Soil Biology and Biochemistry 191 (2024) 109352

Contents lists available at ScienceDirect

Soil Biology and Biochemistry

journal homepage: www.elsevier.com/locate/soilbio

Urbanisation shapes microbial community composition and functional attributes more so than vegetation type in urban greenspaces across climatic zones

Bangxiao Zheng [a,b,i,1], Lantian Su [a,j,k,1], Nan Hui [a,b,*], Ari Jumpponen [c], D. Johan Kotze [b], Changyi Lu [b,h], Richard Pouyat [d], Katalin Szlavecz [e], David A. Wardle [f], Ian Yesilonis [g], Heikki Setälä [b]

[a] School of Agriculture and Biology, Shanghai Jiao Tong University, Shanghai 200240, PR China
[b] Faculty of Biological and Environmental Sciences, Ecosystems and Environment Research Programme, Niemenkatu 73, FI-15140, Lahti, University of Helsinki, Finland
[c] 433 Ackert Hall, Division of Biology, Kansas State University, Manhattan KS66506, USA
[d] Emeritus USDA Forest Service, NRS, Affiliate Faculty Department of Plant and Soil Sciences, University of Delaware, Newark, DE 19716, USA
[e] Department of Earth and Planetary Sciences, Johns Hopkins University, 3400 N. Charles St, Baltimore, MD 21218, USA
[f] Department of Ecology and Environmental Science, Umeå University, Umeå, Sweden
[g] USDA Forest Service, Baltimore Field Station, Maryland, USA
[h] Institute of Urban Environment, Chinese Academy of Sciences, Xiamen, China
[i] School of Environmental Science and Engineering, Xiamen University of Technology, Xiamen, China
[j] Shanghai Yangtze River Delta Eco-Environmental Change and Management Observation and Research Station, Ministry of Science and Technology, Ministry of Education, Shanghai, China
[k] Shanghai Urban Forest Ecosystem Research Station, National Forestry and Grassland Administration, Shanghai, China

ARTICLE INFO

Keywords:
Biomes
Urban greenspace
Plant functional types
Microbial communities
Microbial functional traits
Geochip
Illumina Miseq

ABSTRACT

Urbanisation, as a global driver of change, modifies the natural environment with well-known consequences to biological communities. Under natural conditions, vegetation drives soil processes in concert with the soil microbial community in their rhizosphere. It remains unclear whether and how vegetation influences these communities in heavily disturbed urban systems where many ecosystem services are also strictly linked to soils and their biota. Here, we used amplicon sequencing and GeoChip arrays to study soil microbiota responses to urbanisation and tree functional types across climatic zones. Our data show that soil microbial communities vary widely across biomes, yet urban parks have compositionally unique microbial communities that are distinct from semi-natural forests. Neither functional trait richness nor functional gene relative abundances responded clearly to urbanization or vegetation type. Despite functional redundancy, vegetation type did affect soil communities compositionally. Soils under trees producing recalcitrant litter had a higher richness of fungal species than the labile ones, whereas lawns, despite of their structural simplicity, had an unexpectedly high diversity of bacteria and fungi. In summary, despite distinct differences in the soil microbiota across biomes, urbanisation and vegetation type have similar effects on structuring microbial communities within biomes. However, the urban soil microbiota, irrespective of the plant functional type they associate with, are functionally comparable to those in semi-natural forests, suggesting functional redundancy within this unique microbiota.

1. Introduction

Environmental change is clearly expressed in the urban milieu where management has created significant alterations within, e.g. the plant-soil feedback system (Kotze et al., 2021). In natural systems, vegetation links above and belowground environments and drives soil processes that govern carbon (C) sequestration and biogeochemical cycling (Wardle et al., 2004). Litter quality is an essential plant functional attribute that modulates both soil characteristics and processes, including soil organic matter (OM) turnover and net primary

* Corresponding author. School of Agriculture and Biology, Shanghai Jiao Tong University, Shanghai 200240, PR China.
E-mail address: nan.hui@sjtu.edu.cn (N. Hui).
[1] These authors have contributed equally to this work.

https://doi.org/10.1016/j.soilbio.2024.109352
Received 14 June 2023; Received in revised form 24 December 2023; Accepted 4 February 2024
Available online 9 February 2024

productivity (Grime 2006; Meier and Bowman 2008; Schneider et al., 2012). Even though plant-soil feedbacks regulate processes above and below ground in natural ecosystems (Bardgett and Wardle 2010), these feedbacks may also be important in human-disturbed ecosystems. For example, previous studies (Lu et al., 2021; Setälä et al., 2016) have shown that plants producing recalcitrant or labile litter in managed urban greenspaces differ in their ability to modify soil physiochemical properties in the boreal, temperate and tropical regions (Kotze et al., 2021).

Vegetation regulates soil microbial communities as well. Rhizosphere microbiota respond to plant diversity, e.g., arbuscular mycorrhizal fungi increase with plant diversity in grassland (Koziol and Bever, 2017; van der Heijden et al., 1998), and in forested and agricultural systems (Mohanram and Kumar 2019). Soil microbiomes are also sensitive to plant functional types in urban environments. In the boreal biome, fungal and bacterial communities diverged compositionally in soils under different vegetation types (evergreen tree, deciduous tree, and lawn) in urban parks (Hui et al., 2017a), likely because of the divergent soil properties under these plants (Kotze et al., 2021; Setälä et al., 2016; Taylor et al., 1989) and/or the predominant litter type produced by them. However, whether observations from boreal biomes can be generalized across climatic zones remains unknown. Since plant functional type can modulate soil properties irrespective of the climatic zone (Kotze et al., 2021), the soil microbiome should respond similarly to plant functional types across biomes. However, vegetation and soil type, which are climate dependent (Crowther et al., 2019; Jansson and Hofmockel 2020), may mask such plant effects on the soil microbiota (Pugnaire et al., 2019). Finally, urbanisation itself (here, urbanisation refers to management practices and extensive human use in urban parks) may override natural processes (Pouyat et al., 2010), thus masking the effects of vegetation and climate on the microbiota.

Irrespective of the climatic zone, soil processes, such as C and N dynamics, are largely influenced by the functional trait composition of soil microbiota (Delgado-Baquerizo et al., 2021; Nielsen and Wall 2015). Composition of the microbiota, in turn, depends on plant identity and soil characteristics (Andreote and Silva 2017; Tkacz et al., 2015). For example, soils under plants producing recalcitrant litter harbour relatively more fungi than bacteria (Habtewold et al., 2020; Hui et al., 2017a). However, urbanisation with continual human management (Grimm et al., 2008), high N inputs (Allen et al., 2020) and high soil pH (Pouyat et al., 2015) can distort this dependency, e.g., by affecting the biomass and composition of soil microbial communities (Abrego et al., 2020). Although it has been postulated that plant functional type is important in controlling soil biota and processes across terrestrial ecosystems (Cornwell et al., 2008; Pugnaire et al., 2019; Wardle et al., 2004), it remains poorly known whether this hypothesis is supported for soil microbiomes (especially communities and their functions) in urban settings where strong and repeated human activities may override natural constraints (Pouyat et al., 2015; Schmidt et al., 2017).

In this study, as a continuation of Kotze et al. (2021), we investigated how three plant functional types (trees producing recalcitrant and labile litter, and labile lawns) affect soil microbial communities in young and old urban parks, and compared to semi-natural reference forests. We included three cities representing three climatic regions: Lahti (boreal), Baltimore (temperate) and Singapore (tropical). We investigated (i) whether urbanisation influences microbial communities by comparing soils under trees of similar age between reference forests and old parks, (ii) whether and how plant functional types affect soil microbial community composition and functional traits, and (iii) how plant-type effects observed in soil communities develop with time by comparing young and old parks. We expected the overall soil microbial community composition to be affected by urbanisation, plant functional type and park age across climatic regions. More specifically, we tested four distinct hypotheses, grouped into two themes:

Parks versus reference forests (habitat types). First, we expect microbial richness and diversity in old urban parks to differ from reference forests (***hypothesis 1***) (Fig. 1a), considering both the effects of park management activities, such as mowing and raking (Mgelwa et al., 2019; Thompson and Kao-Kniffin 2019), and the competition between microorganisms influenced by nutrient inputs, as highlighted in recent studies (Wang et al., 2018). This complexity might suggest that microbial diversity could be comparably lower in reference forests because of more intense competition for nutrients. Second, we hypothesized that, within both old parks and reference forests, trees producing recalcitrant litter would harbour more diverse microbial communities and trait compositions compared to those producing labile litter (***hypothesis 2***) (Fig. 1b, the tree type comparison). This hypothesis accounts for the influence of both litter type and associated factors like root exudates and their corresponding microbial species, which are intrinsically linked to the functional types of plants, either producing recalcitrant or labile litters.

