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## Urban forest soils harbour distinct and more diverse communities of bacteria and fungi compared to less disturbed forest soils

**Running title:** Urban forests harbour diverse soil microbiota

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Accepted Article

## Abstract

Anthropogenic changes to land use drive concomitant changes in biodiversity, including that of the soil microbiota. However, it is not clear how increasing intensity of human disturbance is reflected in the soil microbial communities. To address this issue, we used amplicon sequencing to quantify the microbiota (bacteria and fungi) in the soil of forests ( $n=312$ ) experiencing four different land uses, national parks (set aside for nature conservation), managed (for forestry purposes), suburban (on the border of an urban area) and urban (fully within a town or city), which broadly represent a gradient of anthropogenic disturbance. Alpha diversity of bacteria and fungi increased with increasing levels of anthropogenic disturbance, and was thus highest in urban forest soils and lowest in the national parks. The forest soil microbial communities were structured according to the level of anthropogenic disturbance, with a clear urban signature evident in both bacteria and fungi. Despite notable differences in community composition, there was little change in the predicted functional traits of urban bacteria. By contrast, urban soils exhibited a marked loss of ectomycorrhizal fungi. Soil pH was positively correlated with the level of disturbance, and thus was the strongest predictor of variation in alpha and beta diversity of forest soil communities, indicating a role of soil alkalinity in structuring urban soil microbial communities. Hence, our study shows how the properties of urban forest soils promote an increase in microbial diversity and a change in forest soil microbiota composition.

**Keywords:** Bacteria, Fungi, Biodiversity, Urban, National park, Forest management

## Introduction

An accelerating rate of habitat conversion is a prominent feature of the Anthropocene. This change in landscape typically comes at the cost of degradation and loss of natural habitats and biodiversity (McDonald et al., 2020; Seto et al., 2010). The effects of habitat conversion on the soil are often neglected but are of vital importance given that healthy ecosystem functioning depends on the communities of soil-associated microbes, via processes such as primary production, decomposition, carbon cycling and nutrient mineralisation (Fierer, 2017). Hence, changes in land use might impact microbial biodiversity and ecosystem functions.

Two important drivers of habitat conversion are expansion of urban areas and the increase of natural resource exploitation (Kuipers et al., 2021). To mitigate against habitat loss, land can be set aside, for example, as national parks or urban greenspaces, to provide putative benefits of recreational use (Li et al., 2021; Marselle et al., 2021; Siikamäki et al., 2015), climate regulation (Mexia et al., 2018), and/or acting as biodiversity refugia (Lehmann, 2021; Mills et al., 2017; Siikamäki et al., 2015). Of course, the emphasis on type of land use differs among national parks and urban greenspaces, with the former areas primarily directed towards biodiversity conservation (Siikamäki et al., 2015) and the latter more towards recreational use and human health/well-being (Li et al., 2021). Inevitably, urban greenspaces also experience greater anthropogenic impacts through direct use and by virtue of being located within or adjacent to an urban area. Indeed, it is, for example, known that not all (macro)species can tolerate or adapt to life in an urban area (Faeth et al., 2011; Parsons et al., 2018; Spotswood et al., 2021) and that the (macro)biodiversity of urban habitats typically differs from that in more natural areas (*e.g.* national parks). Such changes in assemblages

associated with cities might spread to adjacent suburban areas to create a gradient of biodiversity and associated ecosystem services (Spotswood et al., 2021). Outside urban areas, intensive forest management practices, such as clear-cutting, cause a disturbance that degrades forests and impacts various groups of biodiversity (Fisher & Wilkinson, 2005; García-Tejero et al., 2018; Thompson et al., 2013). Even after decades of recovery, certain managed forests can harbour different communities of (macro)species when compared to more pristine forests (Fisher & Wilkinson, 2005; García-Tejero et al., 2018; Thompson et al., 2013).

Urban habitats are also associated with a change in the composition of bacterial and fungal soil microbiota. In soil bacteria, changes manifest in an increased alpha diversity (Hui et al., 2017; Naylo et al., 2019; Tan et al., 2019) and an apparent lack of convergence (*i.e.* process that makes communities become more similar) in the bacterial community composition (Schmidt et al., 2017). By contrast, there is a lack of consensus about the differences among soil fungal communities in urban and natural areas, with studies reporting either negative (Abrego et al., 2020; Andrew et al., 2019) or neutral (Tan et al., 2019; Tedersoo et al., 2020) impacts of urban habitat on fungal diversity. Nonetheless, Schmidt et al. (2017) reported convergence of fungal communities within cities, but only when natural reference sites were compared with construction sites within the urban matrix. Such changes in fungal soil microbiota were associated with the loss of ectomycorrhizal taxa (ECM) in cities (Schmidt et al., 2017), supporting the idea of fungal convergence in urban areas due to the loss of sensitive species (McKinney, 2006). Also, forest management outside cities is thought to have a weak positive effect on the divergence (*i.e.* process that makes communities become more dissimilar or dispersed) of bacterial and fungal communities (Lee-Cruz et al., 2013).

However, the impacts of anthropogenic disturbance upon the soil microbiota in urban forests are expected to outweigh those in managed forests (Lee-Cruz et al., 2013; Lee & Eo, 2020).

A key limitation with many studies that have attempted to quantify effects of urban land use on soil microbial community composition is that land use is either ambiguously defined or confounded with habitat type. For example, studies examining urban soil microbiota do not clearly describe the habitat type (such as whether the soils were grassland or forest) (Andrew et al., 2019; Pouyat et al., 2015; Schmidt et al., 2017; Tedersoo et al., 2020), or the habitat types differ between the urban and non-urban sample locations (such as sampling gardens and parkland within urban areas and sampling forests outside urban areas) (Abrego et al., 2020; Hui et al., 2017; Tan et al., 2019; H. Wang et al., 2018). Sampling comparable habitats is an important issue to consider given the general association between habitat type and the composition of soil microbiota (Baruch et al., 2021, 2020; Hui et al., 2017; Mills et al., 2020).

In this study, we used amplicon sequencing to examine the impacts of anthropogenic disturbance on the forest soil microbiota (bacteria and fungi) where we specifically define disturbance as human induced changes to the environment that affect the natural structure of ecosystems, including that of microbial communities (adapted from Sergio et al., 2018). To capture the multifactorial nature of anthropogenic impacts, we first calculated a standardised geospatial index - the Human Influence Index (HII, Wildlife Conservation Society et al. 2015) under the assumption that greater proximity to humans and their built settlements associates with a higher degree of chronic disturbance to natural systems (Arnan et al., 2018). The HII differentiates among three forest types (Table S1, Fig. S1): urban forests, suburban forests, and forests located away from the built environment. Because the HII does not

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account for the effects of commercial forestry (Danneyrolles et al., 2019), we further partitioned the latter category of forests into either managed forests or national parks. As protected areas, forests in national parks have some of the lowest levels of chronic anthropogenic disturbance and forest management that is possible to find in Northern Europe. National Park samples thus serve as an ideal contrast to quantify possible legacy effects of commercial forestry (Hartmann et al., 2013) and to examine effects of biodiversity conservation (Siikamäki et al., 2015) independently from urban impacts. Hence, here we combined quantitative and qualitative information to make a distinction between four levels of human induced forest disturbance which broadly represent a gradient: (1) urban forests (areas located entirely within an urban area), (2) suburban forests (commercially managed forests that are adjacent to urban areas), (3) managed forests (commercially managed forests located away from the built environment), and (4) national parks (unmanaged and protected forests away from the built environment). To the best of our knowledge, no study has placed the urban soil microbiota in a wider context of extensive nature conservation areas with long-term protection status such as national parks.

