisons based on Tukey's HSD test. Indicator species analyses (*indicspecies* R package; De Cáceres and Legendre, 2009) was performed to assess possible indicative species for different green space types (indicator value, stats > 0.5, alpha $p < 0.001$). We performed permutational multivariate analysis of variance (PERMANOVA) using distance matrices with function 'adonis2' from *vegan* (Oksanen et al., 2019) to test the effect of green space type (B, O and F) on plant community composition. To visualize the plant communities in different green space types, we used nonmetric multidimensional scaling (NMDS) with function 'metaMDS' from *vegan* with Bray-Curtis dissimilarity indexes to make an ordination.

2.10.2. Microbial analyses

All figures were created using R packages *phyloseq* (McMurdie and Holmes, 2013) or *ggplot2* (Wickham, 2016). Fungal richness and Shannon diversity was obtained with *microbiome* package (Lahti and Shetty, 2017) and bacterial in *vegan*. Differences in means of fungal (OTUs) and bacterial (read count for each taxon) richness and Shannon index and fungal guilds (saprotrophs, pathotrophs, symbiotrophs) as well as N cycle gene count ratios between the green space types were investigated with linear model with function 'lm' (package *R stats*). The significance of the linear model was tested using analysis of variance with the function 'anova.'. Pairwise analyses and significant differences of farming systems were tested with function 'lsmeans' (package *lsmeans*) (v2.30-0; Lenth, 2016) (alpha $p < 0.05$). Similarly, as for plant communities, we performed a PERMANOVA to test the effect of green space type (B, O and F) on bacterial and fungal community composition. To avoid including collinear environmental variables in the nonmetric multidimensional scaling (NMDS), we conducted a Principal components analysis with function 'principal' in package *psych* (Revelle, 2024). In general, only one variable from the variables with highest correlation value that showed high collinearity per principal component was selected into the NMDS (Supplementary Table S2). We also conducted NMDS from relative abundance data with species scores as for vegetation data and plotted the NMDS with representative significant environmental variables (Supplementary Table S3) from function 'envfit' in *vegan*. To identify bacterial taxa obtained from 16S rRNA gene derived metagenomic data and fungal OTUs indicative of groupings, we performed indicator species analyses using the *indicspecies* package. The relative abundances of the most dominant families ($n = 20$) among green space types were determined from the metagenomic bacterial 16S rRNA gene derived read data and fungal ITS2 OTU data, discarding reads that were observed fewer than 10 times. The difference of 16SrRNA gene derived bacterial taxa from metagenomes and fungal OTUs containing all data and the most abundant bacterial and fungal families between green space types were tested with pairwise multilevel comparison method using wrapper function 'pairwise.adonis' in *adonis* from package *vegan*. First, 'vegdist' was used to calculate the similarity matrix and then bray was used as similarity method and bonferroni as the p-value correction method with 999 permutations.

3. Results

3.1. Soil physico-chemistry

The urban forests (green space type F) had the lowest pH in both cities and pH was significantly lower than that of the built and open green spaces (B and O, Supplementary Fig. S2). In Oulu, the lowest pH was recorded in the commercial forest F4 subtype differing significantly from the shelter forest F3 subtype (Supplementary Fig. S3). In the built green space, no significant differences were found between the subtypes. In Helsinki, the lowest pH was found in the recreational forest F2 subtype, but the pH did not differ significantly among the urban forest

subtypes, nor within the built and open green space subtypes. In both cities the CEC values in the urban forests were significantly higher than in the built and open green spaces (Supplementary Fig. S2). Within the urban green space types, the F2 subtype had a significantly higher CEC value compared to near-by F1 and F3 forest subtypes in Helsinki. In both cities the C% (as also the OM; data not shown) and N% were the highest in the urban forests and differed significantly from the built and open green spaces (Supplementary Fig. S4). Within the urban green space subtypes in Helsinki the F2 subtype had a statistically significantly higher C% and N% when compared to the other urban forest subtypes except the N% did not differ between F2 and valued forest F5 subtypes (Supplementary Fig. S5). Similarly, the C/N-ratio was the highest in the urban forests. Within the built and open green spaces no significant differences could be detected. In Oulu, the highest C/N-ratio was detected in the F4 subtype, and in Helsinki, in the F2 subtype. In Oulu, the difference in the C/N-ratio was statistically significantly higher in F4 subtype, than in F3 and F5 subtypes. In Helsinki, the F2 subtype had a significantly higher C/N-ratio when compared to F3. In both cities, the sand, silt, and clay contents (given as %) in soils of OM less than 40 % were equal between the urban green spaces (Supplementary Fig. S6). The only exception was within the open green spaces in Helsinki where the landscape field O1 subtype had a significantly higher clay content than the recreational meadow O2 subtype (Supplementary Fig. S7).

3.2. Vegetation

3.2.1. Plant species richness and their functional types

A total of 263 plant species were detected across all the surveyed study plots, with 188 species observed in Oulu and 199 species in Helsinki. Correspondingly, there were a total of 32 tree species and 25 woody shrubs detected in the study plots, of which 20 tree and 16 shrub species in Oulu, and 27 tree and 14 shrub species in Helsinki. The number of plant species, trees not included, where the highest in the open green spaces (Supplementary Fig. S8), and this was more pronounced in the City of Oulu than in Helsinki where the open and built green spaces did not significantly differ from each other. When the subtypes were compared separately within each green space type the representative B1 subtype of the built green space had significantly fewer plant species than the recreational B2 and shelter B3 subtypes in Oulu (Supplementary Fig. S9). In Helsinki the subtype landscape fields O1 had significantly fewer species than the recreational meadows O2 subtypes. Naturally the number of different tree species and the canopy cover were significantly higher in the urban forests in both cities when compared to the built and open green spaces with no significant differences between the subtypes within each green space type (Supplementary Figs. S8, and S9). Also, the number of shrubs and the ground layer vegetation cover area % was the highest in the urban forests in both cities and differed significantly from the built green spaces (Supplementary Figs. S8 and S10). Significant differences between the respective subtypes could not be detected except for Oulu, where commercial forests F4 subtype showed the highest ground layer vegetation cover area % (Supplementary Fig. S9 and S11). The number of dwarf shrub species was also highest in the urban forests, and it differed significantly from the built and open green spaces in Helsinki and from open spaces in Oulu (Supplementary Fig. S8). The field layer vegetation cover % showed an opposite trend to the ground layer vegetation cover area %, the urban forests having significantly lower field layer vegetation cover % in both cities (Supplementary Fig. S10) compared to the built and open green spaces. Comparison of the respective subtypes revealed no significant differences (Supplementary Figs. S9 and S11). The number of forbs was significantly higher in the open green spaces in both cities when compared to the other green spaces (Supplementary Fig. S10), and in Oulu the number of forbs in the urban forests was even significantly lower than in the built green spaces. For the subtypes significant differences were detected within urban forests in both cities. In Oulu, the F4 subtype had the lowest number of

forbs, whereas in Helsinki, the shelter forest F3 subtype had the highest number of forbs and differed significantly from the recreational forest F2 subtype (Supplementary Fig. S11). The number of graminoids was significantly lower in urban forests in both cities compared to the built and open green spaces (Supplementary Fig. S10) and no significant differences could be detected in comparison of the respective subtypes (Supplementary Fig. S11).

In both cities, there were significant differences between the plant community composition of built, open and urban forest green spaces (Fig. 3, Table 2a and Supplementary Table S4a). As visualized by NMDS, the difference between urban forest compared to other green spaces where more pronounced in Oulu, whereas community compositions of built and open green spaces where closer to each other (Fig. 3). Based on PERMANOVA the green space type explained 35 % ($R^2=0.346$, $p < 0.001$) and 17 % ($R^2=0.165$, $p < 0.001$) of the variation of the plant community composition in Oulu and Helsinki, respectively (Supplementary Table S4a). According to the PERMANOVA, there were also significant differences between plant community compositions of the subtypes in both cities (Oulu $R^2 = 0.43392$, $p = 0.001$, Helsinki $R^2 = 0.31032$, $p = 0.001$). In Oulu the plant community composition in commercial forest F4 subtype, and in Helsinki the recreational forest subtype F2 were the most distinct from the community composition of the built and open green spaces, whereas the F3 subtype in Helsinki was the most similar to the built and open spaces (Supplementary Fig. S12).