Old versus young parks. We hypothesized that the soil microbial communities within urban parks would be influenced by plant functional type (Hui et al., 2017a). Specifically, we expected that soil fungi would be more diverse and traits related to C degradation would be more abundant under recalcitrant trees than labile trees or lawns across climates (***hypothesis 3***) (Fig. 1b). This is because a greater variety of traits is required to decompose structurally complex, recalcitrant litter (Baldrian et al., 2012; Šnajdr et al., 2011). We also expected the plant-type effect to be greatest in the boreal zone (Fig. 1c). This is because favourable climatic conditions in warmer regions accelerate biological degradation irrespective of the complexity of substrates (biotic restriction), thereby diminishing the divergence of soil properties resulting from different plant litter chemistries (Garcia-Palacios et al., 2013). Additionally, we hypothesized that these plant-type effects on soil microbial communities would be more pronounced in old than in young parks regardless of climatic zones (***hypothesis 4***) (Fig. 1d) because older vegetation in old parks has modified soils for many decades compared to younger parks with more recently planted trees and lawns (Hui et al., 2017a; Yan et al., 2021).

2. Materials and methods

2.1. Experimental design

The experimental design has been described in detail in Kotze et al. (2021). Briefly, we sampled soils in three cities across three climatic zones (boreal: Lahti, Finland; temperate: Baltimore, USA; tropical: Singapore) (Fig. 1). We refer to boreal, temperate, and tropical zones or cities throughout this contribution while fully acknowledging that these cities within these climatic zones might not represent the entire zones. Information of study sites, including climate, parent material, C and N concentrations, etc., can be found in Table S1 and Kotze et al. (2021). In each zone, we selected five young (trees aged 10–20 yrs) and five old parks (trees aged 60–200 yrs) as well as five natural to semi-natural forest fragments that served as a reference (trees aged ca. 100 y). In parks, we chose three vegetation types based on traits that reflect litter decomposability (Grime 2006): trees producing recalcitrant litter, trees producing labile litter, and lawns without trees but with grasses and forbs that produce highly labile litter. In reference forests, the lawn was absent, but we selected the same recalcitrant and labile tree species as in parks. The C/N ratio was used to classify either recalcitrant or labile tree leaves (Taylor et al., 1989). We analysed the C/N ratio of each leaf litter type by measuring their C and N contents. Labile tree species were linden (*Tilia x vulgaris*, C/N ratio = 31.6) in Lahti, tulip poplar (*Liriodendron tulipifera*, C/N ratio = 43) in Baltimore, and tembusu (*Cyrtophyllum fragrans*, C/N ratio = 69.4) in Singapore; recalcitrant tree species were Norway spruce (*Picea abies*, C/N ratio = 72), oak (*Quercus* spp., C/N ratio = 75.9), and rain tree (*Samanea saman*, C/N ratio = 20) in the boreal, temperate, and tropical cities, respectively. In the case of rain tree in Singapore, the exceptionally high polyphenolic content makes the leaves decompose slowly (Rita et al., 2018). All the park plots,

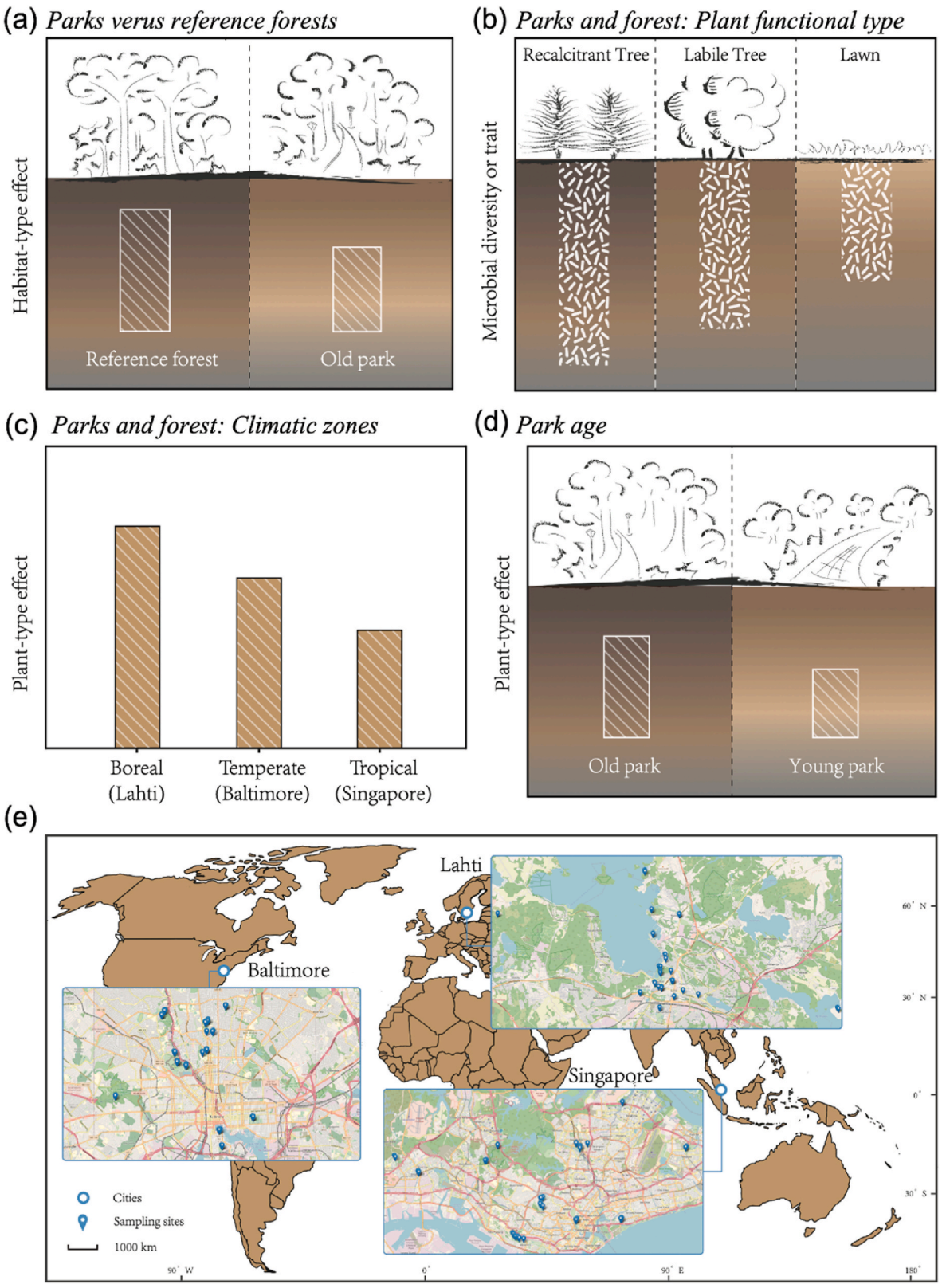

Fig. 1. Hypotheses tested in this study. (a) The microbial community is hypothesized to be more diverse in reference forests compared to old parks (***hypothesis 1***), and (b) trees producing recalcitrant litter would host a more diverse soil microbial community than labile trees (***hypothesis 2***). (b) In parks, we expected fungal diversity to be higher under trees producing recalcitrant litter, compared to trees producing labile litter and lawns (***hypothesis 3***), and (c) we also expect a greater microbial community response to plant type in the boreal than in the temperate and tropical climatic zones. (d) We expect that the old parks would harbour more diverse microbial communities than young parks (***hypothesis 4***). (e) Location of the 120 plots in 43 parks and forests in three cities across three climatic zones. The detailed locations of all plots, including vegetation type and park age, are shown in Extended Figs. 1–3.

including those with trees, were covered with lawn. Therefore, our design included a total of 120 plots across three climatic zones, including 5 replicates of 3 vegetation types in each old and young park, and 5 replicates of recalcitrant and labile tree species in the reference forests (see https://www.google.com/maps/d/u/0/viewer?mid=1XLYE2s4-PTbWzW8lAsqdqxxH6cb9UUzV&ll=34.83162044 2620356%2C13.62469550000003&z=2). We considered all parks to be disturbed systems. In this study, urban anthropogenic disturbances mainly refer to park use and management activities, although some variation in, e.g., air quality or air pollution likely existed as well. All parks were frequently mowed, with leaves raked and removed and grass clippings left on site, but were subject to neither irrigation, fertilization, nor pesticide application.