Building upon results of previous studies on soil microbiota, we hypothesised that (1) proximity to urban areas will increase the alpha diversity of soil bacteria (Hui et al., 2017) while forestry practices will have little long-term impact (Lee-Cruz et al., 2013). In contrast, we did not expect to find clear associations between the fungal alpha diversity and the level of forest disturbance (Tedersoo et al., 2020). Additionally, we predicted to find (2) distinct soil microbiota profiles between forests of different disturbance levels, where the dispersion in beta diversity changes between soil fungal communities but not between soil bacterial communities (Schmidt et al., 2017). Specifically, we expected to see greater dissimilarities between communities in suburban and managed forests in comparison to communities in



cities and national parks for soil fungi. Finally, we predicted that changes in community composition would elicit (3) distinct functional traits in soil microbiota from forests that differ in disturbance levels, with a noticeable decline of ECM fungi in urban soils.

## Material and Methods

### *Study sites*

As explained in the Introduction, we studied four forest groups of land use that differ in their level of anthropogenic disturbance: (1) urban forests, (2) suburban commercially managed forests, (3) non-urban commercially managed forests, and (4) natural forests located in national parks. We note here that although urban forests were not used for commercial harvest, they are not exempt from low impact management practices. All soil samples (total  $n=312$ , urban  $n=47$ , suburban  $n=48$ , managed forest  $n=112$ , national park  $n=105$ ) were collected from twenty sample locations (3 urban and 3 suburban forests, 7 managed forests and 7 national parks, Fig. 1) where each sample location was represented by multiple soil replicates (12 to 22 soil samples per sample location) (see Table S2-S3 for metadata). All soil samples were collected in a period of four weeks in July-August 2019. Urban and suburban forest sites were located within and around three Finnish cities: Jyväskylä, Kuopio and Mikkeli (Fig. 1). All urban forest sites were at least 500 m<sup>2</sup> in area and enclosed by houses and roads. The suburban forest sites were located on the periphery of the urban areas with a minimum distance of 200 m to the nearest detached house and at least 500 m from multi-house settlements. The distance between managed forest sites and their corresponding protected forest sites within national parks ranged from 5 to 19 km. All forest sites were located within the boreal forest zone, with habitats dominated by Norway spruce (*Picea abies*), Scots pine (*Pinus sylvestris*) and silver and downy birch (*Betula pendula* and *B.*

*pubescens*), with bilberry (*Vaccinium myrtillus*) and lingonberry (*V. vitis-idaea*) as undergrowth.

Twenty-three structural habitat factors were quantified for 178 sites during our habitat survey at the time of soil sampling using a method similar to that described by Ecke et al. (2002) (see Table S2-S3). These data aimed to describe the biotic and abiotic properties of the habitat using categorical or numerical scales, including the above ground vegetation (ferns, grass, lichens), abundance of coarse and fine woody debris and stones. To examine the levels of anthropogenic habitat disturbance in our sampling sites, we have calculated the Human Influence Index (HII) for each sample location using the data from the Global Human Influence Index Dataset (Wildlife Conservation Society et al. 2015) in ArcGIS v.10.8.1 software. HII summarises nine data layers, including population density, land use/land cover, built environment, roads, railroads, and other factors reflecting anthropogenic habitat disturbance. The HII differentiates between three out of four forest types with HII levels being the highest in urban areas, intermediate in suburban forests and lowest in managed forests ( $p < 0.01$ , for details on statistics see the Methods section, Table S1, Fig. S1). In contrast, managed forests and national parks had similar values for HII.

#### *Sample collection and processing*

At each sampling site, soil samples (total of ~30 g) were collected at a depth of 10 cm below the ground surface using a metal core instrument (diameter=3 cm). The precise sample location was picked at random in the sparsely vegetated part of the forest patch (away from roads, forest paths, big trees and/or other dense vegetation). After discarding the upper leaf litter layer and the lower mineral layer, the intermediate organic soil layer was sealed into a sterile plastic bag and mixed thoroughly. When necessary, several cores were taken next to

each other to provide enough material per sampling site. Samples were collected wearing gloves and all the equipment was surface sterilised with ethanol before use. All the samples were immediately put on dry ice and stored at -80°C until further processing.

The total genomic DNA was extracted from 100-200 mg of soil homogenate ( $n=312$ ) using the Qiagen DNeasy PowerSoil Pro Kit following the manufacturer's instructions. Measures were taken to avoid contamination (*i.e.* working under a laminar flow hood, sterilisation of surfaces and tools by UV light, usage of sterile filter tips and plastic ware (according to (Eisenhofer et al., 2019))). Soil samples from different study sites were processed in a random order to avoid any systematic bias and possible batch effects. Negative controls containing sterile water ('blanks') were included during DNA extraction. The remaining soil of each original sample ( $n=306$ ) was oven dried at 38°C for at least 72h. Ten grams of dried soil were diluted in deionised water (1:3, soil to water ratio) and mixed thoroughly using a shaker platform for 1 h prior to measuring pH using a combination pH electrode (Mettler Toledo, InLab® Expert Go, Vantaa, Finland).

#### *Amplicon sequencing and read data processing*

The DNA samples were amplified and sequenced using an Illumina HiSeq at the Beijing Genomics Institute (BGI, <https://www.bgi.com/global/>). Briefly, the 515F/806R (Caporaso et al., 2011) and the ITS3/ITS4 (White et al., 1990) primer pairs were used to amplify the V4 region of the 16S ribosomal RNA (rRNA) gene in bacteria (with 250 bp paired-end (PE) reads), and the ITS2 region in fungi (with 300 bp PE reads) The PE reads were demultiplexed by BGI before being processed with QIIME2 v.2020.8 (Bolyen et al., 2019). We used CUTADAPT (Martin, 2011) to remove adaptor sequences and any resulting short reads. The DADA2 plugin (Callahan et al., 2016) was used to trim primers, truncate the 3' end of the

reads when the median quality score dropped below 39 for bacterial reads and 35 for fungal reads (forward and reverse reads at 227 bp for bacteria, reverse read at 257 bp for fungi), merge reads, filter out potential chimeric sequences with the consensus chimera detection method, and to call amplicon sequence variants (ASVs) using default parameters in QIIME2. We removed ASVs that did not get a taxonomic designation, and the ASVs that were classified as archaea, mitochondria, or chloroplasts. Next, we filtered the low frequency (<10 reads) ASVs from the dataset. After filtering, a total of 27 815 087 reads (48 797-233 051 reads/sample) and 40 924 ASVs were recovered for soil bacteria, and 35 829 831 reads (44 457-292 035 reads/sample) and 18 894 ASVs were recovered for soil fungi. The final ASV feature-tables were generated after rarefaction (Weiss et al., 2017) to 48 979 and 44 457 reads per sample in bacterial and fungal datasets, respectively, and were used for analyses unless stated otherwise.

The Naive Bayes classifiers (Bokulich et al., 2018) were trained to assign taxonomy to representative sequences using the V4 region of the 16S rRNA gene (matching the 515F/806R primers) of the SILVA database v.138 for bacterial ASVs (Quast et al., 2012), and the full-length ITS region from the UNITE v.8.0 database for fungi (Nilsson et al., 2019). Both bacterial and fungal reference sequences were clustered at 99% sequence similarity threshold. We parsed the ASVs through FAPROTAX v.1.2.4 (Louca et al., 2016) and FUNGUILD v.1.1 (Nguyen et al., 2016) to infer diversity of functional traits of bacteria and fungi, respectively. FAPROTAX assigned functional traits to 9 251 out of 40 726 bacterial ASVs (22.72%), while FUNGUILD assigned fungal functional traits to 6 723 out of 18 735 ASVs (35.88%) with a confidence level of 'Probable' or 'Highly Probable'.

## Statistical analyses

The soil microbiota alpha diversity was estimated using three different metrics (ASV richness (Fig. 2), Shannon diversity index (referred to as Shannon diversity for clarity), and Faith's Phylogenetic diversity (Fig. S2)) in QIIME2. Phylogenetic diversity was not calculated for fungi as the ITS2 region evolves too rapidly to be useful for phylogeny-based analyses (Nilsson et al., 2008). Changes in alpha diversity (and Human Influence Indices) between different forest disturbance levels were assessed using the Kruskal-Wallis (KW) tests, with a Benjamini-Hochberg adjustment for multiple comparisons using the DUNN.TEST v.1.3.5 package (Dinno, 2015) in R v.4.0.2 (R Core Team, 2020).