3.2.2. Characteristic/Typical plant species

The indicator species analysis identified 15 typical plant species across all green space types in Oulu, and in Helsinki, four typical species (Supplementary Table S5). In Oulu, the typical plant species for built green space were *Plantago major*, *Taraxacum sp.*, *Festuca sp* *Trifolium repens* and *Scorzonerooides autumnalis*, and for the open green spaces *Elymus repens*, *Phleum pratense*, *Trifolium pratense*, *Anthriscus sylvestris* and for the urban forests *Vaccinium myrtillus*, *V. vitis-idaea*, *Pleurozium schreberi* and *Hylocomium splendens*, respectively. In addition, the indicator species analyses identified *Poa sp.* and *Agrostis sp.* as typical plant species for both built and open green spaces in Oulu. In Helsinki, the typical plant species for built green space were *Poa sp.*, *Festuca sp.* and *Plantago major*, and for urban forests *Vaccinium myrtillus*.

3.3. Soil microbiome

3.3.1. Richness and diversity

The bacterial richness (bacterial taxa derived from metagenomic 16SrRNA gene data) in Oulu was significantly lower in the urban forests

Table 2

Results of the pairwise multilevel comparison to test the effect of green spaces (build, B; open, O; forest, F) on relative abundances of a) vegetation, b) the most abundant bacterial (16SrRNA gene) and fungal (ITS) families (family n = 20), c) all 16SrRNA gene derived bacterial taxa and fungal ITS amplicon derived OTUs from the soil samples in the city region of Oulu and Helsinki. Differences are considered significant if p adjusted ≤ 0.05 .

a)		Pairs	Df	SS	F	R2	p	p adjusted
Oulu	F vs B	1	6.4	30.3	0.332	0.001	0.003	
	F vs O	1	2.15	8.3	0.144	0.001	0.003	
	B vs O	1	1.14	9	0.257	0.001	0.003	
Helsinki		Pairs	Df	SS	F	R2	p	p adjusted
Helsinki	F vs B	1	3.86	14.3	0.237	0.001	0.003	
	F vs O	1	2.26	6.7	0.109	0.001	0.003	
	B vs O	1	1.58	5.3	0.091	0.001	0.003	
b)		Pairs	Df	SS	F	R2	p	p adjusted
Oulu	F vs B	1	1.03	42.7	0.412	0.001	0.003	
	F vs O	1	0.38	14.1	0.223	0.001	0.003	
	B vs O	1	0.01	1.18	0.043	0.3	1	
ITS	F vs B	1	3.03	24.6	0.287	0.001	0.003	
	F vs O	1	0.95	7.28	0.129	0.001	0.003	
	B vs O	1	0.35	3.22	0.11	0.01	0.02	
Helsinki		Pairs	Df	SS	F	R2	p	p adjusted
Helsinki	F vs B	1	0.49	17.6	0.276	0.001	0.003	
	F vs O	1	0.4	14	0.203	0.001	0.003	
	B vs O	1	0.05	2.29	0.041	0.07	0.2	
ITS	F vs B	1	2.26	18.2	0.283	0.001	0.003	
	F vs O	1	1.5	10.9	0.166	0.001	0.003	
	B vs O	1	0.27	1.88	0.034	0.05	0.1	
c)		Pairs	Df	SS	F	R2	p	p adjusted
Oulu	F vs B	1	1.88	41.2	0.403	0.001	0.003	
	F vs O	1	0.64	13.4	0.215	0.001	0.003	
	B vs O	1	0.06	1.71	0.062	0.06	0.2	
ITS	F vs B	1	2.77	9.34	0.133	0.001	0.003	
	F vs O	1	1.07	3.42	0.065	0.001	0.003	
	B vs O	1	0.51	2	0.072	0.002	0.006	
Helsinki		Pairs	Df	SS	F	R2	p	p adjusted
Helsinki	F vs B	1	0.82	15.6	0.253	0.001	0.003	
	F vs O	1	0.68	12.5	0.186	0.001	0.003	
	B vs O	1	0.09	2.07	0.038	0.06	0.2	
ITS	F vs B	1	1.65	5.86	0.113	0.001	0.003	
	F vs O	1	1.31	4.53	0.076	0.001	0.003	
	B vs O	1	0.59	2.25	0.041	0.001	0.003	

compared to the built and open green spaces (Fig. 4a). Furthermore, within the Oulu urban forests the commercial forest F4 subtype had significantly the lowest bacterial richness compared to F1 near-by and F3 shelter forest, whereas the built subtypes did not differ

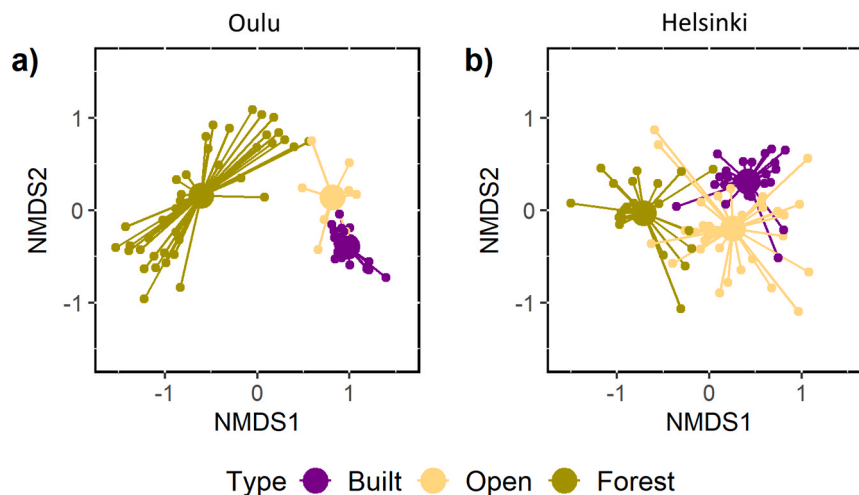


Fig. 3. Non-metric multidimensional scaling (NMDS) analysis showing the variation in plant community composition in a) Oulu and b) Helsinki between the three main green space types: built (B), open (O) and urban forests (F).