2.2. Soil sampling

Soil sampling and the measurement of edaphic properties, including pH, bulk density, percentage organic matter (OM), carbon (C), nitrogen (N), and the C/N ratio, have been described in detail in Kotze et al. (2021). For soil DNA extraction, we sampled a total of 120 soils using sampling protocols described in previous studies (Kotze et al., 2021; Hui et al., 2017b). Soils were collected in 2019 between 2–8 March in Singapore, 7–13 May in Lahti, and 6–16 June in Baltimore with mean annual temperatures of 31.7 °C, 9.2 °C, and 24.2 °C, respectively. Seasonality can influence soil microbial community composition and function, especially in boreal and temperate sites (Carson et al., 2019; Santalahti et al., 2016; Uroz et al., 2016). However, our analyses focus explicitly on the differences among habitats (parks of different ages or reference stands) or vegetation types within each climatic region. Therefore, temporal variation should have minor impacts on our interpretation and - particularly - on the conclusions we draw from our data. As to the soil sampling, briefly, soils were sampled beneath trees at the outer edge of the tree canopy projection. All reference sites had closed canopies, whereas trees in the parks were widely spaced without a closed canopy. To minimize the effects of trees on one another and lawns in the parks, we ensured that plots were placed at a distance greater than the height of the nearest tree. We collected three sub-samples from each plot, using a metal soil corer (3.5 cm diameter × 10 cm deep), followed by sieving the soil through a 2 mm sieve to remove roots, rocks, and large debris. The three sub-samples were pooled, gently mixed manually and then transferred to a sterile plastic bag. The tools for sampling, mixing, and transferring were washed and cleaned with 70% ethanol between samplings. An average of 7 g fresh soil was preserved from the ~200–300 g of thoroughly mixed fresh soils in 20 mL LifeGuard Soil Preservation Solution (QIAGEN, Maryland, USA) in a cooler box and stored at −20 °C in the laboratory.

2.3. DNA sequencing and quantitative PCR

The LifeGuard solution was removed before DNA extraction (centrifuged at 2500 g for 5 min with the supernatant discarded). The total soil DNA was extracted, quantified, and quality estimated at the Environmental Laboratory of the University of Helsinki (https://www2.helsinki.fi/en/infrastructures/environmental-laboratory). We used the DNeasy PowerMax Soil Kit protocol (QIAGEN, Maryland, USA) as per the manufacturer's protocol to extract DNA from the soil. DNA concentration was measured using a Qubit 4 Fluorometer together with Qubit RNA IQ Assay Kit (Invitrogen, Massachusetts, USA). DNA extracts were visualized by 1% agarose gel electrophoresis, diluted to 1.5 ng uL^{-1} for amplicon sequencing, and stored at −20 °C until sequencing.

Amplicon sequencing. A hypervariable region of the bacterial 16 S rRNA gene (V4–V5 region) was amplified using primer pair 515 F/907 R (Table S2) amended with barcode and adaptor sequences. The fungal ITS1 region was amplified using the primer pair ITS1F/ITS2 (Table S2). The amplicons were sent to the Novogene UK Sequencing Center for library construction and sequencing (Illumina MiSeq 2500 platform, California, USA). Illumina sequencing returned ~22 million 16 S rRNA sequences, and ~23 million ITS sequences. We used QIIME2-2020.2 to demultiplex the imported paired-end sequences, followed by DADA2 denoise algorithm to delineate ASVs (amplicon sequence variants). A maximum-likelihood tree was generated using FastTree2 (Fig. S1). We used the Greengenes database at 99% similarity, to delineate a total of 14 536 462 16 S rRNA ASVs, including 19 117 archaeal and 14 517 345 bacterial ASVs. We removed archaea ASVs in this study because only few available functional genes can be retrieved based on GeoChip gene categories. In addition, we delineated 17 816 252 fungal ASVs by matching to the 'dynamic' representative sequences of the UNITE database. All pipeline analyses were executed using the *Puhti* supercomputer (Finnish IT Center for Science, https://research.csc.fi/csc-s-servers). The ASV sequence counts are listed in Table S3. The paired fastq files are available at the Sequence Read Archive (SRA) of the NCBI (National Center for Biotechnology Information, www.ncbi.nlm.nih.gov) under accession number PRJNA633748.

The 16 S rRNA and ITS primers (Table S2) used for sequencing were also used in real-time quantitative PCR (RT-qPCR) to estimate copy numbers as a proxy for bacterial and fungal abundances, respectively (see Table S3 for term clarification). Triplicate DNA samples were prepared on plates that included 10-fold serial dilutions of standard plasmid DNA for calibration curves (LightCycler 480, Roche, Basel, Switzerland). The reactions included 0.3 μM of each primer, 10 ng template DNA, 0.1 mg mL^{-1} BSA (bovine serum albumin), and 1 × SYBR premix *Ex Taq* (TaKaRa, Shanghai, China). The reactions were initiated with 3-min denaturation (98 °C), followed by 40 cycles of 15 s at 98 °C and 30 s at 58 °C for an extension. A dissociation curve indicated one peak at a melting temperature of 92.3 °C. Reactions with an efficiency between 90 and 110% were accepted.

2.4. Gene abundance

We measured functional gene intensities using GeoChip 5.0 (Glomics Inc., Oklahoma, USA). In each hybridization, 1 μg of purified DNA per plot was labeled, hybridized, and imaged. We used a positive spot with qualified signals to represent an effective probe hybridization indicating that a functional gene of a taxon was effectively detected. Before qualifying the signals, outliers and unreliable signals from raw signal data were removed using a standard analysis pipeline (http://ieg.ou.edu/microarray). We filtered the positive spots with qualified signals by the following standards: (1) a signal-to-noise ratio of no less than 2.0, (2) the coefficient of variation (*c.v.*) of the background was more than 0.8, (3) signal intensity in the spot was higher than 200 and at least 1.3 times higher than the background. We observed a total of 126 663 positive spots across all 120 plots, and the average gene intensity was 392.19 ± 1.64 million per plot. Because we focused on C, N, P, and S cycles in this study, the gene intensities of these functional categories from GeoChip results were extracted for further analyses (Table S4). Although there are challenges in using GeoChip, including limited probe coverage, a need for continuous updates, and issues with specificity, sensitivity, and quantification, it remains a valuable tool for microbial community functional gene analysis (He et al., 2015). Its high specificity and advanced probe design allow for accurate detection of known gene variants, while its capacity for quantitative analysis provides clear, reliable data on microbial community dynamics (He et al., 2015; Robinson et al., 2018). GeoChip's precision, coupled with its suitability for consistent community comparisons, provides a means for reliable analysis of annotated gene functions in certain contexts of microbial ecology.

Community-weight-mean (CWM) method transformation. We multiplied the gene intensity data from GeoChip with soil DNA concentrations to acquire an absolute value, i.e., the intensity of functional genes per gram of dry soil (Table S3). Among trait-based approaches, the community-weight-mean (CWM) transformation results in fewer statistical errors and greater stability. We utilized a modified CWM method to

transform gene abundance to functional trait abundance based on the following three assumptions: (1) the spot intensity for each functional gene can be considered a measure of gene abundance per gene per taxon; (2) a major taxon with the function of interest can be sufficiently detected by both amplicon sequencing and GeoChip array; (3) the 16 S and ITS amplicon sequencing can be used as a credible measure of the relative abundances of taxa, though the technology itself may have reproducibility issues (Buzzard et al., 2019). For the CWM transformation, both taxa-assigned ASV and GeoChip intensity tables are needed. The trait j indicates a function within a GeoChip category (a functional gene of a specific taxon). The CWM value for each trait j per plot k was calculated as:

$$CWM_{jk} = \sum \varphi_{ik}\lambda_{ijk}$$

where φ_{ik} is the abundance of taxon i in plot k, λ_{ijk} is the average trait abundance j of taxa i in plot k. ASV frequencies do not need to be rarefied for CWM calculation since rarefying may underestimate CWM values (Buzzard et al., 2019). Notably, we used the CWM method to measure functional traits, even though it is still debatable whether the CWM method is optimal for all research backgrounds (Muscarella and Uriarte 2016). Functional genes from the GeoChip array are indirect markers for functions of the microbial community, and the transformed trait abundance that we used should be considered as the (meta-)genomic potential that may perform specific physiological or biochemical functions. Whether a direct causal relationship exists between traits and ecosystem processes requires further investigation (Klassen 2018). Data files are available at the Gene Expression Omnibus (GEO) database of NCBI under accession number PRJNA722405.

2.5. Statistical analyses

Statistical analyses were performed using the R statistical software (version 3.6.3, R Core Team, 2021). Taxon and trait abundances were normalized using Hellinger or z-score transformations when necessary (*decostand*, vegan).

Compositinal analyses and diversity indices. Microbial community composition and traits were visualized using NMDS (non-metric multidimensional scaling) ordinations (*metaMDS*, vegan, R). Between-group differences (e.g., old parks vs. reference forests; young vs. old parks) were analysed by permutational multivariate analysis of variance (PERMANOVA, *adonis*, vegan). Observed ASV richness and the Shannon-diversity were iteratively calculated and rarefied at the lowest number of sequences/functional genes across the whole dataset, for bacterial 16s, fungal ITS, and the GeoChip data. Diversity indices were analysed separately using two different strategies: (1) to test ***hypotheses 1–2***, the dataset included only tree samples in old parks and reference forests because of the absence of lawn in reference forests. (2) To test ***hypotheses 3–4***, a dataset including microbial composition, trait abundance and diversity was used to compare all vegetation types in young and old parks.