The soil microbiota beta diversity for each forest disturbance level was visualised by the Principal Coordinate Analysis (PCoA), based on Bray-Curtis (Fig. S3) and Jaccard (Fig. S4) distances generated by the PHYLOSEQ v.1.34.0 package in R (McMurdie & Holmes, 2013). In order to verify potential bias due to geolocation (latitudinal or longitudinal clines), we analysed a subset of samples ( $n=195$ ) that included all urban and suburban samples but only the three nearest (in relation to cities) pairs of national parks and managed forests (Konnevesi, Pyhä-häkki and Leivonmäki; Fig. 1). The Bray-Curtis distances were recalculated for this subset of samples and the spread of variance was assessed by the *betadisper* (Fig. 3) and *permutest* ( $n$  permutations=9 999) functions in VEGAN v.2.5-7 (Oksanen et al., 2020). PERMANOVA tests implemented by the *adonis2* and *pairwise.adonis2* functions in VEGAN ( $n$  permutations=9 999) were then used to examine whether forests with different disturbance levels differ significantly in terms of sample grouping and to determine if disturbance level had a higher explanatory value ( $R^2$ ) than location *per se* (*i.e.* latitude and longitude). The prerequisite of dispersion homogeneity for

the *adonis2* function was not met (*i.e.* the within-group variation differed between the four disturbance levels which could confound the results). However, our results are still valid since we analysed a subset of samples with a balanced design (Anderson & Walsh, 2013). To further examine whether geographical distance between soil samples may explain variation in the beta diversity patterns, we fitted bacterial and fungal distance-decay models per disturbance level with a negative exponential function by implementing the *decay.model* function using the BETAPART v.1.5.6 R package ( $n$  permutations = 9 999) with both the Bray-Curtis and Jaccard metrics (Baselga et al., 2022).

Differential abundance of bacterial and fungal ASVs (from the most abundant phyla with >0.01 relative abundance only) between forest disturbance levels was calculated using the *deseq* function in DESEQ2 v.1.30.1 (Love et al., 2014) using the unrarefied feature-tables (Fig. 4). We also calculated the relative proportions of ASVs grouped at the phylum level to identify significant differences in proportions of the most abundant phyla (with >0.01 relative abundance) between forest groups. This was done by first constructing General Linear Models with a quasibinomial distribution and then applying the Tukey's multiple comparison test with the *glht* function in the MULTCOMP v.1.4.20 R package (Hothorn et al., 2008). Successfully assigned bacterial (FAPROTAX) and fungal functional traits (FUNGUILD) were converted to relative abundances, with low relative abundance traits (<0.05 for all four disturbance levels) removed to aid plotting (Fig. 5). Differences in the relative abundance of these most abundant functional traits between the four forest groups were examined with the same statistical methods as for taxonomical proportions.

To examine how environmental variables (23 structural habitat factors and soil pH, Table S2-S3) correlate with alpha and beta diversity, we first assessed their collinearity. We used the Spearman's rank correlation coefficient in R to identify correlations involving ranked categorical variables, whereas the Pearson's correlation coefficient was used for correlations between numerical variables (Table S4). To identify variables with the strongest impact on the community structure, we used Constrained Analysis of Principal Coordinates (CAP) through the *ordinate* function within the PHYLOSEQ v.1.34.0 R package (Fig. 6, Fig. S5) and extracted the values from the first CAP axis (Table S5). Correlated variables with little impact on community structure were removed, and six remaining structural habitat factors (grass, boulders, fine woody debris, stumps, lichens and shrubs for bacteria; stone holes instead of boulders for the fungal analyses; Table S2-S3) and soil pH were included in the subsequent analyses. For the analyses of alpha diversity, we verified that the linear model had a Variance Inflation Factor that was lower than 2 for all these selected variables (Johnston et al., 2017). The alpha diversity model including these seven variables was inserted into the model selection tool provided by the *dredge* function within the MUMIN v.1.43.17 R package (Barton, 2020) and was based on the subset of the samples for which environmental data were available ( $n=178$ ). The most parsimonious model that was within two AIC (Akaike Information Criterion) units of the model with the lowest AIC value was considered the best model. Additionally, either Spearman's rank correlations (involving categorical variables) or Pearson's correlations were used (involving continuous variables) to test for significant associations between the variables in the final model and soil microbiota alpha diversity (Fig. 6). We also simultaneously examined the effects of the seven environmental variables selected above on the soil microbiota beta diversity (Bray-Curtis dissimilarity,  $n=178$ ) using a PERMANOVA test implemented by the *adonis2* function in VEGAN v.2.5-7 ( $n$  permutations=9 999).

Given a well-established association between pH and soil microbiota (Fierer & Jackson, 2006), we have examined the relative importance of soil pH and habitat disturbance (*i.e.* Human Influence Index) in explaining the variation in alpha and beta diversity. We compared the Pearson's correlation coefficients for soil microbiota alpha diversity. For beta diversity, we ran PERMANOVA tests with the *adonis* function to compare the amount of variation ( $R^2$ ) explained by either the soil pH or the Human Influence Index in separate models. These analyses were run on two datasets: (1) on all soil samples with pH data ( $n = 306$ ), and (2) on a subset of soil samples from two contrasting habitat disturbance levels that had comparable levels of soil pH ( $n = 50$ ). Specifically, we selected the 25 soil samples collected from managed forests with the highest pH (range: 4.34 - 5.08) and the 25 soil samples from urban forests with the lowest pH (range: 3.52 - 5.01) (a comparable subset of national parks did not result in equivalent levels of soil pH with urban soil samples). The comparable levels of soil pH between the two forest groups were confirmed by a non-significant *wilcox.test* ( $p > 0.05$ ). Thus, this smaller dataset allowed us to examine the effects of habitat disturbance in isolation from the effects of soil pH.

## Results

### *Forest soil microbiota alpha diversity is positively associated with anthropogenic disturbance*

The level of alpha diversity (*e.g.* ASV richness) present in forest soil samples varied greatly between bacteria and fungi with the former having a range of 504-3 078 ASVs and the latter having a range of 101-966 ASVs. For both bacteria and fungi, we found that the forest disturbance level was an important explanatory factor for the variation in soil microbiota alpha diversity (*e.g.* ASV richness:  $\chi^2=74.5207$ ,  $df=3$ ,  $p < 0.001$  for bacteria;  $\chi^2=76.5905$ ,



*df*=3,  $p < 0.001$  for fungi). Specifically, we found that all three alpha diversity metrics differed significantly between forest groups ( $p < 0.02$ , KW, Table S6), except between the forests in national parks and managed areas, and between urban and suburban forests for fungal ASV richness ( $p > 0.025$ , KW, Table S6). Interestingly, alpha diversity of both bacterial and fungal soil microbiota was lowest in the national parks and managed forests, intermediate in suburban forests, and highest in urban forests (Fig. 2, Fig. S2, Table S6). Urban forest soil samples had on average 58% more bacterial and 57% more fungal ASVs than the soil samples from national parks.

#### *Urban forest soil microbiota communities look alike irrespective of geolocation*

Both bacterial and fungal soil microbiota were separated principally along the first PCoA axis, but it explained a greater amount of variation for bacteria (17.2%) than for fungi (4.5%) (Fig. S3-S4). Urban soil samples formed a separate cluster with distinct sample grouping in the PCoA ordinations (Fig. S3-S4). In contrast, the soil samples from national parks and managed forests tend to overlap considerably in the ordination space. The importance of anthropogenic disturbance level was greater than that of geolocation, which had little notable impact on variation in beta diversity (Table S7). For example, disturbance level explained almost 10% of the variation in bacterial beta diversity ( $F=6.865$ ,  $R^2=0.094$ ,  $p < 0.001$ , based on Bray-Curtis) which is six times greater than the explanatory power of latitude ( $F=3.28$ ,  $R^2=0.015$ ,  $p < 0.001$ ), while longitude did not explain a statistically significant portion of variation. Similarly, 4% of the variation in beta diversity of forest soil fungal microbiota was explained by the habitat disturbance level ( $F=2.767$ ,  $R^2=0.041$ ,  $p < 0.001$ , based on Bray-Curtis) which is five times more than the variation explained by latitude ( $F=1.552$ ,  $R^2=0.008$ ,  $p < 0.002$ ) and almost seven times more than the variation explained by longitude ( $F=1.221$ ,

$R^2=0.006$ ,  $p<0.05$ ). Similar patterns were observed when analyses were run based on the Jaccard distance metric (Table S7, Fig. S4).