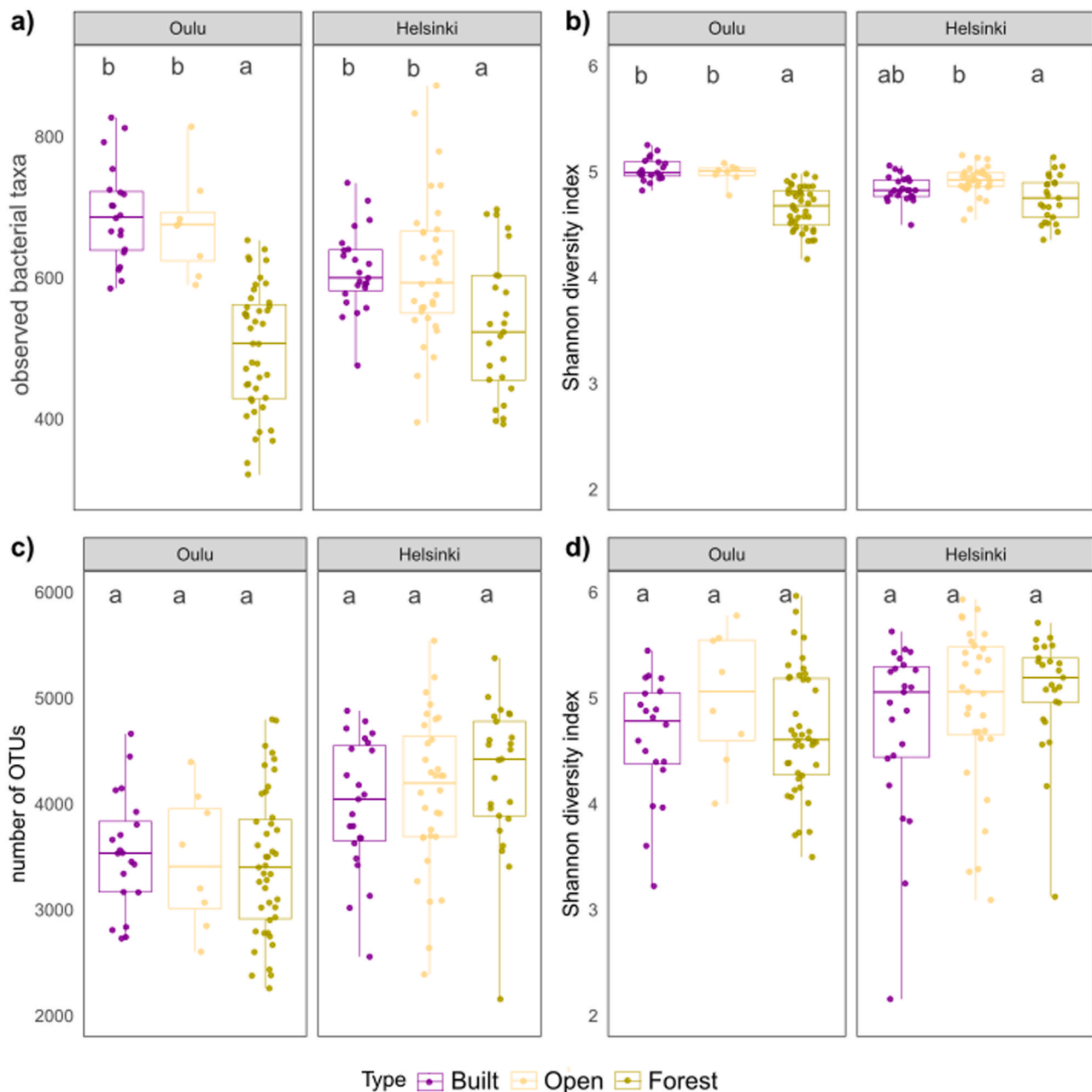


Fig. 4. Bacterial a) and b) and fungal c) and d) richness and Shannon diversity between three main green space types built (B), open (O) and urban forests (F) in Oulu and Helsinki. Different letters indicate statistical differences ($p < 0.05$) between types. Bacterial 16S rRNA gene reads from metagenomic data sets are investigated at genus level and fungal ITS derived OTU data are shown as “species” level with 97 % similarity.

(Supplementary Fig. S13a). The bacterial diversity in Oulu was significantly lower in the urban forests than in built or open green spaces and it was the lowest in the F4 subtype and differed from F1, F3 and F5 valued forest significantly (Fig. 4b, S13b). In Helsinki, the bacterial richness was significantly lower in the urban forests when compared to the built and open green spaces (Fig. 4a). The bacterial diversity of the urban forests was significantly lower than in the open green space but not compared to the built green space (Fig. 4b). Neither bacterial richness nor diversity did differ between green space subtypes in Helsinki (Supplementary Fig. S13a, S13b). Either in Oulu or in Helsinki, the fungal richness (number of OTUs) or diversity (Shannon index) did not differ between urban green space types or their subtypes (Figs. 4c, 4d, Supplementary Fig S13c and S13d).

3.3.2. The most abundant microbial taxa

The most abundant bacteria at family level varied significantly between the urban forests when compared to the built and open green spaces (Table 2b, Fig. 5a) in Oulu and in Helsinki. Families *Acetobacteraceae*, *Acidobacteriaceae* and *Mycobacteriaceae*, and order Acidobacteriales were more abundant in urban forests than in built or open green space types. In Oulu also class Alphaproteobacteria and family *Isosphaeraceae* were abundant in urban forests. In the built and open green space types Solirubrobacterales bacterium ‘67–14’, order Gaiellales, Chloroflexi of group KD4–96, family *Nocardioidaceae*, and order Vicinamibacterales were typically more abundant than in urban forests. The difference in the most abundant families between the urban forest and open and built green space types was clear, since it explained more than 20 % of the variation (Table 2b).

In both Oulu and Helsinki, the most abundant fungal families

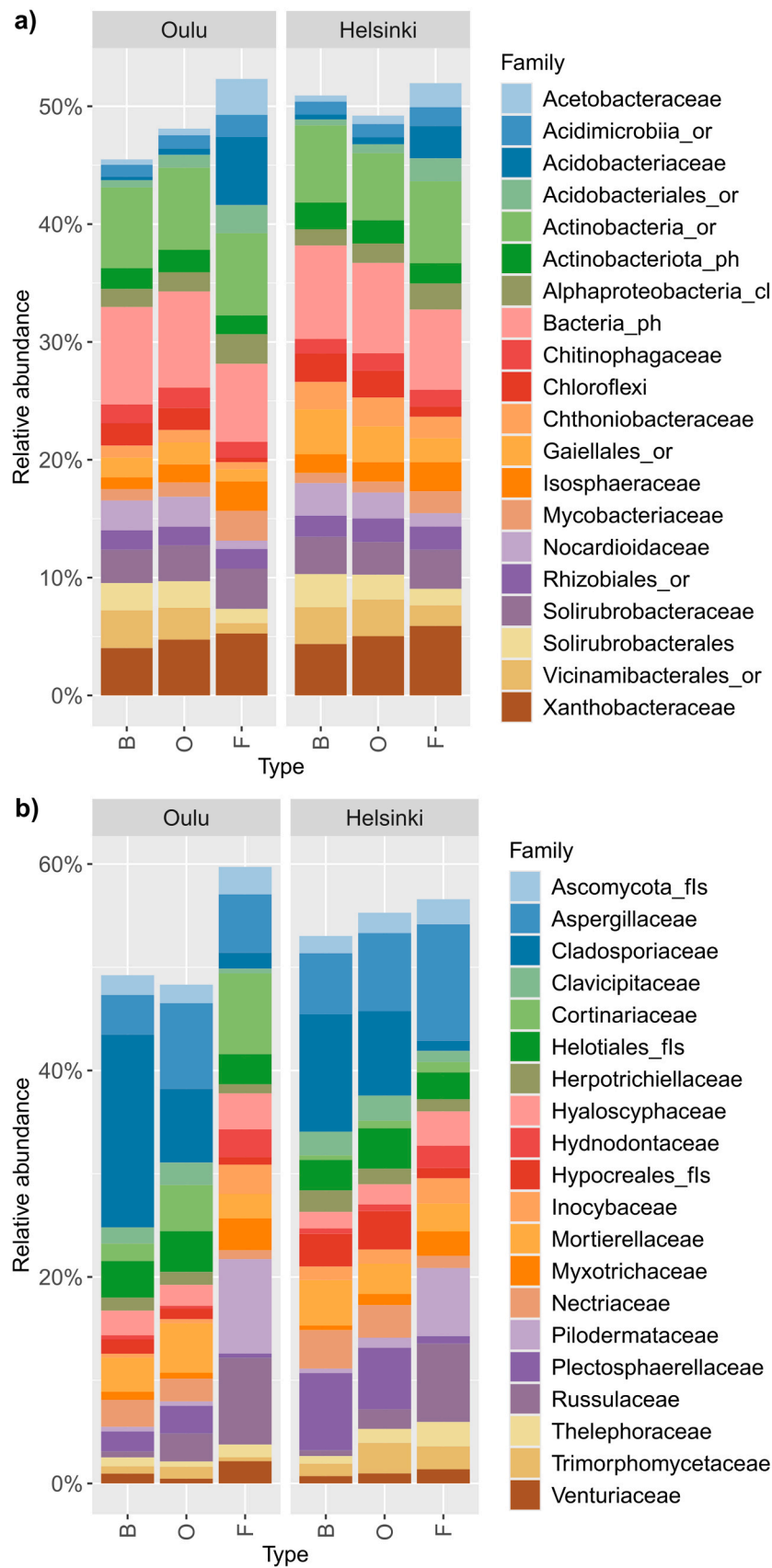


Fig. 5. Relative abundances of twenty of the most abundant a) bacterial and b) fungal families of the three main green space types built (B), open (O) and urban forests (F) in Oulu and Helsinki.

diverged between urban forests and built and open green space types (Table 2b). The family *Cladosporiaceae* was particularly abundant in the built green spaces, and *Aspergillaceae*, *Clavicipitaceae*, *Mortierellaceae*, *Plectosphaerellaceae* and *Trimorphomycetaceae* were more abundant in the O3 subtype (the landscape meadow and grazing area O3 was the only O representative in Oulu), than in built and forested spaces (Fig. 5b). In the urban forest *Cortinaceae*, *Hyaloscyphaceae*, *Inocybaceae*, *Myxotrichaceae*, *Pilodermataceae* and *Russulaceae* were more abundant than in built or open spaces. In Helsinki only the urban forest had a significantly different composition and included the most abundant fungal families *Aspergillaceae*, *Hyaloscyphaceae*, *Hydnodontaceae*, *Inocybaceae*, *Myxotrichaceae*, *Pilodermataceae*, *Russulaceae* and *Thelephoraceae*, whereas families *Cladosporiaceae* and *Plectosphaerellaceae* were scarce compared to the built and open spaces (Fig. 5b).