Generalized linear mixed model (GLMM). GLMMs were conducted, using the *lmer* function in the lme4 library (Bates et al., 2015) in R, to compare soil microbial diversity and traits under labile or recalcitrant trees between habitat types (old parks vs. reference forests) (Extended Table 1). The model was constructed based on predictor variables including vegetation type (excluding lawn), habitat type and climatic zone and their interactions, as well as C, C/N and pH (OM and N content was not included in the models because they are highly correlated with C content). We performed model selection by removing non-significant terms, starting with the term with the highest p-value. C, C/N and pH were initially subject to model simplification until only terms with p-values <0.1 were left. If the habitat type × vegetation type × climatic zone interaction, including their two-way interactions, remained non-significant (p-values >0.1) after this procedure, it was also removed. However, to remain true to our experimental design, the main effects (habitat type, vegetation type, and climatic zone) were always retained in the model irrespective of their significance. Secondly, GLMM was used to evaluate how soil microbial diversity and traits under vegetation producing very labile litter (lawn), labile litter (labile tree) or recalcitrant litter (recalcitrant tree) change as parks age (young vs. old parks), and whether this relationship is influenced by climatic zone (Extended Table 2). Predictor variables included vegetation type, park age and climatic zones as factors and their interactions, as well as C content of the soil, the C/N ratio and soil pH. We performed the same model selection procedure as described above. Since our samples were from three climatic zones and different vegetation types were present in each park, park identity nested within climatic zone was added as a random term to the models.

3. Results

Soil microbiomes differed compositionally among climatic zones, as the soil bacterial ($r^2 = 0.255$, $p < 0.001$) and fungal ($r^2 = 0.337$, $p < 0.001$) community and functional trait ($r^2 = 0.129$, $p < 0.010$) compositions differed across biomes, based on PERMANOVA analyses. Soil bacterial ASV richness was lowest in the boreal zone ($r^2 = 0.112$, $p < 0.001$), whereas fungal ASV richness ($r^2 = 0.092$, $p = 0.133$) and functional trait richness ($r^2 = 0.013$, $p = 0.249$) did not differ among biomes.

3.1. The effects of urbanisation on soil microbial communities

Microbial communities differed compositionally between the reference forests and old parks across climate zones (Fig. 2, Table S5). This habitat type effect was climate dependent: communities in the reference forests (grey ellipses) and old parks (red ellipses) were more dissimilar in the boreal than in temperate and tropical zones, particularly so for fungi (Fig. 2 a-f). Habitat type affected functional trait composition in the boreal and temperate zones, but less so in the tropical zone (Fig. 2 g-i). Soil microbial richness mostly differed between reference forests and old parks, but these differences varied between bacteria and fungi. Fungal richness was mostly higher in the reference forests than in the old parks across climatic zones, whereas bacterial richness was higher in old parks only in the boreal zone (Fig. 3 a, b, see also Fig. S2 a, b, d, e for similar trends in diversity indices). Similarly, dominant fungal and bacterial phyla differed in their abundances (Fig. S5). For example, the relative abundance of Basidiomycota was higher in the reference forest than in the old parks across climatic zones, whereas this was reversed for the Ascomycota (Fig. S5 g, h). For bacteria, the relative abundance of Actinobacteria was greater in parks, whereas Bacteroidetes was more abundant in reference forests (Fig. S5 a, e).

Microbial functional trait richness, however, did not differ among habitat types or climates (Fig. 3c, Fig. S2 c, f, i, Table S5), suggesting functional redundancy despite compositional differences. Additionally, bacterial and fungal communities did not differ in their qPCR-inferred abundances between the two habitat types or climatic zones (Fig. S2 g, h). Habitat type had no consistent effect on specific functions, except for C and N fixation, methane metabolism and assimilatory N reduction that tended to be higher in reference forests than in old parks (Fig. 3 d-i). Climate also strongly impacted these functions, e.g., the genes for C degradation were most abundant in the tropical soil, whereas genes for C fixation, methane metabolism, assimilatory N reduction, N fixation and denitrification were least abundant in these soils (Fig. 3 d-i).

Vegetation type affected the microbial community composition only in the boreal zone (Fig. 2 a-f, compare triangles with circles). There was no evidence of a vegetation type effect on functional trait composition across climatic zones (Fig. 2 g-i). However, microbial richness and abundance differed underneath the two tree types that produced divergent litter (Fig. 3 a, b). Irrespective of habitat type or climatic zone, fungal Shannon-diversity and evenness were always higher under recalcitrant trees than labile ones (Fig. S2 b, e). In contrast, bacterial richness and Shannon-diversity were generally higher under labile trees

Table 1
GLMM evaluations of effects of climate, habitat-type, and vegetation types on microbial diversities and traits.

	Interc	Temp	Trop	Recal	Reference	pH	Carbon content	C/N ratio	Temp × Reference	Trop × Reference	Temp × Recal	Trop × Recal	Reference × Recal
Richness													
Bacterial taxa	2554.727 (±155.922) ***	−286.332 (±218.845)	−122.252 (±223.822)	−227.181 (±171.211)	−683.133 (±235.75) **			90.801 (±51.186) •	1083.827 (±290.892) ***	872.955 (±290.293) **	154.351 (±243.515)	485.47 (±249.167) •	−292.684 (±283.758)
Fungal taxa	425.874 (±29.658) ***	−13.303 (±35.022)	−66.944 (±34.551) •	89.887 (±23.606) ***	153.595 (±26.577) ***	50.319 (±13.943) ***							
Microbial traits (× 103)	17.701 (±1.338) ***	1.553 (±1.974)	1.388 (±1.979	−3.539 (±1.680) *	1.266 (±2.007)		−1.376 (±0.640) *	0.848 (±0.495) •	−0.196 (±2.662)	1.047 (±2.574)	2.452 (±2.466)	4.229 (±2.376) •	5.315 (±2.365) *
Shannon-diversity													
Bacterial taxa	7.002 (±0.092) ***	0.022 (±0.117)	−0.151 (±0.131)	−0.062 (±0.058)	−0.019 (±0.079)	0.153 (±0.035) ***	−0.183 (±0.048) ***	0.119 (±0.042) **					
Fungal taxa	2.762 (±0.33) ***	0.824 (±0.453) •	0.265 (±0.446)	0.848 (±0.452) •	1.371 (±0.446) **	0.313 (±0.102) **			−1.398 (±0.633) *	−1.223 (±0.602) *	−0.59 (±0.612)	−0.819 (±0.614)	−0.464 (±0.616)
Microbial traits	8.866 (±0.103) ***	−0.088 (±0.151)	−0.536 (±0.151) ***	−0.286 (±0.131) *	−0.043 (±0.151)	0.091 (±0.035) **		−0.098 (±0.036) **	0.222 (±0.205)	0.173 (±0.194)	0.298 (±0.192)	0.357 (±0.186) •	0.412 (±0.184) *
Evenness													
Bacterial taxa	0.904 (±0.008) ***	−0.006 (±0.011)	−0.035 (±0.012) **	0.005 (±0.005)	0.006 (±0.007)	0.017 (±0.003) ***	−0.016 (±0.004) ***	0.01 (±0.004) **					
Fungal taxa	0.46 (±0.045) ***	0.126 (±0.064) *	0.049 (±0.063)	0.117 (±0.063) •	0.188 (±0.063) **	0.039 (±0.015) **			−0.205 (±0.09) *	−0.187 (±0.087) *	−0.073 (±0.088)	−0.121 (±0.088)	−0.072 (±0.089)
Microbial traits	0.904 (±0.005) ***	−0.012 (±0.007) •	−0.058 (±0.006) ***	0.001 (±0.004)	0.004 (±0.005)	0.009 (±0.002) ***		−0.01 (±0.003) ***					
Abundance													
Bacterial taxa (× 1013)	25.676 (±11.657) *	−13.702 (±13.220)	0.951 (±13.207)	2.411 (±9.813)	−10.454 (±1.011)								
Fungal taxa (× 1012)	15.48 (±2.884) ***	−8.939 (±3.533) *	−8.84 (±3.451) *	−1.106 (±2.024)	−3.958 (±2.435)								
Microbial traits	338.911 (±61.31) ***	44.686 (±73.715)	665.098 (±71.833) ***	−12.372 (±51.42)	−107.494 (±62.856) •	−117.615 (±29.46) ***		81.098 (±31.218) **					
Taxa relative abundance													
Actinobacteria	0.404 (±0.026) ***	−0.101 (±0.036) **	−0.024 (±0.036)	−0.038 (±0.032)	−0.09 (±0.038) *	−0.02 (±0.008) **			0.031 (±0.052)	0.107 (±0.051) *	0.004 (±0.045)	0.011 (±0.045)	−0.042 (±0.047)
Proteobacteria	0.269 (±0.021) ***	0.01 (±0.028)	−0.02 (±0.03)	0.028 (±0.023)	0.044 (±0.033)	0.011 (±0.006) •	−0.021 (±0.01) *	0.015 (±0.008) •	0.018 (±0.04)	−0.035 (±0.042)	−0.025 (±0.032)	−0.04 (±0.032)	0.014 (±0.035)
Acidobacteria	0.107 (±0.014) ***	0.089 (±0.02) ***	0.035 (±0.02) •	0.012 (±0.019)	0.043 (±0.022) *	−0.009 (±0.005) •		0.009 (±0.005) •	−0.079 (±0.029) **	−0.071 (±0.029) *	−0.018 (±0.026)	−0.003 (±0.026)	−0.003 (±0.027)
Verrucomicrobia	0.079 (±0.014) ***	−0.009 (±0.019)	−0.041 (±0.02) *	−0.004 (±0.012)	−0.021 (±0.018)			−0.009 (±0.004) *	0.038 (±0.023) •	0.025 (±0.024)	0.017 (±0.016)	0.001 (±0.016)	0.024 (±0.018)