Differences in dispersion of soil samples between the forest groups was found for both bacteria ( $F=8.562$ ,  $p<0.001$ , based on Bray-Curtis) and fungi ( $F=5.375$ ,  $p<0.002$ , Bray-Curtis). Permutation tests with pairwise comparisons revealed that dispersion in bacterial communities was significantly higher between soil samples from urban forests (in comparison to all the other forest groups (Fig. 3, Table S7). In case of fungi, the highest levels of dispersion were found for soil samples from national parks (Fig. 3, Table S7). This pattern differed significantly from the dispersion of soil samples from managed and suburban forests ( $p<0.002$ ) but not in comparison to the dispersion of soil samples from urban forests ( $p>0.05$ ).

Additionally, we found evidence of a significant increase in assemblage dissimilarity occurring with greater distance separating samples for bacteria and fungi ( $p<0.05$  for all disturbance levels, except urban fungi). The strength of the slopes representing this relationship gradually decreased with anthropogenic disturbance (steepest slopes were found in national parks for bacteria and fungi, Fig. S6-S7, Table S8). However, the strength of all slopes can be considered very weak (all slopes  $<3.10^{-6}$ ) making microbial communities located further apart only slightly more dissimilar than nearby located microbial communities.

#### *Impacts of anthropogenic disturbance on forest soil microbiota composition*

Five phyla of bacteria (Acidobacteriota, Proteobacteria, Planctomycetota, Actinobacteriota and Verrucomicrobiota) and two phyla of fungi (Ascomycota, Basidiomycota) comprised ~80-90% of the total microbial community. Soil microbiota composition varied markedly

among forest disturbance levels, with greater taxonomic changes observed in bacteria than in fungi. The proportions of all five dominant bacterial phyla differed significantly between the two most contrasting forest groups (national parks and urban forests), supporting the observed variation in beta diversity (Fig. 4A, Fig. 4C, Table S9). For example, when compared to national parks, the urban soil comprised relatively less Acidobacteriota (-37.3%) and Planctomycetota (-25.4%) and relatively more Actinobacteriota (+37.8%), Proteobacteria (+23%) and Verrucomicrobiota (+81.5%). Interestingly, suburban forest soils reflected a composition of bacterial phyla that is intermediate to the soils of natural forests and urban forests, thus reinforcing the patterns observed for alpha and beta diversity.

The frequencies of 1 306 bacterial ASVs represented by a total of 13 174 114 sequences were significantly different between national parks and urban forests (3.2% of the total 40 924 non-rarified ASVs and 47.36% of the total 27 815 287 non-rarified reads), with the majority of these ASVs assigned to one of the five dominant phyla, and thus driving the inter-phyla differences between national parks and urban forests (Fig. 4B, Fig. 4D). The orders belonging to Acidobacteriota and Planctomycetota that experienced the greatest reduction in urban areas were Acidobacteriales (Acidobacteriota) and Isosphaerales (Planctomycetota).

Simultaneously, the orders belonging to Actinobacteria, Proteobacteria and Verrucomicrobiota that had the highest gains in urban areas were Gaiellales, Microtrichales and Solirubrobacterales (Actinobacteria), Burkholderiales and Rhizobiales (Proteobacteria) and Chthoniobacterales (Verrucomicrobiota) (Table S10).

In contrast, the two dominant fungal phyla had no significant changes in their proportion between national parks and urban forests although there was a trend towards a decline in proportion of Basidiomycota (-11.7%) in urban forests (Fig. 4A, Fig. 4C, Table S9).

Nonetheless, we identified 325 fungal ASVs represented by a total of 10 528 620 sequences that were differentially abundant between national parks and urban forests (1.7% of the total non-rarified 18 894 ASVs and 29.39% of the total 35 829 831 non-rarified reads), and 80 ASVs were assigned to the phylum Basidiomycota (Fig. 4B, Fig. 4D). The orders of Basidiomycota that experienced the highest reduction in urban areas were Agaricales, Atheliales and Russulales (Table S10).

### *Impacts of urban forests on the composition of functional traits associated with soil microbes*

The five most common functional traits of bacterial communities (>5% relative abundance for at least one of the four disturbance levels) were aerobic chemotrophs, chemotrophs, intracellular parasites, animal symbionts and cellulolytic species whereas the two chemotrophic groups account for ~60% of the present functional traits (Fig. 5). The fungal functional traits were dominated by ECM (ranging from 47 to 67%), with the other most common traits being either saprotrophs and/or endophytes (Fig. 5). The relative proportion of the five most abundant predicted functional traits showed more apparent changes for fungi than for bacteria when compared between different forest groups (Fig. 5, Table S11). For clarity, only the forest groups with the highest difference in disturbance level (*i.e.* national parks and urban forests) will be further discussed in details. Urban soils had a significantly lower proportion of cellulolytic bacteria (-25.3%) and intracellular parasites (-60.41%) than soil from national parks. Urban soil fungal microbiota had significantly less ECM (-27.84%) and ECM-endophytes (-69.6%), whereas the proportions of endophytes–litter saprotrophs (+84.9%) and undefined saprotrophs (+152.6%) were significantly increased in comparison to national parks (Table S11).

## *Impacts of environmental factors on soil microbiota alpha and beta diversity*

Much of the variation in the alpha and beta diversity of bacteria and fungi was associated with changes in the soil pH. Although this was true for both bacteria and fungi, the effect of soil pH was much stronger for bacteria than for fungi (Fig. 6, Fig. S8, Table S12-13).

Soil pH was the sole explanatory factor for bacterial ASV richness and bacterial Shannon diversity (Table S12). The best models explaining fungal ASV richness included pH and the abundance of shrubs while pH, the abundance of shrubs and the amount of stumps determined the fungal Shannon diversity (Table S12). Using Spearman's and Pearson's correlations, we found that the soil pH is the only significant variable correlating with the fungal ASV richness and the fungal Shannon diversity, making the effect of the other explanatory variables (*i.e.* shrub abundance and amount of stumps) negligible. More specifically, the soil pH and all studied alpha diversity metrics are characterised by a strong positive correlation for both bacteria (*e.g.* ASV richness:  $r=0.73$ ) and fungi (ASV richness:  $r=0.61$ ) (Fig. 6A, Fig. 6C, Table. S13).

In terms of explaining the observed variation in beta diversity for both bacteria and fungi, soil pH also appeared to be the most important environmental variable. About 16% of the total variation in the bacterial beta diversity could predominantly be explained by soil pH ( $F=29.3185$ ,  $R^2=0.136$ ,  $p<0.001$ , based on Bray-Curtis), and less by the abundance of shrubs ( $F=1.421$ ,  $R^2=0.026$ ,  $p<0.05$ , Bray-Curtis) (Table S13). In contrast to bacteria, only ~6% of variation in fungal beta diversity was explained by the combination of soil pH ( $F=5.758$ ,  $R^2=0.031$ ,  $p<0.001$ , Bray-Curtis) and the abundance of grass ( $F=1.175$ ,  $R^2=0.025$ ,  $p<0.01$ , Bray-Curtis) (Table S13). These patterns were consistent when analyses were run based on the Jaccard distance metric (Table S13).