The urban forest subtypes, F2 recreational forest and F4 commercial forest, had a distinct pattern of dominant bacterial and fungal taxa in Oulu. Bacterial families *Acetobacteraceae*, *Acidobacteriaceae*, Alphaproteobacteria and *Mycobacteriaceae*, and fungal families *Cortinaceae*, *Hyaloscyphaceae*, *Inocybaceae* (F4), *Pilodermataceae*, and *Russulaceae* were abundant (Supplementary Fig. S14). In Helsinki, F2 had highly abundant taxa including bacterial *Acetobacteraceae*, *Acidobacteriaceae*, Actinobacteria, Alphaproteobacteria and *Mycobacteriaceae*, and fungal families *Hyaloscyphaceae*, *Inocybaceae*, *Pilodermataceae*, *Myxotrichaceae*, *Russulaceae*, and *Venturiaceae*. On the contrary, bacterial taxa Chloroflexi, Solirubrobacterales and Vicinamibacterales, and fungal taxa *Cladosporiaceae* and *Plectosphaerella* were scarce in respective forest subtypes both in Oulu and Helsinki.

3.3.3. Bacteria and fungi characterizing different green spaces

To further investigate about the differences in microbial communities between urban forest and built and open green spaces an indicator species analysis was performed (Supplementary Tables S6 and S7). Only the bacterial community of the built green spaces and urban forests in Oulu showed indicative taxa (nr of families >1 in brackets) (Table S6a). Built green spaces had members from four phyla: Actinobacteriota, Chloroflexota, Desulfobacterota, and Bacillota with 16 families (Table S6a). For urban forest only Pseudomonadota (2) were significantly indicative. As in Oulu also in Helsinki, only built and forested spaces had significantly indicative species (Table S6b). For built green spaces Bacteroidota, Bacillota and Methylomirabilota were indicative phyla, and for urban forests phyla Acidobacteriota, Actinobacteriota, candidate division FCP426, Planctomycetota, Pseudomonadota (2), Verrucomicrobiota, and candidate phylum WPS-2. Common indicative bacteria in built spaces of both cities were *Thermoflavimicrobium* belonging to Bacillota, and in forests genus *Acidocella* of the family *Acetobacteriaceae*.

Indicative fungal phyla were found for all three urban green space types in Oulu. The built green space had members only from Ascomycota and Basidiomycota (Supplementary Table S7a). The only representative of open green spaces, the landscape meadow and grazing area O3 subtype, had most indicative fungi in Oulu: Ascomycota (12 families), and Basidiomycota (4), Chytridiomycota (3), Glomeromycota (2), Mucoromycota, Rozellomycota and one uncertain phylum (Table S7a). In urban forests only one Ascomycota was highly significantly indicative. In Helsinki, the highly significantly indicative phyla were in built green spaces: Ascomycota (5), Basidiomycota (3), Blastocladiomycota, Chytridiomycota, Rozellomycota and one uncertain phylum. For open green spaces only Ascomycota (2) were indicative (Table S7b). In urban forests the two large phyla belonged to Ascomycota including 10 families, and Basidiomycota including 9 families (Table S7b). Also, some members of uncertain phylum, Mortierellomycota, Mucoromycota, and Rozellomycota were identified. Common indicative fungi for urban forests in both cities were *Archaeorhizomyces*.

3.3.4. C and N-cycling genes

The *mcrA* and *pmoA* values were very low in both towns varying

between 2.9×10^{-8} to 1.4×10^{-6} and 4.9×10^{-7} to 1.1×10^{-6} , respectively, and therefore no statistics were conducted. The $\delta nrfA$ -*nir* values for the built, open and forest green spaces of both cities were negative (Fig. 6a) indicating denitrification over ammonification for all soils. Urban forest values significantly ($p < 0.05$) differed from the built and open green space values indicating higher ammonification compared to built and open green spaces. The *nir/nosZ*-ratio of the urban forests of both cities was significantly ($p < 0.05$) higher when compared to the built and open green spaces (Fig. 6b) indicating a higher loss of gaseous N as N_2O than N_2 .

3.3.5. Community composition

The bacterial and fungal community composition (β -diversity), as visualized by NMDS, was in both cities clearly different in the urban forests compared to the built and open green spaces (Figs. 7a, 7c and 8a, 8c) whereas in the built and open green spaces the bacterial and fungal community were alike. This later statement was also verified with a data subset from which the forest communities were removed (data not shown). According to PERMANOVA, the green space type explained 42 % ($R^2=0.417$, $p < 0.001$) and 21 % ($R^2=0.214$, $p < 0.001$) of the variation in bacterial community composition data (metagenomic data) and 15 % ($R^2=0.146$, $p < 0.001$) and 10 % ($R^2=0.098$, $p < 0.001$) of the variation in fungal community composition data (OTUs) in Oulu and Helsinki, respectively (Supplementary tables S4b and S4c). According to the pairwise multilevel comparison the total bacterial community differed mostly between urban forest and built green spaces explaining 40 % and 25 % of the variation in Oulu and Helsinki, respectively (Table 2c). For the total fungal community, the comparison showed that OTU composition differed between all main green space types but, like bacteria, mainly differed between urban forests and built open green spaces explaining 13 % and 11 % of the variation in Oulu and Helsinki, respectively (Table 2c).

The PERMANOVA showed that subtypes explained even more of the variation in community compositions in Oulu (for bacteria $R^2 = 0.531$, $p = 0.001$; for fungi $R^2 = 0.248$, $p = 0.001$) and in Helsinki (for bacteria $R^2 = 0.359$, $p = 0.001$; for fungi $R^2 = 0.245$, $p = 0.001$) than the green space type. For both the bacterial and fungal community composition the urban recreational F2 and commercial forest F4 subtypes were the most distinct from the community compositions of the built and open green spaces in Oulu. In Helsinki the recreational forest subtype showed the same clear separation (Figs. S15 and S16).

3.3.6. Relative abundance of fungal guilds

Oulu showed no differences in the relative abundance of saprotrophic fungi between the green spaces, but in Helsinki the urban forests had significantly more saprotrophs than the built green spaces (Fig. 9a). No differences in the relative abundance of saprotrophic fungi were observed between the respective subtypes of each green space type (Supplementary Fig. S17a). The urban forest fungal community exhibited a significantly higher relative abundance of symbiotrophic fungi in both cities, with no differences found among the urban forest subtypes in Oulu or Helsinki (Fig. 9b, S17b). But within the open green spaces in Helsinki the landscape meadow and grazing area O3 subtype had higher abundance of symbiotrophic fungi than the landscape fields O1 subtype (Supplementary Fig. S17b). In both cities the urban forests had significantly lower relative abundances of pathotrophic fungi than were observed in the built and open green spaces (Fig. 9c). Of the green space respective subtypes significant differences could be detected only in Helsinki where the recreational and shelter green space B3 subtype showed higher relative abundance of pathotrophic fungi than measured in representative green space B1 subtype (Supplementary Fig. S17c). Furthermore, from all the other O subtypes pathotrophic fungi were higher in O1 subtype in Helsinki.