(*continued on next page*)

Table 1 (*continued*)

	Interc	Temp	Trop	Recal	Reference	pH	Carbon content	C/N ratio	Temp × Reference	Trop × Reference	Temp × Recal	Trop × Recal	Reference × Recal
Bacteroidetes	0.041 (±0.009) ***	−0.016 (±0.012)	−0.034 (±0.013) **	0.002 (±0.013)	0.021 (±0.013)	0.008 (±0.003) **			−0.012 (±0.018)	−0.006 (±0.018)	0.004 (±0.018)	−0.003 (±0.018)	0.017 (±0.018)
Firmicutes (× 10–2)	0.47 (±1.427)	0.895 (±1.941)	5.954 (±1.948) **	−0.064 (±9.970e-03)	0.143 (±1.621)				−0.509 (±1.992)	−4.672 (±2.045) *	−0.201 (±1.293)	−0.324 (±1.334)	0.328 (±1.529)
Ascomycota	0.685 (±0.051) ***	−0.104 (±0.072)	0.222 (±0.071) **	0.107 (±0.071)	−0.353 (±0.07) ***			0.032 (±0.017) •	0.003 (±0.099)	0.173 (±0.099) •	0.027 (±0.102)	−0.086 (±0.101)	0.099 (±0.102)
Basidiomycota	0.152 (±0.056) **	−0.06 (±0.079)	−0.123 (±0.079)	−0.06 (±0.078)	0.312 (±0.076) ***				−0.128 (±0.109)	−0.203 (±0.109) •	0.038 (±0.11)	0.055 (±0.111)	−0.104 (±0.112)
Mortierellomycota	0.12 (±0.04) **	0.047 (±0.057)	−0.136 (±0.057) *	−0.067 (±0.056)	0.03 (±0.055)			−0.026 (±0.014) •	0.135 (±0.078) •	0.014 (±0.078)	0.003 (±0.081)	0.067 (±0.08)	−0.022 (±0.081)
Ectomycorrhiza	0.022 (±0.042)	−0.01 (±0.06)	0.025 (±0.061)	0.003 (±0.059)	0.137 (±0.061) *	−0.026 (±0.015) •		0.041 (±0.016) *	−0.048 (±0.083)	−0.221 (±0.083) **	0.018 (±0.085)	−0.021 (±0.085)	−0.072 (±0.085)
Trait relative abundance													
Carbon degradation	0.646 (±0.018) ***	0.033 (±0.025)	0.172 (±0.023) ***	−0.032 (±0.022)	−0.012 (±0.023)				−0.06 (±0.034) •	0.014 (±0.031)	0.009 (±0.031)	0.025 (±0.029)	−0.054 (±0.029) •
Carbon fixation	0.116 (±0.008) ***	−0.015 (±0.011)	−0.078 (±0.01) ***	0.019 (±0.01) •	0.007 (±0.01)				0.03 (±0.015) *	−0.006 (±0.014)	−0.008 (±0.014)	−0.017 (±0.014)	0.015 (±0.013)
Methane (× 10–4)	23.67 (±3.838) ***	1.949 (±5.532)	−16.93 (±5.101) ***	−5.149 (±4.701)	10.13 (±5.053) *	2.085 (±1.127) •			−1.672 (±7.258)	−9.761 (±6.658)	6.922 (±6.523)	5.157 (±6.219)	13.81 (±6.095) *
Assimilatory N reduction (× 10–3)	19.98 (±1.670) ***	−2.842 (±2.401)	−12.44 (±2.253) ***	2.649 (±1.937)	4.654 (±2.477) •		−1.357 (±6.309e-04) *		2.841 (±3.240)	−6.575 (±2.951) *	−0.206 (±2.666)	−2.566 (±2.526)	2.602 (±2.521)
Nitrogen fixation (× 10–2)	4.658 (±0.756) ***	−0.858 (±1.089)	−3.592 (±1.012) ***	−0.577 (±0.963)	−0.193 (±0.995)				2.393 (±1.448) •	0.099 (±1.343)	1.086 (±1.347)	0.628 (±1.296)	3.011 (±1.276) *
Denitrification	0.021 (±0.002) ***	0.005 (±0.003)	−0.014 (±0.003) ***	−0.003 (±0.003)	−0.008 (±0.003) *				−0.002 (±0.004)	0.006 (±0.004)	0.001 (±0.004)	0.002 (±0.004)	0.009 (±0.004) *

Notes: Values presented include the coefficient, the standard error (in parenthesis) and the statistical significance (• $p < 0.10$, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$). Climate is a three-level factor (boreal, temperate, and tropic zones), habitat-type is a two-level factor (reference forest and old park), and vegetation type is a two-level factor (recalcitrant tree and labile tree). Edaphic properties as influencing factors on microbial communities, including soil pH, organic matter, contents of carbon and nitrogen, and the C/N ratio, were included in the model. Young lawn in the boreal biome is in the intercept. Abbreviations: Temp, temperate; Trop, tropical; Recal, recalcitrant.

Table 2
GLMM evaluations of effects of climate, park-age, and vegetation types on microbial diversities and traits.

	Interc	Temp	Trop	Labile	Recal	Old	pH	Carboncontent	C/Nratio	Labile × Temp	Labile × Trop	Recal × Temp	Recal × Trop	Old × Temp	Old × Trop	Old × Labile	Old × Recal
Richness																	
Bacterial taxa	2813.194 (±122.654) ***	48.215 (±177.137)	−60.138 (±183.238)	−171.26 (±153.162)	−388.554 (±145.738) **	−162.659 (±179.21)				−189.262 (±210.78)	−86.617 (±218.301)	307.101 (±205.448)	446.507 (±219.574) *	−161.202 (±242.964)	341.278 (±255.284)	2.246 (±212.538)	−15.293 (±215.8)
Fungal taxa	613.633 (±29.255) ***	−55.945 (±33.51) •	−91.409 (±33.843) **	−116.986 (±25.843) ***	−85.043 (±26.03) **	−8.529 (±25.479)											
Microbial traits (× 103)	20.4 (±0.838) ***	0.768 (±1.088)	0.913 (±1.209)	−0.151 (±0.486)	−1.044 (±0.484) *	−1.29 (±0.622) *		−0.711 (±0.329) *									
Shannon-diversity																	
Bacterial taxa	7.259 (±0.077) ***	−0.065 (±0.113)	0.186 (±0.117)	−0.131 (±0.087)	−0.091 (±0.083)	−0.049 (±0.114)	0.048 (±0.02) *	−0.105 (±0.03) ***	0.042 (±0.021) *	0.038 (±0.12)	−0.154 (±0.122)	0.077 (±0.119)	−0.209 (±0.127)	0.003 (±0.147)	−0.335 (±0.158) *	0.014 (±0.118)	−0.06 (±0.12)
Fungal taxa	4.155 (±0.198) ***	0.042 (±0.218)	−0.211 (±0.22)	−0.541 (±0.183) **	−0.408 (±0.184) *	−0.183 (±0.17)											
Microbial traits	8.953 (±0.059) ***	−0.119 (±0.073)	−0.375 (±0.075) ***	0.051 (±0.044)	−0.003 (±0.044)	−0.079 (±0.052)	0.04 (±0.022) •		−0.036 (±0.02) •								
Evenness																	
Bacterial taxa	0.912 (±0.008) ***	−0.008 (±0.011)	0.031 (±0.012) **	−0.007 (±0.008)	0.005 (±0.008)	0.001 (±0.011)	0.006 (±0.002) **	−0.01 (±0.003) ***	0.004 (±0.002) •	0.01 (±0.012)	−0.017 (±0.012)	−0.001 (±0.012)	−0.045 (±0.012) ***	0.009 (±0.015)	−0.06 (±0.016) ***	−0.001 (±0.011)	−0.008 (±0.012)
Fungal taxa	0.666 (±0.046) ***	−0.048 (±0.066)	−0.075 (±0.065)	−0.131 (±0.064) *	−0.025 (±0.064)	−0.028 (±0.066)				0.171 (±0.089) •	0.174 (±0.088) *	−0.051 (±0.091)	0.03 (±0.088)	0.066 (±0.094)	0.079 (±0.094)	−0.028 (±0.093)	−0.012 (±0.089)
Microbial traits	0.906 (±0.004) ***	−0.02 (±0.005) ***	−0.049 (±0.005) ***	0.005 (±0.003)	0.005 (±0.003)	0.001 (±0.004)	0.005 (±0.002) **		−0.003 (±0.002) •								
Abundance																	
Bacterial taxa (× 1014)	1.38 (±1.775)	−0.529 (±2.522)	5.823 (±2.541) *	−1.105 (±2.461)	−2.079 (±2.449)	−0.426 (±2.448)		1.304 (±0.609) *		−0.369 (±3.585)	−4.842 (±3.468)	2.161 (±3.458)	−3.928 (±3.467)	0.129 (±3.457)	−4.515 (±3.530)	1.702 (±3.465)	1.074 (±3.460)
Fungal taxa (× 1012)	5.229 (±3.449)	−1.33 (±4.963)	−5.53 (±4.780)	−4.365 (±4.546)	−3.207 (±4.518)	−5.618 (±4.961)				3.395 (±6.033)	5.934 (±5.920)	0.371 (±6.226)	3.556 (±5.894)	−0.695 (±7.027)	7.88 (±6.848)	25.291 (±6.649) ***	15.668 (±6.109) *
Microbial traits	356.318 (±90.76)	−39.552 (±119.439)	401.296 (±127.633)	−7.142 (±121.118)	39.121 (±118.365)	−17.731 (±120.344)	−51.017 (±21.561)			52.242 (±151.626)	−272.213 (±159.314)	−20.69 (±145.385)	−55.241 (±155.752)	−67.759 (±156.55)	337.121 (±167.641)	−85.753 (±152.556)	−168.991 (±161.969)