Notably, some environmental factors used in the analyses were confounded within the disturbance level. For example, soil pH showed consistently higher alkaline levels in forest areas with higher levels of anthropogenic disturbance (Fig. 6B, Fig. 6D). This pattern was further confirmed by a strong positive correlation between pH and the Human Influence Index ( $r=0.65$ , Fig. S9). For the complete dataset ( $n=306$ ), we found that these two variables have comparable explanatory power in terms of explaining alpha diversity in fungi and beta diversity in both bacteria and fungi (although the overall explained variation is ~4 times higher for bacteria in comparison to fungi) (Table S14). In contrast, bacterial alpha diversity correlated more strongly with soil pH ( $r=0.70$  for ASV Richness) than with the Human Influence Index ( $r=0.54$  for ASV richness) (Table S14). We found the same trend with our smaller pre-selected dataset ( $n=50$ ) where the two variables explained the beta diversity within bacteria and fungi more or less equally. Interestingly, the bacterial alpha diversity correlated more strongly with soil pH ( $r=0.39$  for ASV richness) while the fungal alpha diversity was more influenced by the Human Influence Index ( $r=0.25$  for ASV richness) (Table S15).

## Discussion

Forest soil microbes are important as they are essential for proper ecosystem functioning (Fierer, 2017). Here, we used amplicon sequencing to characterise bacterial and fungal communities in forest soils that differ in their level of anthropogenic disturbance (national parks, managed, suburban and urban forests). In accordance with our hypotheses, we found that (1) the alpha diversity of forest soil bacteria increases with proximity to urban areas with no apparent impact of forest management. However, we unexpectedly also found the same pattern for the diversity of soil fungi. The prediction that (2) the communities of bacteria and

fungi would be impacted by anthropogenic disturbance is compatible with our data, although the underlying patterns of dispersion were not foreseen. The greatest dispersion in beta diversity for soil bacterial communities was found in urban forests while the pattern for fungi was the opposite, as we observed the greatest dispersion in national parks. We found (3) little evidence that variation in bacterial communities of urban and other forest soils elicits a major change in functional traits, but the variation in fungal communities was indeed associated with a decline in relative abundance of ECM in urban areas. Additionally, our data uncovers a strong association between the intensity of habitat disturbance (measured by the Human Influence Index) and soil pH, identifying important factors underlying the variation in forest soil microbiota diversity and composition.

*Urban soil microbiota have a higher level of alpha diversity in comparison to less disturbed soil microbial communities*

That the level of anthropogenic disturbance is positively associated with both bacterial and fungal diversity (Fig. 2, Fig. S2) is in accordance with earlier studies on soil bacteria (Hui et al., 2017; Naylo et al., 2019; Tan et al., 2019), but not with many studies on soil fungal communities (Abrego et al., 2020; Andrew et al., 2019; Tan et al., 2019; Tedersoo et al., 2020). A possible reason for this discrepancy is that other studies often confounded habitat type and urban location (*i.e.* sampling soil from forests in non-urban areas but from gardens and parks in urban areas). Suburban forests characterised by an intermediate level of alpha diversity, lend support to the concept of an apparent biodiversity gradient from natural to urban forests (Spotswood et al., 2021). Our data from soils of managed forests are consistent with the idea that long-term effects of forest management do not interfere much with microbial alpha diversity (Lee & Eo, 2020; Lee-Cruz et al., 2013). On the other hand, it is

possible that the immediate effects of commercial forestry management (*i.e.* compaction and removal of the upper organic layer) can cause disruptions in the soil microbial communities shortly after timber harvest.

Although, Finnish national parks were established with the principal aim to act as a refuge for (macro)diversity (Siikamäki et al., 2015), it is unclear whether such biodiversity policy is equally efficient at conserving microbial biodiversity. Our data shows that the microbiota communities in soils from national parks have the lowest degree of alpha diversity, which indicates that the biodiversity of macro- and microspecies are not necessarily following the same patterns. This also suggests that certain physico-chemical properties of urban soil enable a wider range of microbes to coexist on smaller geospatial scales (Wang et al., 2017; Tedersoo et al., 2020).

#### *Soil microbial communities exhibit parallel shifts in urban forests*

The anthropogenic disturbance of forests alters soil microbial communities, with urban forests consistently harbouring the most distinct communities (relative to other areas, Fig. S3-S4). This implies that there are strong parallel environmental stressors associated with urban forests that markedly shape both bacterial (Wang et al., 2017) and fungal (Tedersoo et al., 2020) microbial communities.

Furthermore, we highlight the contrasting patterns for bacteria and fungi in terms of the dispersion in their community profiles. In contrast to our hypotheses, we observed that urban landscapes promote higher dissimilarities between bacterial communities, while national parks increase the dissimilarities between fungal communities (Fig. 3). Generally, there are two potential mechanisms supporting higher dispersion patterns: (1) greater dispersal limitation between fragmented forest patches, and (2) a higher variety of microhabitats with



different selection pressures for colonisation (Wang et al., 2016). Interestingly, the distance decay analyses did not show strong support for dispersal limitation being a major contributing factor in explaining the observed patterns in bacteria and fungi (Fig. S6-S7, Table S8). Thus, it is more likely that urban forests and national parks provide diverse microhabitats for bacteria and fungi, respectively. These results challenge the idea that the concept of urban biotic convergence (McKinney, 2006) can be generally applied to model urban impacts on soil microbes. Additionally, it also underlines the potential conservation value that national parks have by sustaining natural variation among fungal communities, likely through variation in the accumulation of deadwood (different decay stages and/or plant species) (Dudley & Vallauri, 2005).

#### *Urban forests associate with changes in bacterial and fungal species and functional traits*

The composition of microbial communities differs markedly between urban and natural forests (Fig. 4). For bacteria in cities, these changes associate with shifts in the proportions of several phyla characteristic of forest degradation such as the decreased ratio of Acidobacteriota : Proteobacteria (Zhou et al., 2018). One of the major changes in urban soil fungal communities included a relative (but non-significant) decrease in Basidiomycota, a phylum that has many ECM members (Tedersoo et al., 2020). This reduction in Basidiomycota might reflect the lower percentage of conifers in urban forests (*i.e.* ‘forest order’; Fig. S5, Table S3).

From the viewpoint of functional traits, urban forests have higher proportions of fungal saprotrophs while having lower proportions of cellulolytic bacteria and ECM in comparison to national parks (Fig. 5). Only small changes in the relative proportion of bacterial functional

traits were detected among the four forest groups which implies little change in diversity of functional traits. In contrast, the proportion of ECM in the total community is much lower in urban areas (47%) in comparison to national parks (64%). Such community changes resulted in a higher level of fungal saprotrophy in urban soil. The main limitation of these findings lies in the predictive nature of this method, and that the greater part of the sequences was not assigned any functional traits. While we cannot validate these results with the available data, the major decline in the proportion of ECM fungi in our samples from urban areas is indeed consistent with previous studies (Abrego et al., 2020; Schmidt et al., 2017).

#### *Association between soil pH, habitat disturbance and changes in soil microbiota*

Soil pH is important in explaining the differences in soil microbiota between forests of different anthropogenic disturbance levels (Fig. 6, Table S12-13) although we did find that bacteria are more responsive to changes in soil pH than fungi (Rousk et al., 2010; Shen et al., 2020). Despite this strong relationship, it remains difficult to distinguish the individual effects of environmental factors since many are collinear (Fig. S5). For example, we found that soil pH and the Human Influence Index correlate strongly with one another (Fig. S9) with higher soil pH found in forests with higher values for HII. As such, our data uncovers an important association between the proximity to the built environment and soil pH. Interestingly, even when accounting for differences in soil pH between forest groups (*i.e.* selecting samples with similar pH ranges), our analyses suggest that the HII and soil pH are still relevant in shaping the alpha and beta diversity of soil microbiota (Table S15). This suggests that although soil pH and HII generally correlate, they both have independent effects on the soil microbiota.

Future experimental studies are needed to establish directionality of the intriguing association between soil pH and the proximity to the built environment and detect the underlying mechanisms to the changes observed in bacterial and fungal communities in urban areas. One potential explanation for this interaction could be that high soil alkalinity is an inherent part of a typical built environment (Pouyat et al., 2015) due to rainwater passage through concrete materials such as pipes and street gutters (Davies et al., 2010; Nugent & Allison, 2022). Although no causal inferences could be made with the available data, our study raises important questions in relation to any attempt to implement the ‘Microbiome Rewilding Hypothesis’, which postulates that degradation of microbial communities in cities can simply be counteracted by restoration and rewilding of urban green spaces (Mills et al., 2017).