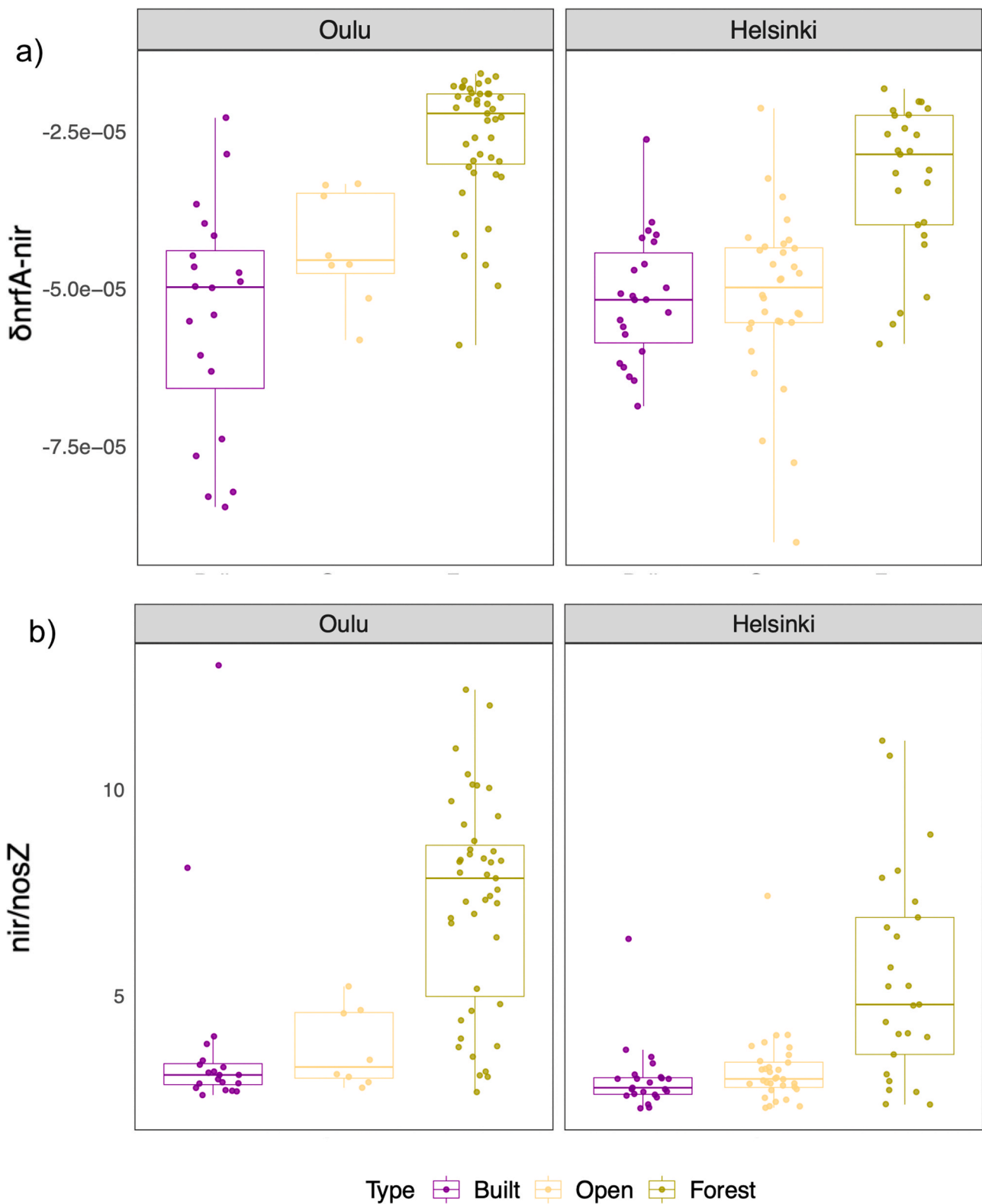


Fig. 6. The $\delta nrfA-nir$ (a) and $nir/nosZ$ -ratio (b) values of the three main green space types built (B), open (O) and urban forests (F) in Oulu and Helsinki. Different letters indicate statistical differences ($p < 0.05$) between types.

3.3.7. Environmental variables affecting the microbial community composition

In both cities, the soil pH indicated positive correlation with the bacterial community composition of the built and open green spaces apart from the urban forests (Figs. 7b and 8b, Supplementary Tables S3a,

S3c). The field vegetation cover (FieldCov), the coverage of grasses (GrassCov), and the coverage of herbs (HerbCov) characterized the bacterial community composition of the built and open green space in both cities, particularly in Oulu. In Oulu also the amount of grass species (GrassN) increased in the built and open green spaces. The cover of

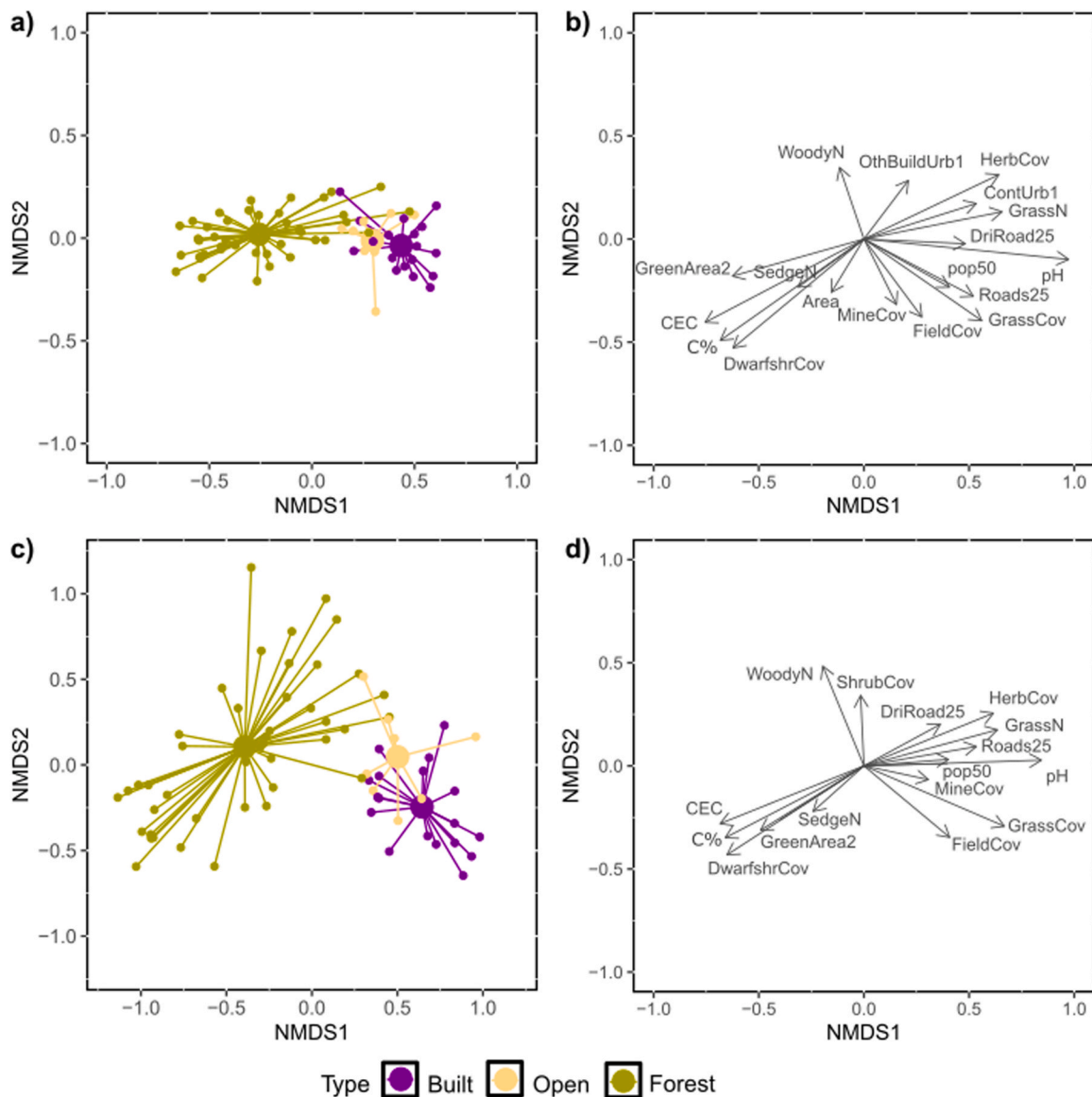


Fig. 7. Non-metric multidimensional scaling (NMDS) analysis showing the variation in a) bacterial and c) fungal community composition between three main green space types built (B), open (O) and urban forests (F) obtained from soil samples in Oulu city region. Vectors are representing some of the significant environmental factors affecting b) bacterial and d) fungal for variation in the ordination ($p < 0.05$). Overlapping factors with similar scores or lower r^2 values are not shown, but they are listed in the [Supplementary Table S3](#). See abbreviations for environmental variables in [Supplementary Table S1](#).

mineral ground (MineCov) was slightly associated with the built and open green spaces in both cities. In both cities, the soil CEC, C%, and the cover of dwarf shrubs (DwarfshrCov) characterized the bacterial community of urban forest soils. In Oulu also the number of sedges (SedgeN), and in Helsinki the number of coniferous tree species (ConTreeN) were slightly associated with the urban forest soil bacterial community. In both cities, the number of woody plant species (WoodyN) was more strongly associated with the forest bacterial community than with the built or open green spaces. Of the vectors symbolizing urbanization, the area of continuous urban fabric (ContUrb1), length (m) of driving roads within 250 m (DriRoad25) and the length (m) of roads within 250 m (Roads25), and to some extent also the area of other built area (such as industrial and commercial units and construction sites) within 1 kilometer (OthBuiltUrb1) and the population size living within 500 m (pop50) were associated with the soil bacterial community of the built and open spaces in Oulu. The OthBuiltUrb1 and pop50 were slightly associated with the soil bacterial community of the built and open spaces also in Helsinki. The total area of the green spaces including all

green space types within 200 m (GreenArea2) characterized the urban forest soil bacterial community in Oulu, whereas the size of the studied green space type (Area) was slightly associated with the urban forest soil bacterial community in Helsinki. In Helsinki, the built and open green spaces were also associated with the number of different green spaces within 100 m (GreenN1).