(*continued on next page*)

Table 2 (*continued*)

	Interc	Temp	Trop	Labile	Recal	Old	pH	Carbon content	C/N ratio	Temp × Labile	Trop × Labile	Temp × Recal	Trop × Recal	Temp × Old	Trop × Old	Labile × Old	Recal × Old
	***		**				*				•				*		
Taxa relative abundance																	
Actinobacteria	0.296 (±0.026) ***	0.006 (±0.039)	0.062 (±0.038)	−0.035 (±0.035)	0.007 (±0.04)	0.028 (±0.039)		0.018 (±0.011) •		0.021 (±0.054)	0.008 (±0.05)	−0.046 (±0.054)	−0.019 (±0.054)	−0.121 (±0.057) *	−0.032 (±0.057)	0.095 (±0.051) •	0.011 (±0.053)
Proteobacteria	0.311 (±0.011) ***	−0.006 (±0.012)	−0.007 (±0.012)	−0.001 (±0.01)	−0.004 (±0.01)	−0.021 (±0.009) *											
Acidobacteria	0.14 (±0.011) ***	0.022 (±0.016)	−0.023 (±0.016)	0.008 (±0.015)	−0.008 (±0.016)	−0.023 (±0.016)				−0.007 (±0.022)	−0.004 (±0.022)	0.011 (±0.022)	0.015 (±0.022)	0.07 (±0.023) **	0.033 (±0.023)	−0.022 (±0.022)	0.001 (±0.022)
Verrucomicrobia	0.078 (±0.007) ***	−0.007 (±0.009)	−0.038 (±0.009) ***	0.001 (±0.005)	0.001 (±0.005)	0.003 (±0.006)	−0.008 (±0.002) ***										
Bacteroidetes	0.056 (±0.004) ***	−0.015 (±0.004) ***	−0.042 (±0.005) ***	−0.004 (±0.003)	−0.003 (±0.003)	−0.001 (±0.003)	0.003 (±0.002) *	−0.006 (±0.002) **	0.006 (±0.002) **								
Firmicutes (× 10–2)	0.004 (±0.003)	0.003 (±0.003)	0.022 (±0.003) ***	−0.001 (±0.002)	−0.002 (±0.002)	0.004 (±0.002) •											
Ascomycota	0.363 (±0.059) ***	−0.065 (±0.084)	0.464 (±0.084) ***	0.284 (±0.084) ***	0.293 (±0.084) ***	0.044 (±0.084)				0.002 (±0.118)	−0.292 (±0.118) *	−0.011 (±0.118)	−0.205 (±0.118) •	0.127 (±0.118)	−0.053 (±0.118)	−0.017 (±0.118)	0.084 (±0.118)
Basidiomycota	0.287 (±0.032) ***	−0.103 (±0.031) **	−0.175 (±0.032) ***	−0.092 (±0.031) **	−0.125 (±0.032) ***	−0.012 (±0.027)	−0.031 (±0.014) *										
Mortierellomycota	0.217 (±3.249) ***	0.105 (±3.225) **	−0.126 (±3.246) ***	−0.09 (±3.223) **	−0.083 (±3.265) *	−0.043 (±2.774)	0.029 (±1.428) *										
Ectomycorrhiza	0.015 (±0.021)	−0.015 (±0.030)	−0.015 (±0.030)	−0.005 (±0.026)	0.027 (±0.029)	0.059 (±0.029) *				0.008 (±0.039)	0.005 (±0.035)	−0.021 (±0.038)	−0.028 (±0.038)	0.002 (±0.041)	−0.06 (±0.041)	−0.056 (±0.036)	−0.098 (±0.038) *
Trait relative abundance																	
Carbon degradation	0.589 (±0.016) ***	0.099 (±0.023) ***	0.201 (±0.028) ***	0.043 (±0.019) *	0.042 (±0.022) •	0.001 (±0.023)				−0.017 (±0.027)	−0.046 (±0.03)	−0.02 (±0.029)	−0.042 (±0.035)	−0.087 (±0.033) **	0.009 (±0.036)	0.016 (±0.028)	−0.024 (±0.029)
Carbon fixation	0.145 (±0.007) ***	−0.046 (±0.011) ***	−0.088 (±0.013) ***	−0.023 (±0.009) **	−0.019 (±0.01) •	−0.003 (±0.011)				0.011 (±0.013)	0.022 (±0.014)	0.01 (±0.013)	0.015 (±0.016)	0.043 (±0.015) **	−0.005 (±0.016)	−0.004 (±0.013)	0.014 (±0.013)
Methane (× 10–4)	33.1 (±2.383) ***	−1.276 (±2.967)	−19.39 (±2.994) ***	−3.119 (±1.620) •	−4.986 (±1.673) **	−2.499 (±1.977)											
Assimilatory N reduction	0.024 (±0.001) ***	−0.007 (±0.002) ***	−0.012 (±0.002) ***	−0.003 (±0.002)	−0.001 (±0.002)	0.002 (±0.002)				0.001 (±0.002)	0.003 (±0.003)	0.001 (±0.002)	0.002 (±0.003)	0.006 (±0.003) *	−0.002 (±0.003)	−0.003 (±0.002)	−0.001 (±0.002)
Nitrogen fixation (× 10–3)	7.915 (±0.616) ***	−4.426 (±0.854) ***	−6.945 (±1.070) ***	−1.571 (±0.626) *	−3.071 (±0.785) ***	−2.565 (±0.828) **		−0.491 (±0.200) *		1.017 (±0.882)	1.721 (±1.015) •	2.588 (±1.020) *	3.198 (±1.250) *	5.155 (±1.140) ***	2.521 (±1.247) *	1.265 (±0.899)	2.726 (±1.015) **
Denitrification	0.019	0.006	−0.011	0.001	0.001	−0.001			0.001								

(*continued on next page*)

Table 2 (*continued*)

	Interc	Temp	Trop	Labile	Recal	Old	pH	Carbon content	C/N ratio	Temp × Labile	Trop × Labile	Temp × Recal	Trop × Recal	Temp × Old	Trop × Old	Labile × Old	Recal × Old
	(±0.002)	(±0.002)	(±0.002)	(±0.001)	(±0.001)	(±0.001)			(±0.001)								
	***	***	***						*								

Notes: Values presented include the coefficient, the standard error (in parenthesis) and the statistical significance (• $p < 0.10$, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$). Climate is a three-level factor (boreal, temperate, and tropic zones), park-age is a two-level factor (old and young parks), and vegetation type is a three-level factor (recalcitrant tree, labile tree, and lawn). Edaphic properties as influencing factors on microbial communities, including soil pH, organic matter, contents of carbon and nitrogen, and the C/N ratio, were included in the model. Young lawn in the boreal biome is in the intercept. Abbreviations: Temp, temperate; Trop, tropical; Recal, recalcitrant.

(Fig. 3 a, Fig. S2 a). Dominant fungal and bacterial phyla did not respond consistently to plants producing labile or recalcitrant litter (Fig. S5).