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### **Data Accessibility**

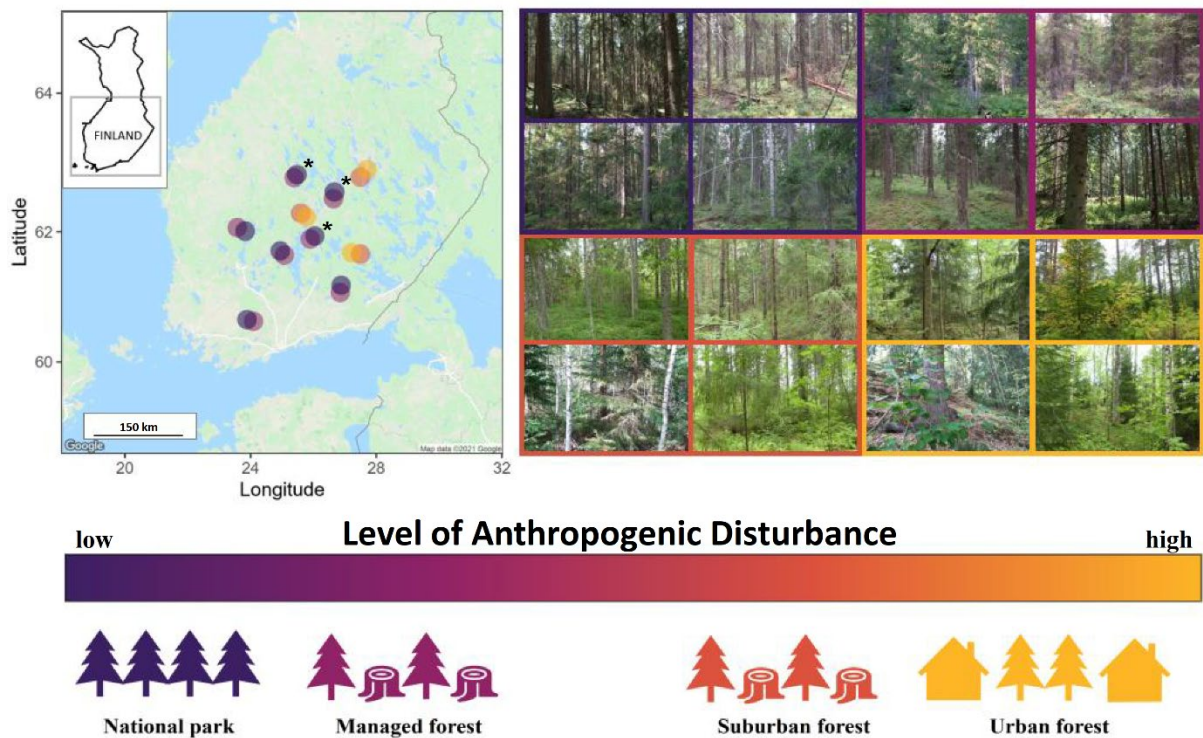
The raw sequences and associated metadata have been deposited to the National Center for Biotechnology Information (NCBI) under accession no. PRJNA823643 (<https://www.ncbi.nlm.nih.gov/bioproject/PRJNA823643/>).

### **Author Contributions**

All authors took part in conceiving the project design; TS, TM, AL, RH, ET and IB conducted field surveys and sample collection for the experiments. TS, AL and AV carried out work necessary for the pH measurements. TS performed laboratory work and completed data analysis, with additional bioinformatics support provided by AL and PCW. TS wrote the manuscript with significant contributions from AL and PCW and critical input from all other authors. The funders had no role in this study design, data collection and analysis, decision to publish, or preparation of the manuscript.

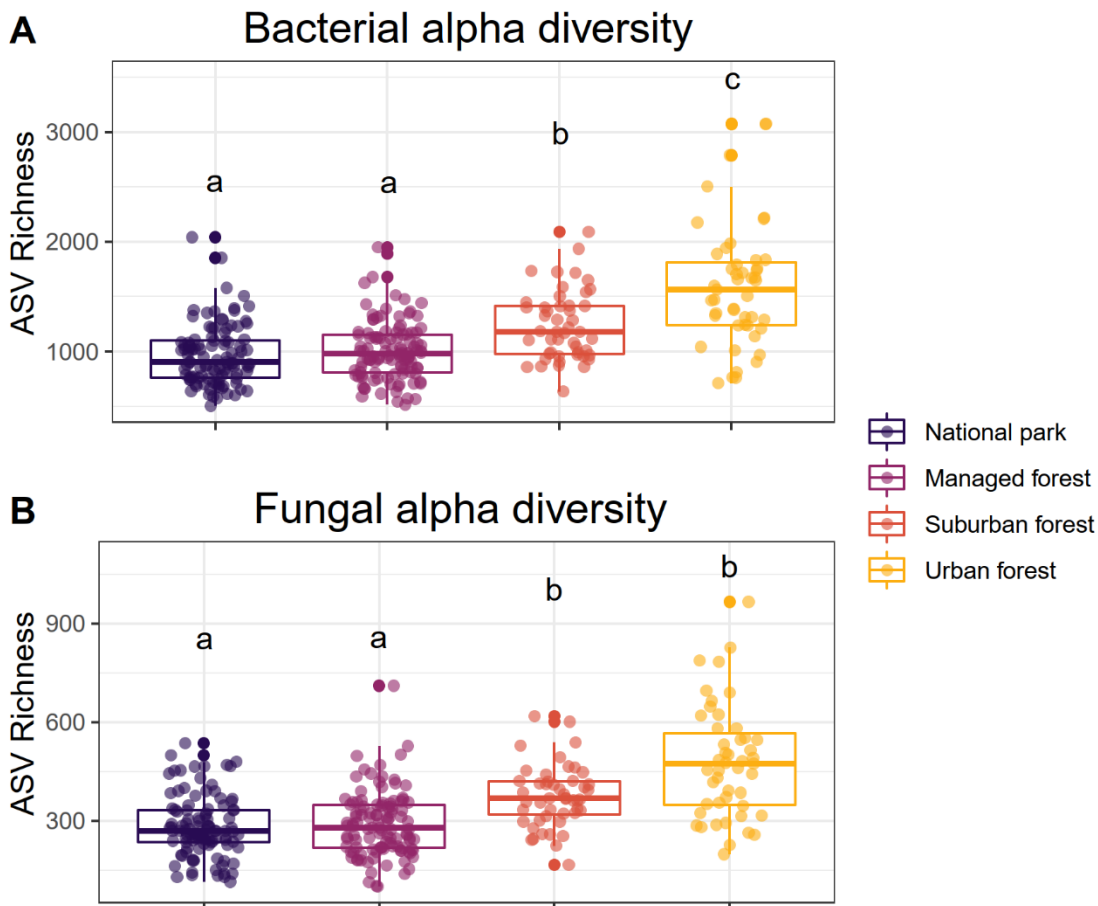
### **Competing interests**

The authors declare no competing interests.