As with the soil bacterial community the soil fungal community composition of the built and open green spaces of both cities was characterized by pH, the field vegetation cover (FieldCov), the coverage of grasses (GrassCov) and the coverage of herbs (HerbCov) and in the urban forests by CEC, C% and the cover of dwarf shrubs (DwarfshrCov) (Figs. 7d and 8d, [Supplementary Tables S3b, S3b](#)). In both cities the number of woody plant species (WoodyN) indicated more towards the forest fungal community than the built and open one. In Oulu the number of sedges (SedgeN) and in Helsinki the number of ferns (FernN) were slightly associated with the fungal community of urban forests. Shrub coverage (ShrubCov) in Oulu and tree root cover (TreeroCov) in Helsinki) represented the second dimension. The urbanization factors,

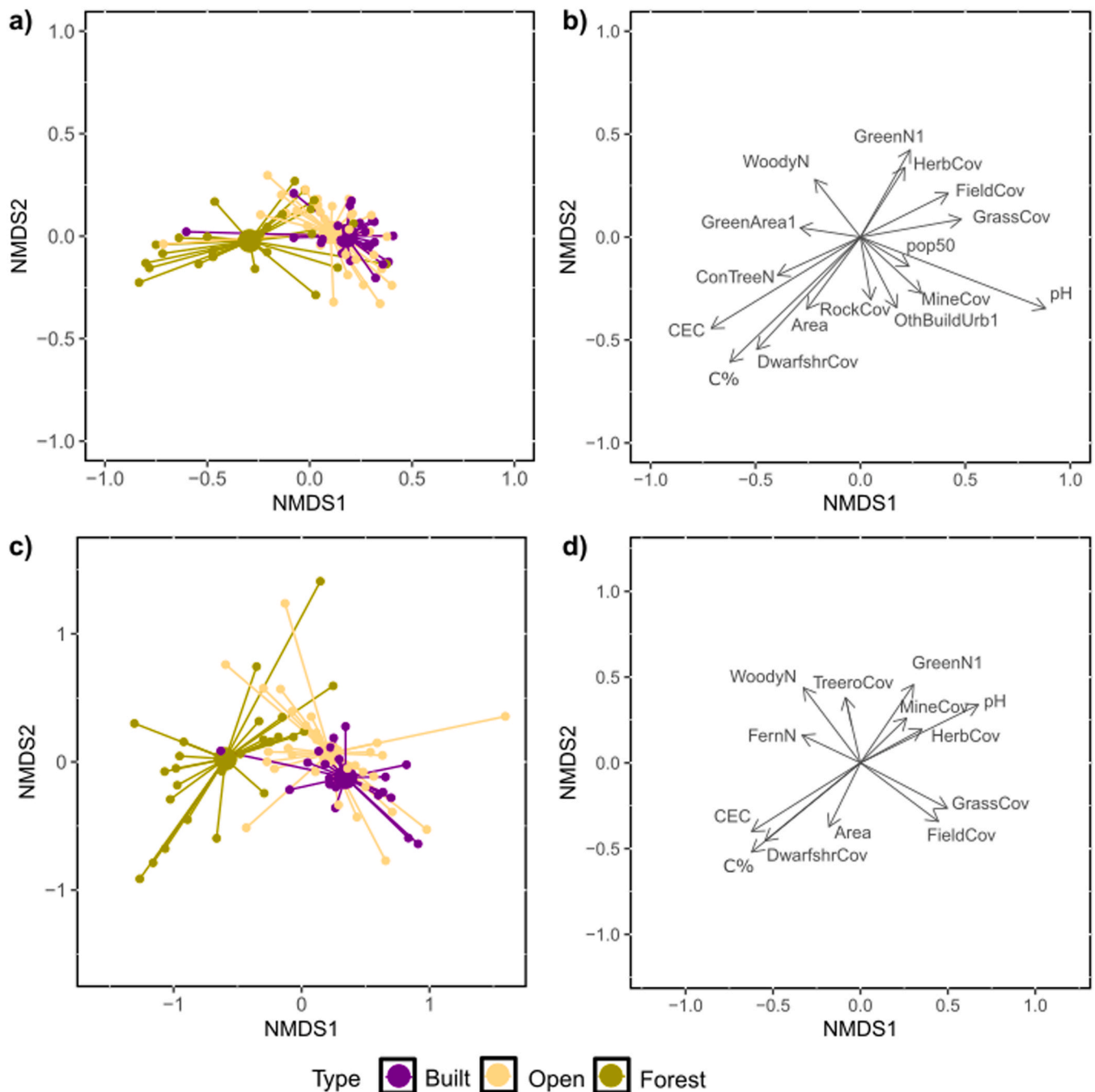


Fig. 8. Non-metric multidimensional scaling (NMDS) analysis showing the variation in a) bacterial and c) fungal community composition between three main green space types built (B), open (O) and urban forests (F) obtained from soil samples in Helsinki city region. Vectors are representing some of the significant environmental factors affecting b) bacterial and d) fungal for variation in the ordination ($p < 0.05$). Overlapping factors with similar scores or lower r^2 values are not shown, but they are listed in the [Supplementary Table S3](#). See abbreviations for environmental variables in [Supplementary Table S1](#).

the length (m) of roads within 250 m (Roads25), and to some extent also length (m) of driving roads within 250 m (DriRoad25) and the population size living within 500 m (pop50) characterized the soil fungal community of built and open green spaces in Oulu. Similarly, as with bacterial communities, the total area of the green spaces including all green space types within 200 m (GreenArea2) characterized the urban forest soil fungal community in Oulu, whereas in Helsinki the size of the green space (Area) was slightly associated with the urban forest soil fungal community and the number of different green spaces within 100 m (GreenN1) with the built and open green spaces.

In Oulu, on the subtype level, both the bacterial and the fungal community compositions were most clearly associated with C%, CEC, the cover of dwarf shrubs (DwarfshrCov), and the total area of the green spaces including all green space types within 200 m (GreenArea2) in the urban recreational F2 and commercial forest F4 subtypes, which were most distinct from the community compositions of the built and open green spaces. In Helsinki, a similar trend was observed, as both the bacterial and the fungal community compositions were most clearly associated with C%, CEC, the cover of dwarf shrubs (DwarfshrCov), and the size of the green space (Area) in the recreational forest subtype.