Overall, vegetation type did not influence functional trait richness in the two habitat types across climates but affected specific functions (Fig. 3). For instance, traits for C degradation were more numerous under labile trees (Fig. 3 d) whereas C- and N-fixation traits, or assimilatory N reduction traits, were generally more abundant under recalcitrant trees (Fig. 3 e, g, h).

3.2. The effect of vegetation type and park age on soil microbial communities in urban parks

Park age affected the composition of both bacterial and fungal communities and their functional traits (Fig. 4 a-f, Table S5). This was particularly true in the boreal city. In contrast, park age had little effect on bacterial and fungal richness (Fig. 5 a, b), qPCR-inferred abundances (Fig. S3 g, h), or the dominant bacterial and fungal phyla (Fig. S6). However, functional trait richness was generally greater in young than in old parks (Fig. 5 c). Further, park age had a weak effect on the relative abundance of C- and N-process related genes (Fig. 5 d-i).

Vegetation type affected the composition of both bacterial and fungal communities, but not their functional traits (Fig. 4 g-i, Table S5). Vegetation type also affected microbial richness across climatic zones. In both young and old parks, fungal and bacterial richness was highest in lawn soils (Fig. 5 a, b). However, qPCR-inferred fungal abundance was generally higher under recalcitrant trees than under lawns (Fig. S3 h). This was largely attributable to the higher relative abundance of fungi in the phylum Ascomycota under recalcitrant trees than in lawn soils across climates (Fig. S6 g). However, bacterial abundances differed little among vegetation types (Fig. S3 g). In terms of functional genes, lawns had generally lower abundances of traits associated with C-degradation (Fig. 5 d), but higher relative abundances of traits associated with C-fixation, methane processing and assimilatory N-reduction, except in the tropics (Fig. 5 e, f, g). Similarly to the reference forest vs. old park comparison above (Fig. 3 d-h), the relative abundance of C-degradation traits was greatest in the tropics, whereas traits associated with C- and N-fixation and assimilatory N-reduction and methane were lowest there (Fig. 5 d-h).

4. Discussion

We showed that urbanisation affected microbial community composition similarly across three climatic zones. Surprisingly, microbial functional attributes were less responsive to urbanisation and vegetation type. Below we explore our findings in greater detail as it relates to urbanisation/habitat type, vegetation type and park age effects on the soil microbiota.

4.1. Urbanisation effect (hypothesis 1)

Community composition. Our study indicates distinct fungal and bacterial communities as well as distinct functional trait compositions between the (semi-)natural reference forests and old parks across climatic zones (see Fig. 2), supporting our hypotheses and corroborating many other studies (Abrego et al., 2020; Chen et al., 2021; Hui et al., 2017a; Liu et al., 2022; Norton et al., 2019; Ramirez et al., 2014; Reese et al., 2016; Schmidt et al., 2017; Tan et al., 2019). Generally, these studies report that both bacterial and fungal communities differ along urbanisation gradients – an effect likely attributable to anthropogenic drivers often associated with disturbances such as lawn mowing, litter removal and trampling (Pouyat et al., 2010; Shade et al., 2012), but also to differences in edaphic properties (Jesus et al., 2009; Kotze et al., 2021).

In our study, these anthropogenic disturbances proved surprisingly similar in their impacts on soil microbial communities across biomes with vastly different climates and natural histories. This may be because

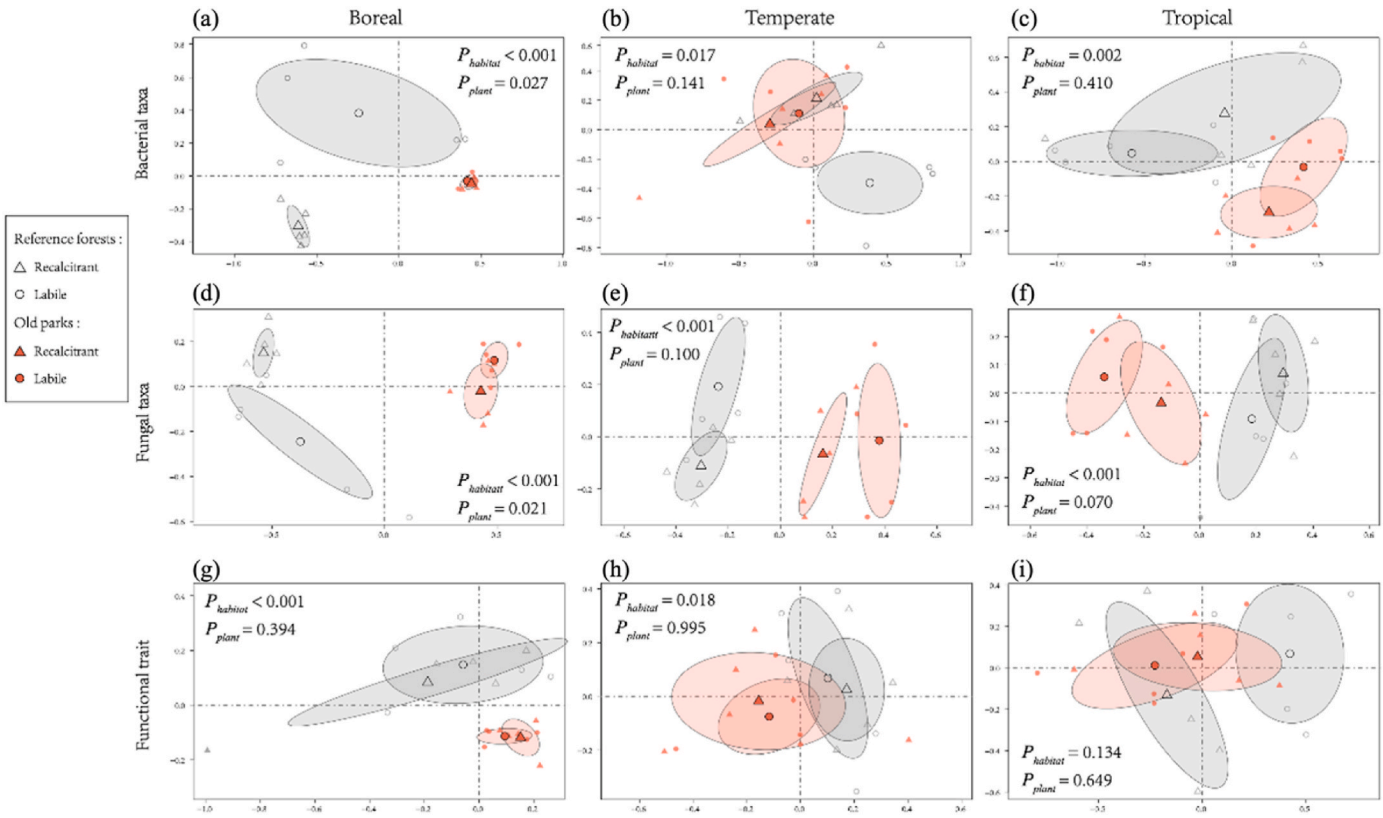

Fig. 2. NMDS ordinations show the composition of the bacterial (a–c) and fungal (d–f) communities, and their functional traits (g–i) among vegetation types (plant) in old parks vs. reference forests across the three climatic zones. The stress values of each figure are listed in Table S4. Ellipses represent 95% confidence intervals. Statistical inference and estimates of significance were calculated using the *envfit* function in the vegan R package.

of the strong anthropogenic effect in urban parks, suggesting that microbial communities are homogenised in urban park soils across biomes (Schmidt et al., 2017) and thus clearly differ from those in reference forests. Besides the across-biome urbanisation effect on soil microbial community composition, urbanisation also strongly affected their functional traits. This corroborates Chen et al. (2021) who reported that soil microbial functional group composition changed along an urbanization gradient in Arizona, USA. They hypothesized that this change is due to root exudates, whose production is greater in non-urban areas with higher plant diversity than in managed urban parks. The greater availability of plant-derived resources positively affects soil microbial biomass (Schops et al., 2018; Solly et al., 2014), whose function (e.g., extracellular enzymatic activities) (Steinauer et al., 2016) also responds to the diverse root exudates.

Community metrics of the microbiota. Our hypothesis of the greater microbial richness in reference forests than in old parks was partly supported as fungal, but not bacterial, richness and abundance were consistently higher in the semi-natural forests than in urban parks across biomes. This was largely attributable to the changes in fungi in the phylum Basidiomycota, which was also reflected in the greatest changes in Mycorrhizal (Fig. S7c). Our results support others (Abrego et al., 2020; Chen et al., 2021; Schmidt et al., 2017) who reported a decrease in fungal diversity in urban systems. As fungi are sensitive to disturbances (Shi et al., 2019; Xu et al., 2021), their greater richness in reference forests across climatic zones seems plausible. This is in line with Kotze and Setälä (2021), who reported faster decomposition of recalcitrant material in semi-natural forests than in parks – a process largely attributable to fungal communities. However, most local to regional studies have showed either no urbanisation effect on fungal richness (Hui et al., 2017b; Norton et al., 2019; Reese et al., 2016; Tan et al., 2019; Xu et al., 2021) or – surprisingly – an increase in richness with urbanisation (Huo et al., 2017; Liu et al., 2022; Naylo et al., 2019), likely resulting from local park management practices (e.g., fertilization, transport and import of soils from other sources).