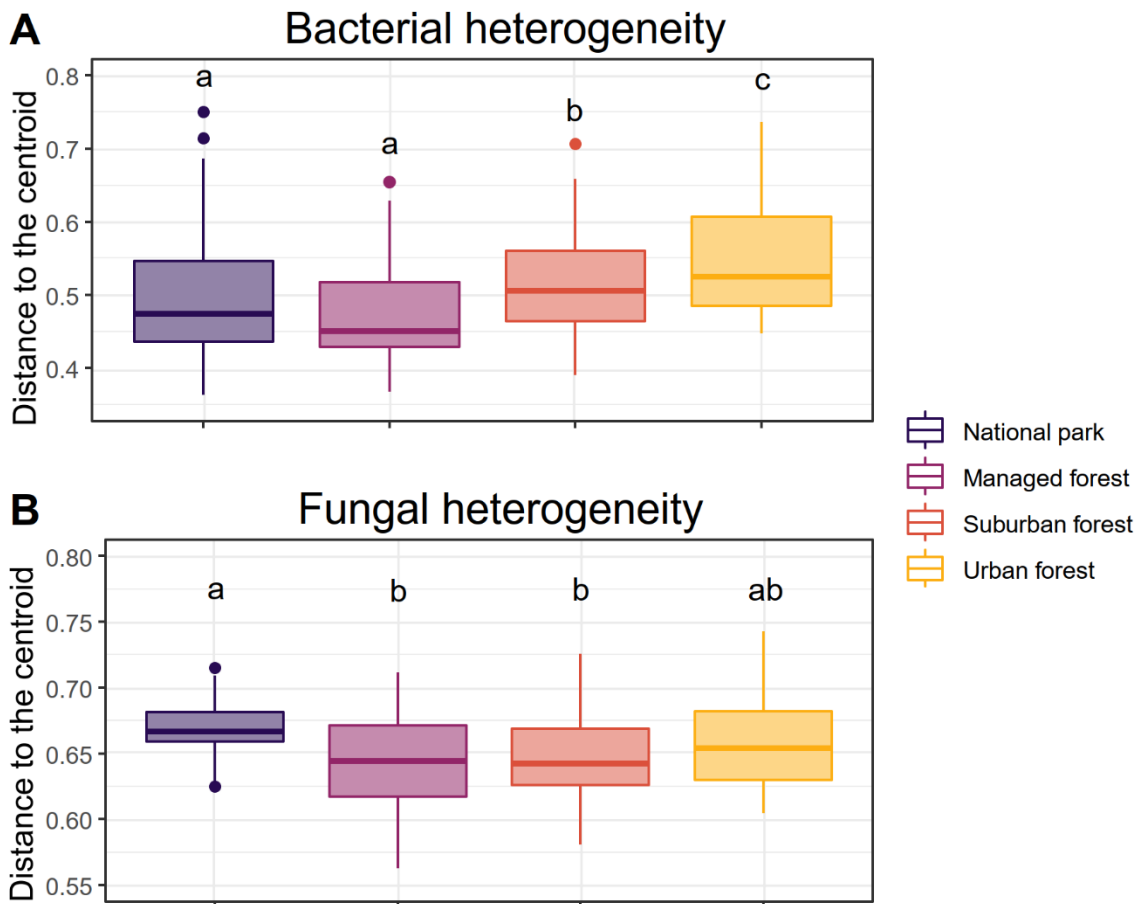
**Fig 1.** Forest soil sampling and study design.

The upper left panel provides an overview of the sample locations: seven national parks (dark purple), their surrounding managed forests (light purple), and suburban (orange) and urban (yellow) forests of three cities (Jyväskylä, Kuopio and Mikkeli). Every point on the map represents 12-22 forest sites where soil samples were collected (total  $n=312$ ). The upper right panel shows a mosaic of forest photographs with colour frames matching the corresponding forest disturbance level. The bottom panel shows the position of the four studied forest types along a gradient of anthropogenic disturbance that increases from national parks, managed forests, to suburban and urban forests. National parks and managed forests marked with an asterisk are included in the subset of data used for beta diversity analyses ( $n=195$ ). The map (upper left) was created with the *ggmap* package in R (Kahle & Wickham, 2013).



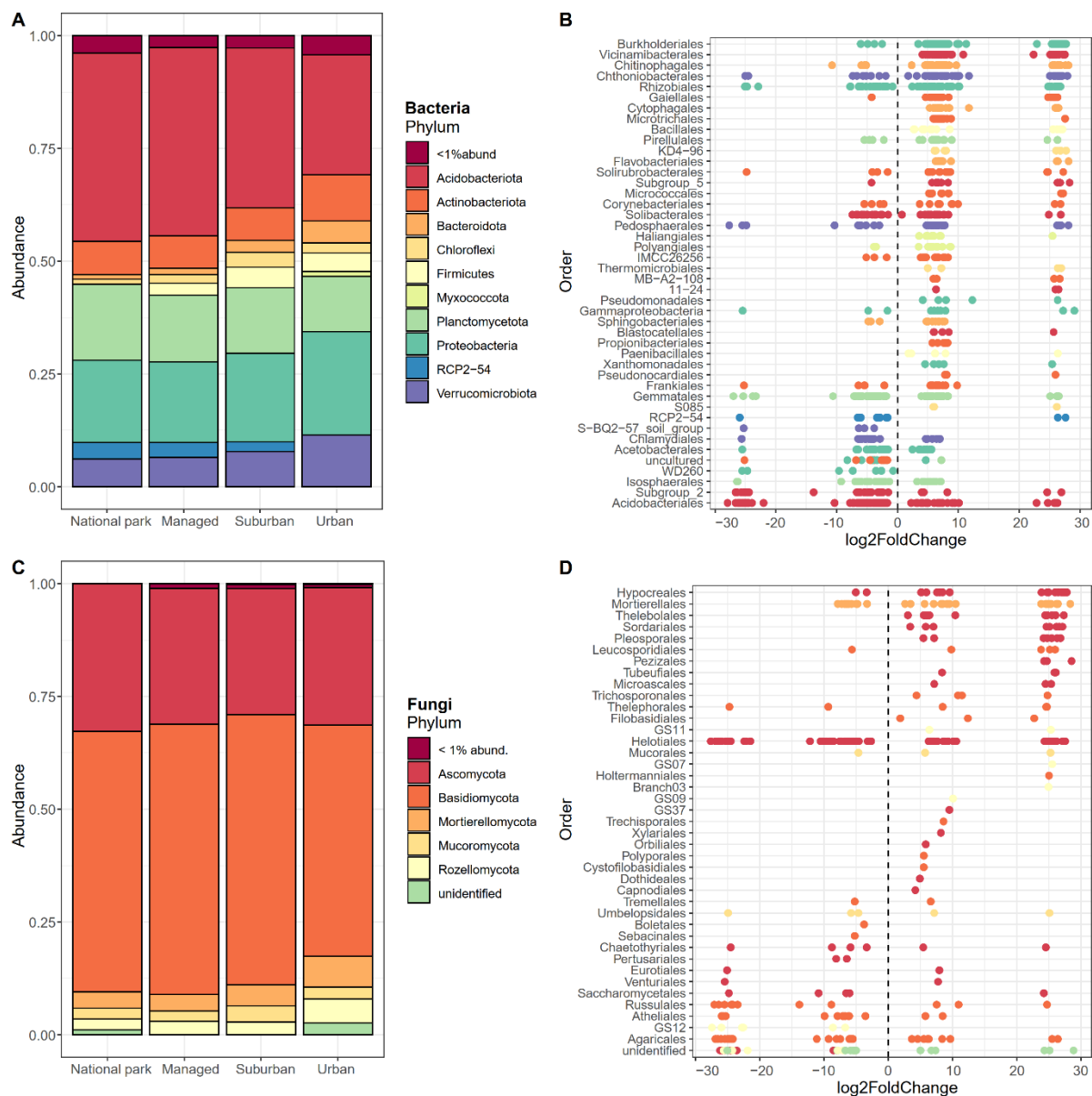
**Fig. 2.** Differences in the alpha diversity of soil microbiota according to the forest disturbance level.

An increase in diversity is visible from national parks (dark purple) towards urban forests (yellow) for bacterial (A) and fungal ASV richness (B). National parks (dark purple) and managed forests (light purple) are not significantly different from one another. Suburban forests (orange) have intermediate levels of microbial alpha diversity when compared to less disturbed forests (*i.e.* national parks and managed forests) and urban forests (yellow). The letters correspond to the significance levels between groups based upon Kruskal-Wallis tests, with a Benjamini-Hochberg adjustment ( $p < 0.025$ ).