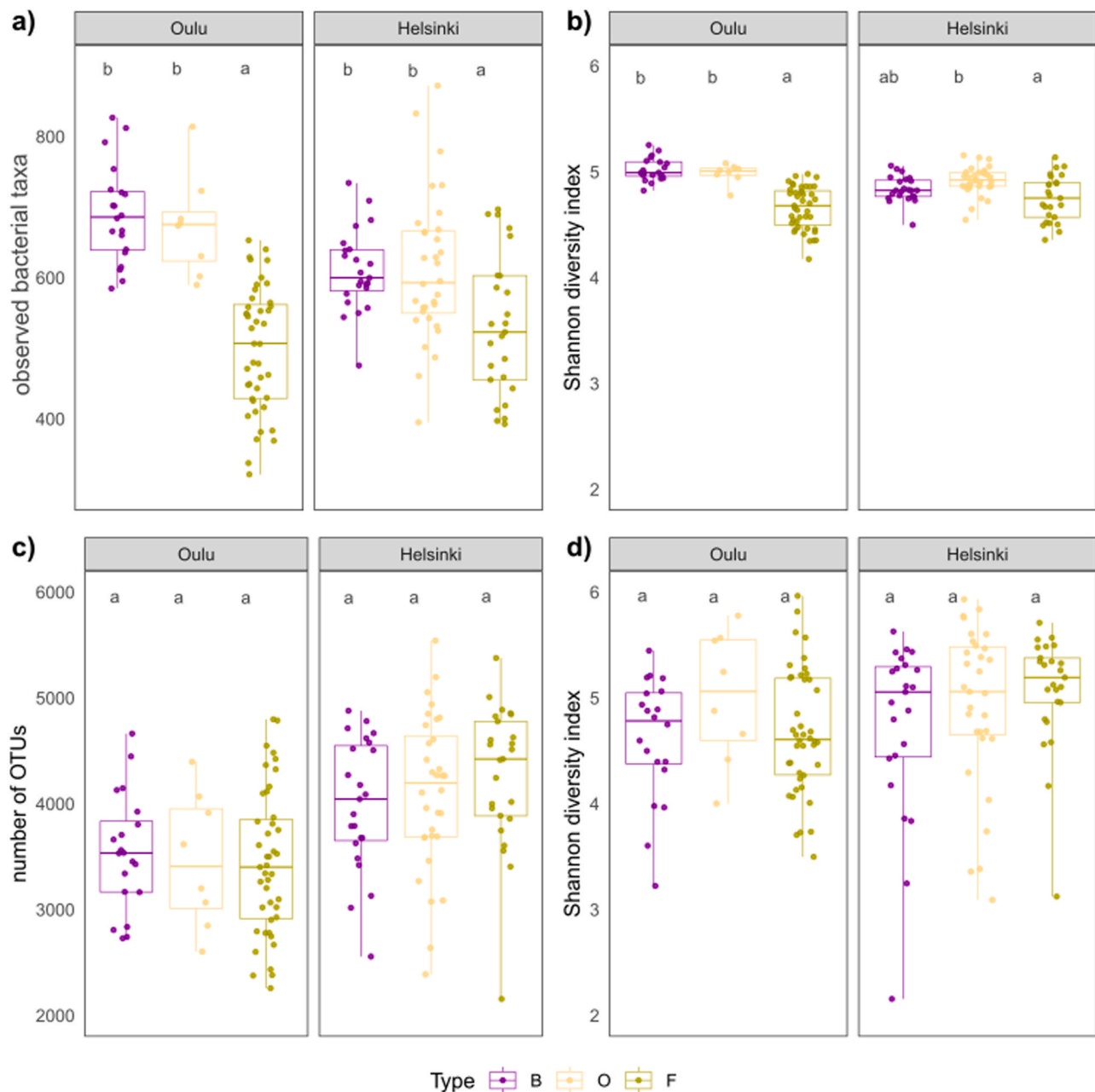


Fig. 9. Relative abundances of the fungal guilds representing potential a) saprotrophic, b) symbiotrophic and c) pathotrophic fungi between three main green space types built (B), open (O) and urban forests (F). Different letters indicate statistical differences ($p < 0.05$) between types.

4. Discussion

It is generally accepted that ecosystem vegetation regulates soil microbial communities through litter deposition and the flow of photosynthetic C from plants into the soil. These processes attract and shape the soil microbiome in relation to the underlying mechanisms. The dominant vegetation, in addition to the parent material, modifies soil pH and organic matter quality, and shapes the composition of soil microbial communities. This also appears to be true for vegetation types in urban green spaces, as in the boreal biome, fungal and bacterial communities in soils under different vegetation types (evergreen tree, deciduous tree, and lawn) in urban parks were compositionally distinct (Hui et al., 2017). Similar patterns have been observed in temperate and tropical urban vegetation types (Zhou and Wang, 2023; Zheng et al., 2024). However, urban green space microbiomes differed from those in control forests (Zheng et al., 2024) likely due to varying soil properties under

the specific vegetation and/or the predominant litter type produced by the plants (Kotze et al., 2021). Thus, our overarching hypothesis was that the complexity of urban green space plant communities controls the soil microbial diversity and function. To test the three more specific hypotheses, various urban green spaces with different history of human influence and management intensities were studied. We sampled green space types commonly found in boreal urban environments: regularly mowed lawns in parks with or without trees and flower beds, which are the dominant form of urban green spaces, especially in temperate regions (e.g. Southon et al., 2017; Norton et al., 2019), open green spaces and meadows, which resulted from past agricultural or pasture practices, or established later to maintain open views, cultural heritage, landscape values and/or biodiversity values, and urban forests, which are remnants of the original soil and vegetation before urbanization but have been altered by human activity.

As expected, urban forest plant communities differed markedly from

other green space types. Urban forests were characterized by a high number of tree, shrub, dwarf shrub, and moss species, and with higher canopy and ground layer cover. Differences between built and open green spaces were smaller, both characterized by high ground vegetation cover and a high number of graminoids. However, open green spaces were more diverse, with many forb and shrub species and a higher total number of plant species (excluding trees). Built green spaces were initially sown with fast-growing plants, mainly grasses, to quickly establish a disturbance-tolerant vegetation. High mowing frequency and trampling in built green spaces appears to prevent the establishment of disturbance-sensitive species, resulting in a more homogenous plant community dominated by graminoids. In contrast, lower management pressure in urban meadows increases the plant species richness and functional groups (Chollet et al., 2018). Increased species richness and vegetation height in meadows may further increase invertebrate diversity and influence the soil microbial communities (Norton et al., 2019).

Although the vegetation analysis separated the three urban green space types into three groups, the hypotheses H1 – the soil bacterial and fungal richness and Shannon diversity are lower in human-built than in open and forested green spaces, and H2 – the soil community composition of the three urban green space types is different from each other, did not hold true. Therefore, the overarching hypothesis did not hold. The bacterial Shannon diversity of the built and open green spaces did not differ significantly from each other but was, unexpectedly, higher than in the urban forests. The bacterial community composition in both cities differed between the urban forests and the built and open green spaces. In Oulu, this separation between forests, built, and open spaces also held for the subtypes, but in Helsinki, the shelter forest (F3) and valued forest (F5) subtypes overlapped with the built and open subtypes. Certain soil chemical characteristics in shelter forests, such as low CEC, were also more similar to those in built and open spaces. It appears that the bacterial communities were more diverse in green spaces where natural soil layers had been disturbed, and new organic material had been introduced. As temperatures are known to be higher in cities compared to the less urbanised areas, it was interesting that a thermophilic *Thermoflavimicrobium* (Goodfellow and Jones, 2015) was found as an indicative bacterial taxon in the built spaces of both cities. For the fungal richness and Shannon diversity measures, the results were even more surprising as they did not differ between urban green space types in either city. The fungal community composition was also similar to the bacterial community in that the urban forests differed from both the built and open green spaces, but there was an overlap of subtypes in Helsinki. Again, the fungal community of the shelter forest subtype resembled the open green space communities, possibly indicating the role of soil physico-chemistry and plant functional types in shaping the urban forest microbiome. The separation of the urban forest fungal community from the other green space communities is mainly due to the increased presence of symbiotic (mycorrhizal) fungi in forest soils, as in temperate and boreal forests, most woody plant species are obligate partners with ectomycorrhizal fungi that provide nutrients (e.g., N) and water to host trees in exchange for photosynthetically fixed C (Smith and Read, 2008). When the forest microbial communities were excluded from the data sets, the microbial communities of the built and open spaces were still similar. This result does not necessarily apply to all urban areas, as differences between private backyards and urban parks have been reported in Tasmania (Grierson et al., 2023). Soil microbial communities are influenced by the degree of urbanization (Zhang et al., 2021; Whitehead et al., 2022) and thus differences in the microbial community structure between urban green spaces may occur when sampling within a small area of the same degree of urbanization.

Soil variables that correlated with changes in the bacterial and fungal community composition between open and forest green spaces in both cities were a decrease in pH and an increase in CEC in urban forests. Soil pH has a strong influence on both bacterial (Lauber et al., 2009) and fungal community composition (Rousk et al., 2010). The increase in soil

CEC in forests is related to the increase in OM (correlating with C%) and acidic pH, which provides negatively charged functional groups of the OM (Ross et al., 2008). Vegetation characteristics of open habitats such as a high cover of grasses, herbs and field layer vegetation cover, separated the soil microbial communities of the built and open green spaces from the urban forest soil community. A notable feature was that coverage of plant functional groups, rather than individual plant species, was often the driving characteristic. Spatial variables indicating a high degree of urbanization (e.g. length of driving roads, area of densely built urban fabric, and to some extent also population density) were associated with the bacterial communities of built and open green spaces, while the size of the green space increased in urban forests. In Oulu, a similar trend was observed in the fungal community, as the length of roads and the population density characterized more built and open green space types, while the size of the studied green space type and the total area of green spaces characterized the forest fungal communities. In Helsinki, the fungal community was not as clearly correlated with the urban structure, although, the increase in area also characterized the urban forests. This is expected, as densely built urban areas tend to have smaller green spaces, such as parks, rather than larger forested areas, which are often further from the urban core. This phenomenon is particularly evident in Oulu, where large forest areas, mainly commercial forests, are located on the outskirts of the city centre. Generally, the urban forests in Oulu were more clearly separated from built and open green spaces in terms of plant, bacterial and fungal communities than in Helsinki. This may be partly explained by the fact that urbanization in Oulu is not yet as strong as in Helsinki. The subtype variation of the soil microbial communities, may reflect the heterogeneity of urban areas which is mentioned as a driving force of urban soil microbiomes (Nugent and Allison, 2022).