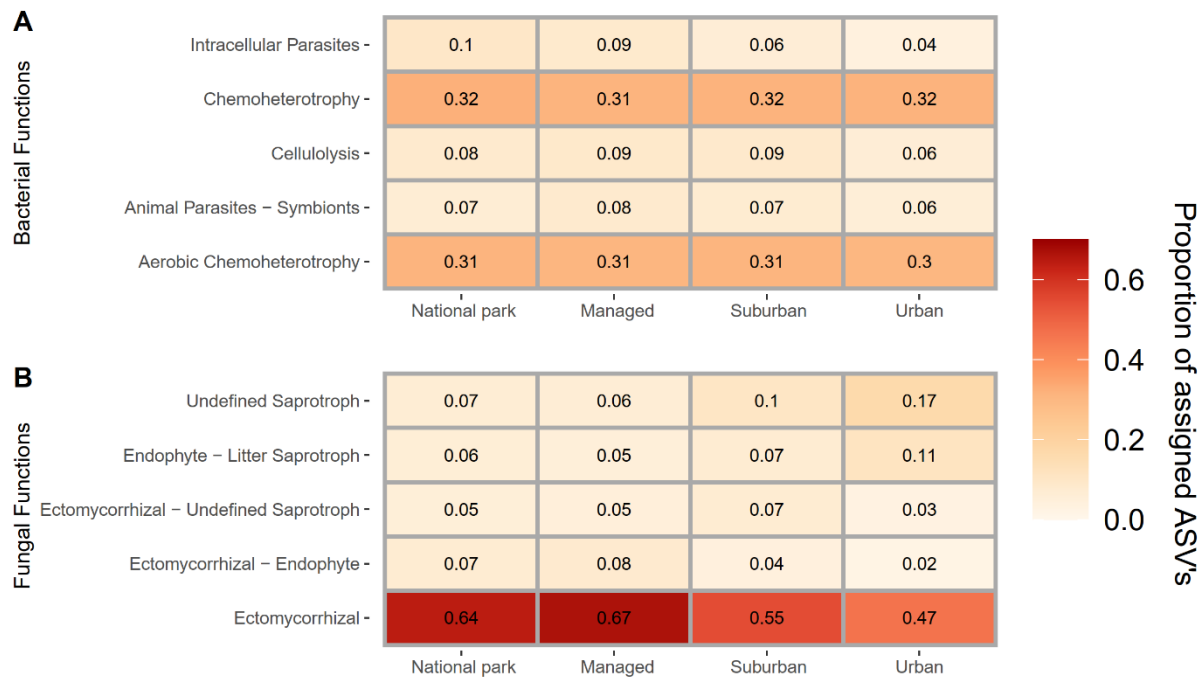
**Fig 3.** Dispersion in microbial communities according to the forest disturbance level.

Box plots represent the dispersion in the microbial communities calculated over the entire multidimensional space (all axes included) and based upon the Bray-Curtis metric ( $n=195$ ). The variation in the bacterial community positively correlates with increasing anthropogenic disturbance reaching its peak in urban forests (A). The lowest variation in the fungal community is found in managed and suburban forests, followed by urban forests and reaching its highest level in national parks (B). The letters correspond to the significance levels between groups based upon permutation-based tests of multivariate homogeneity of group dispersions ( $p<0.05$ ).



**Fig 4.** Taxonomic changes in the soil microbiota according to the forest disturbance level.

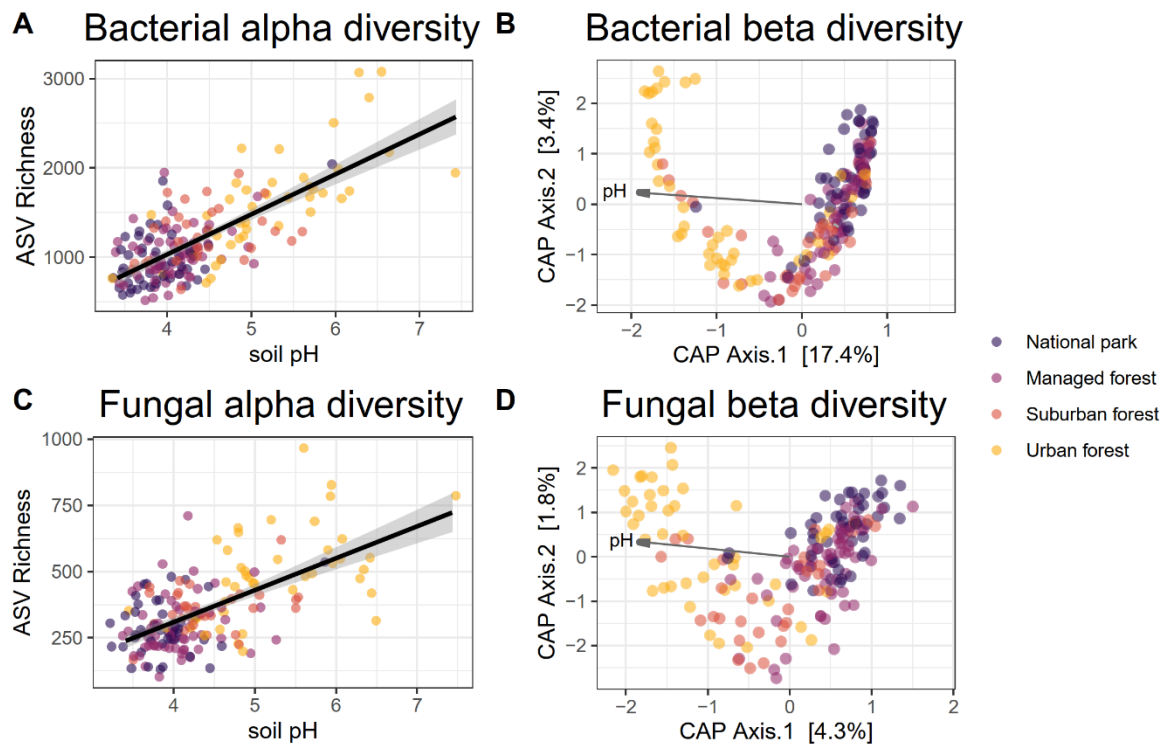
The two stacked bar plots on the left show the average relative abundances of microbial phyla in soils from forests that differ in disturbance level (bacteria (A), fungi (C)). Only the phyla that constitute at least 1 percent of the total abundance are shown. The remaining phyla are summarised under the category ‘<1% abund’. The corresponding differential abundance plots on the right (bacteria (B), fungi (D)) provide an overview of which underlying orders are driving shifts in the microbial community composition. Every order is categorised by one or several ASVs (closed points) that are either significantly more abundant in urban soils (positive values) or significantly more abundant in soil of national parks (negative values). A summation of all ASVs per order makes the order either increase (orders at the top of the graph, e.g. Burkholderiales) or decrease (at the bottom of the graph, e.g. Acidobacteriales) in abundance in urban forests. The colours of phyla between the panels on the left and right are matched for bacteria and fungi. To aid plotting, only the bacterial orders with the highest differential change ( $|\text{Log}_2\text{FoldChange}| > 38$ ) are shown (B).



**Fig 5.** Changes in predicted functional traits of the soil microbiota according to the forest disturbance level.

The heatmaps visualise the relative abundance of functional traits of the soil microbiota according to the forest disturbance level in bacteria (A) and fungi (B). Only the five most abundant functional traits ( $>0.05$  relative abundance for at least one of the four disturbance levels) for both bacteria and fungi are shown for clarity. Numbers represent relative abundance in percentages, ranging from 0.1 (10%) to 0.7 (70%).





**Fig 6.** The association between forest soil microbiota alpha and beta diversity and soil pH.

The Pearson's correlation between the pH of forest soil and the alpha diversity of the forest soil microbiota is shown for bacteria (A) and fungi (C) in terms of ASV richness ( $n=178$ ). The Constrained Analysis of Principal Coordinates (CAP) plots based on the subset of soil samples with the environmental data available are shown for bacteria (B) and fungi (D). The Bray-Curtis distances were used for both ordinations. The length of the arrows corresponds to the strength of its association with the beta diversity of the microbial community. Only the most important arrow (for soil pH) has been added to this graph to facilitate the readability. The CAP plots with arrows for all the 23 recorded environmental variables and pH has been added to the Supplementary Material as Fig. S5. Point colour matches the corresponding forest disturbance level.