The CH₄ cycle involved gene reads were too low and scattered to allow valid data analyses or reflection on the C cycle. However, differences in the microbial community composition are reflected in the potential N cycle processes, supporting H3 – the change in the microbial community is accompanied by a change in the potential ammonification and denitrification process rates as analysed from gene count ratios. All sites showed negative $\delta nrfA-nir$ values, indicating that denitrification dominates over ammonification. The value of the urban forest soils differed significantly from the built and open soils, showing potentially higher ammonification rates. This could mean that more N is immobilized, for example, in microbial biomass in the urban forest soils compared to the built and open green spaces. The *nir/nosZ*-ratio showed that all urban green spaces had the potential to lose N through denitrification, with urban forest soils more so in the form of N₂O than the built and open green spaces. Li et al. (2024) showed that *nrfA* increased in forest soils along an urbanization gradient, but they did not calculate the $\delta nrfA-nir$ values for comparison with our results. However, they did calculate the *NosZ/(nirS + nirK)* ratio, which increased along the urbanization gradient, indicating increased N₂O reduction to N₂ in urban sites. If urbanization is the driving force behind this result, then it also explains our results. However, a better explanation might be the pH difference between our built and open green spaces and the urban forest soil. Shaaban et al. (2023) showed that increasing soil pH increased *nosZ* gene abundance and decreased N₂O emissions. An increase in pH and corresponding N cycle genes in urban green spaces was also observed also by Delgado-Baquerizo et al. (2021).

This study investigated the soil microbiota to which city residents are exposed when passing through or spending time in various urban green spaces. Soils act as a reservoir of microbes to which citizens are exposed through different routes (reviewed in Potter et al., 2023). Outdoors these are mainly aerosols and dust particles and hand-to-mouth behaviour, affecting human skin and gut microflora contributing to the biodiversity hypothesis (Hanski et al., 2012; Roslund et al., 2024). In this respect our results are positive, showing that citizens are exposed to the similar soil microflora in constructed urban green spaces as in more natural open green spaces. While urban forests provide residents with a soil

microbiome similar to that found in built and open green spaces, they also host a distinct soil microbial community. Twenty dominant bacterial and fungal families showed significant differences between urban forests and the other green spaces in both cities. Bacterial families such as *Acetobacteraceae*, *Acidobacteriaceae* and *Mycobacteriaceae*, and the order Acidobacteriales were more abundant in urban forests than in built or open green spaces. This was expected as Acidobacteria include decomposers of polysaccharides such as cellulose, xylan and chitin, which are derived from the woody vegetation that dominates in forests (Ward et al., 2009). In turn, built and open green spaces included the dominant bacteria (e.g. family *Nocardioideae*, group 67–14 of the Solirubrobacterales, group KD4–96 of the order Chloroflexota) which seem to be adapted to anthropogenic disturbances such as heavy metals and complex pollutants (Wegner and Liesack, 2017; Kujala et al., 2018; Huang et al. 2021; Ma et al., 2023). The presence of *Russulaceae*, *Pilodermataceae*, *Myxotrichaceae*, *Hyaloscyphaceae* and *Inocybaceae* supports our finding of symbiotic fungal dominance in urban forest soils. In Oulu we also found significant differences between the fungal families present in the soils of built and open green spaces. This finding may be because Oulu has only landscape meadows and grazing areas (O3) as a representative open green space subtype.

According to Byrd et al. (2018) most of the human skin microbiota belongs to the Kingdom Bacteria, with approximately 75 %, approximately 5–20 % Viruses, and the rest to Eukarya (Fungi). Actinobacteria (36–51 %), Bacillota (24–34 %), Pseudomonadota (syn. Proteobacteria) (11–16 %), and Bacteroidetes (6–9 %) (Carmona-Cruz et al., 2022) are the four major bacterial phyla found on human skin and were all present in all the soils in our study. We are not aware that the bacterial phyla, families, or species, revealed in this study, are directly related to the biodiversity hypothesis, and our results serve future research on this topic. For example, the soil-derived Pseudomonadota community on human skin has been linked to immune regulation (Roslund et al., 2020). According to our results, several genera of *Acetobacteraceae* and Pseudomonadota were indicative of urban forests and were found in all forest subtypes studied. Thus, city residents are associated with Pseudomonadota in all three types of urban green spaces, but in different proportions and probably with a variety of different bacterial representatives of the phylum. *Acetobacteraceae* are acidophilic bacteria and are commonly found in forest soils (Baldrian et al., 2012). Some of them are nitrogen fixers ensuring that atmospheric nitrogen is incorporated into organic matter (Reis and Teixeira, 2015). So far, fungi have not been linked to the biodiversity hypothesis, and thus our results do not provide a deeper insight into this matter. However, human skin has its own mycobiome and by analogy with the animal skin mycobiome, it could be shared between host and habitat (Nguyen and Kalan, 2022). Like bacteria, city residents are associated with different proportions of the same dominant fungal families in different urban green spaces. As the study sites can later be linked to the residential area of the Northern Birth Cohort members whose clinical records are regularly monitored, the next step should be to look for possible connections between human health and the soil microbiome. We are also looking for approaches to identify antibiotic resistance genes, virulence-related genes, and heavy metal resistance genes in this urban soil data set.

5. Conclusion

The soil microbiome plays an essential role in the ecosystem services provided by urban green spaces, such as nutrient cycling, and they can also benefit human health, as recent studies suggest. This study provided new information on soil microbiome communities in built, open and forested green spaces. We found that the bacterial and fungal communities of urban forests differed from those of built green spaces, such as parks, and open green spaces, such as meadows, whereas the microbiome of built and open green spaces were more similar. Bacterial diversity was higher in soils of built and open spaces compared to urban forests, but fungal diversity did not differ.

According to the biodiversity hypothesis humans need contact with diverse environments and this diversity can be achieved in urban environments by maintaining and managing various green space types including urban forests in addition to built and open green spaces. Our results also show that the size of the urban forest has an impact on the microbial community composition and therefore also larger natural forest remnants, where they exist, should be preserved to ensure a diverse contact with environmental microbes. By maintaining the variability of green space types including different built and open green space types, and urban forests, cities can further maintain the overall biological diversity as they increase the diversity of plant species and subsequently other species such as insects, that benefit from them (e.g., Norton et al., 2019).

Residents have different preferences for vegetation in urban green spaces, which can be influenced by park type, with just over half of park visitors suggesting changes (Talal et al., 2021). Many recreational-active and multi-use park visitors wanted more flowers, colour, grasses, mid-growth trees, and food-bearing plants, as well as better vegetation placement, while visitors to natural-passive parks preferred additional removal of invasive/harmful plants. In addition, biodiverse perennial meadows have found to have an aesthetic value and were preferred by residents over herbaceous borders, bedding planting and mown amenity lawns (Southon et al., 2017). These different preferences could be considered in future urban planning, as according to our results, it is possible to design parks with different types of vegetation without altering environmental microbiome contact.

All urban green spaces were found to be potentially denitrifying environments, with genes that convert nitrite to gaseous nitrogen forms, possibly leading to more nitrous oxide (N₂O) in urban forest soils. Our findings indicate the need for greenhouse gas flux measurements combined with metagenomic or transcriptomic analyses in urban green spaces to further investigate the impact of urbanization on soil microbial community functions.

CRedit authorship contribution statement

Leila Korpela: Writing – review & editing, Investigation. **Katja Kangas:** Writing – original draft, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Hannu Fritze:** Writing – original draft, Resources, Project administration, Investigation, Funding acquisition, Conceptualization. **Krista Peltoniemi:** Writing – original draft, Methodology, Formal analysis, Data curation. **Taina Pennanen:** Writing – review & editing, Investigation, Funding acquisition, Conceptualization. **Sannakajsa Velmala:** Writing – original draft, Methodology, Funding acquisition, Formal analysis, Data curation. **Jenni Hultman:** Writing – original draft, Methodology, Formal analysis, Data curation. **Oili Tarvainen:** Writing – review & editing, Investigation, Formal analysis, Data curation. **Jouni Karhu:** Writing – original draft, Methodology, Formal analysis, Data curation. **Marika Laurila:** Writing – review & editing, Investigation.

Declaration of AI and AI-Assisted Technologies in the Writing Process

Use of DeepL's Write and MS Office's Copilot to check spelling, grammar, and punctuation.

Declaration of Competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.ufug.2025.128870](https://doi.org/10.1016/j.ufug.2025.128870).

Data Availability

Data will be openly available from 2026, and until that upon request; the sequence data for this study have been deposited in the European Nucleotide Archive (ENA) at EMBL-EBI under project PRJEB75452, and other used data will become available via Zenodo [10.5281/zenodo.11542875](https://doi.org/10.5281/zenodo.11542875).

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