That neither bacterial richness nor abundance responded to urbanization here is in line with previous reports (Chen et al., 2021; Norton et al., 2019; Reese et al., 2016; Schmidt et al., 2017), although the abundances of Proteobacteria and Bacteroidetes were lower in urban sites. That no bacterial phyla seem to consistently respond to urbanization aligns with Ramirez et al. (2014) who reported that bacteria in New York Central Park soils differed little from those of representative ecosystems across the globe probably because of their greater adaptability to diverse environments.

Surprisingly, functional traits, their richness or abundances did not respond consistently to urbanisation. For instance, C degradation genes were more abundant in old urban parks, whereas many other functional genes tended to be more abundant in reference forests. This implies that microbially driven ecosystem services may be resistant to the effects of urbanisation.

The climate zones differed in their functional gene abundances, with most declining towards the equator. An exception was the genes associated with C degradation, whose abundance was highest in the tropics. Like in natural systems, C and nutrient cycling in urban systems are microbially driven (Pouyat et al., 2010; Szlavecz et al., 2017), and these processes depend on temperature (Davidson and Janssens 2006). Further, labile litter turnover is more sensitive to temperature than that of recalcitrant litter (Stuble et al., 2019). Thus, the increase in genes involved in decomposing labile C compounds could be responsible for the overall increase in C degradation genes in the tropics.

4.2. *Vegetation type effect (hypotheses 2 & 3)*

Community composition. When comparing reference forests to old urban parks, vegetation type (trees producing recalcitrant or labile

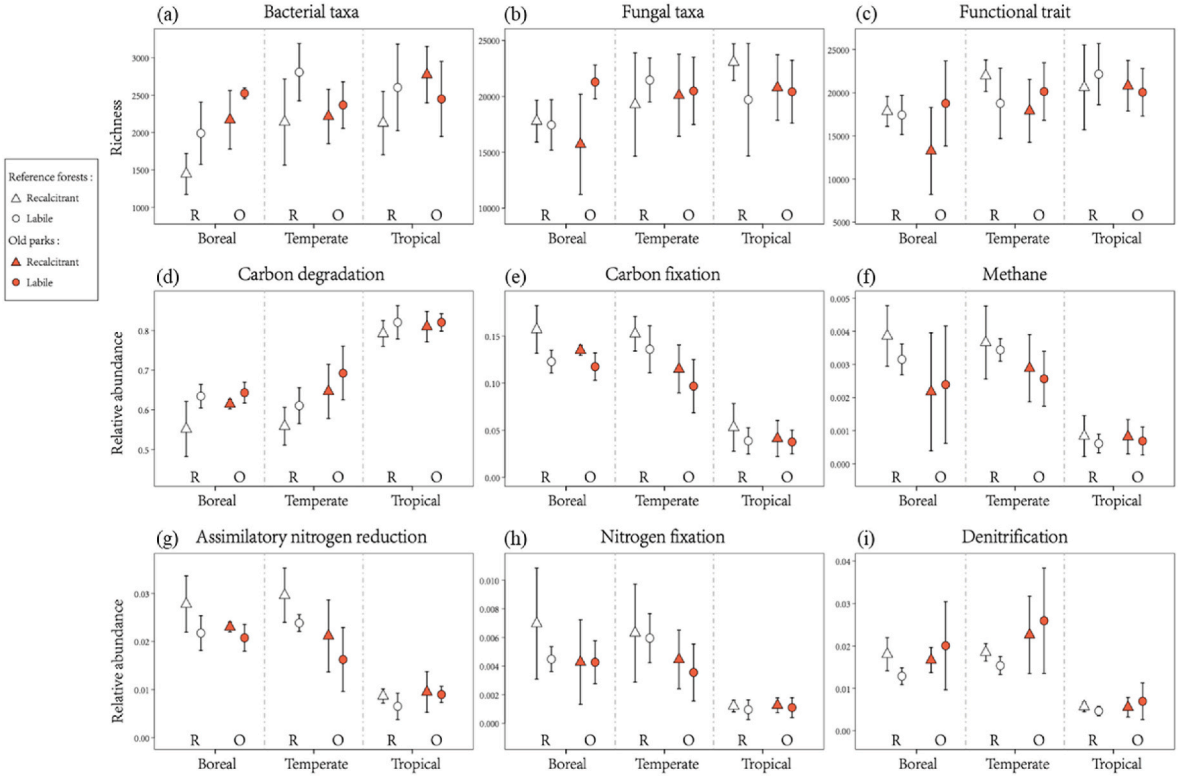

Fig. 3. The effect of habitat type (old parks vs. reference forests) as influenced by vegetation (plant) type (trees producing recalcitrant or labile litter) on microbial communities across the three climatic zones. Bacterial (a), fungal (b), and functional trait richness (c), and the relative abundances of six functional traits (d–i). Other diversity indices (Shannon-diversity and evenness), taxon/trait abundance, and representative polyphosphate degradation and sulfide oxidation functional gene groups can be found in Figs. S2 and S4. GLMM predicted values are listed in Extended Table 1.

litter) affected the fungal community composition only in the boreal zone. These results agree with Hui et al. (2017a, b), who compared fungal communities in general and ectomycorrhizal communities specifically in a similar system. A likely explanation for the observed vegetation type effect in the boreal zone and its absence in the warmer temperate and tropical zones is that OM accumulates under recalcitrant trees (here *Picea* sp.) in the boreal climates due to cold climate, whereas labile litter under linden decomposes quickly (Merilä et al., 2010). In urban parks, litter and OM accumulation and decomposition are further limited because of park management. In contrast, OM does not accumulate in reference forests in the temperate and tropical zones under trees producing labile or recalcitrant litter, similarly to old trees in parks but due to different processes (fast decomposition due, e.g., to the activities of earthworms in natural environments vs. earthworms and management in urban parks) (Hedĕnec et al., 2022).

When only urban parks were considered, vegetation affected the microbiota across climatic zones. This vegetation effect on microbial communities does not necessarily indicate a strong tree type effect, but may be attributable to the vastly different vegetation, *i.e.*, the presence of lawn that affected fungal and bacterial diversity (see below). Similarly, Ramirez et al. (2014) reported differences in both bacterial and eukaryote communities among plant cover types in Central Park, New York, and Norton et al. (2019) reported that microbial communities were sensitive to plant species composition in differently managed urban lawns. Despite differences in community composition, we observed no differences in microbial functional trait composition among vegetation types across the climatic zones, suggesting that soil microbial communities are functionally redundant (Setälä and McLean 2004) across habitat types and climates, even if their compositions differ. We posit that urbanisation is a megatrend that manifests itself in soil communities, although whether these trends are reflected in ecosystem process rates remains unverified.

Community metrics of the microbiota. Our hypothesis of a more species rich and abundant fungal communities under recalcitrant trees was supported in our comparisons of the reference forests and old parks across biomes. We are unaware of any studies that have investigated this, yet there is evidence that plants producing structurally complex recalcitrant litter harbour rich and abundant fungal communities, especially Ascomycota (Baldrian et al., 2012; Šnajdr et al., 2011). This may be attributable to ascomycetes that contribute to the early decomposition of litter and their persistence at high abundance throughout the decomposition process (Vivelo and Bhatnagar 2019). When investigating parks only, lawns (absent in reference forests) appeared to have a surprisingly strong effect on fungal richness (Hui et al., 2017b), but no such effect was observed on overall abundance, or the abundances of dominant phyla. This challenges the view that recalcitrant litter favours more diverse soil microbes than labile litter (Bahram et al., 2018; Crowther et al., 2019; Tedersoo et al., 2012). This may relate to higher root density under lawns and the greater inputs from grass clipping under lawns than under trees. The resultant greater provision of root exudates and greater litter inputs would in turn enrich microbial diversity (Mgelwa et al., 2019; Prada-Salcedo et al., 2022; Schops et al., 2018; Solly et al., 2014).

In general, when comparing reference forests to old parks, bacterial richness was higher, but bacteria were not more abundant under labile trees across climatic zones, reflected especially by the relative abundance of the phylum Actinobacteria. This corroborates our previous work in the boreal zone (Hui et al., 2017a), suggesting bacterial community turnover rather than a change in population size in soils with labile litter. In urban parks, bacterial richness in lawn soils was higher than underneath the two tree types, probably because vegetation producing labile litter favours bacteria that can utilize labile litter effectively (Kotze and Setälä, 2021).

Independent of habitat type or park age, functional trait richness was

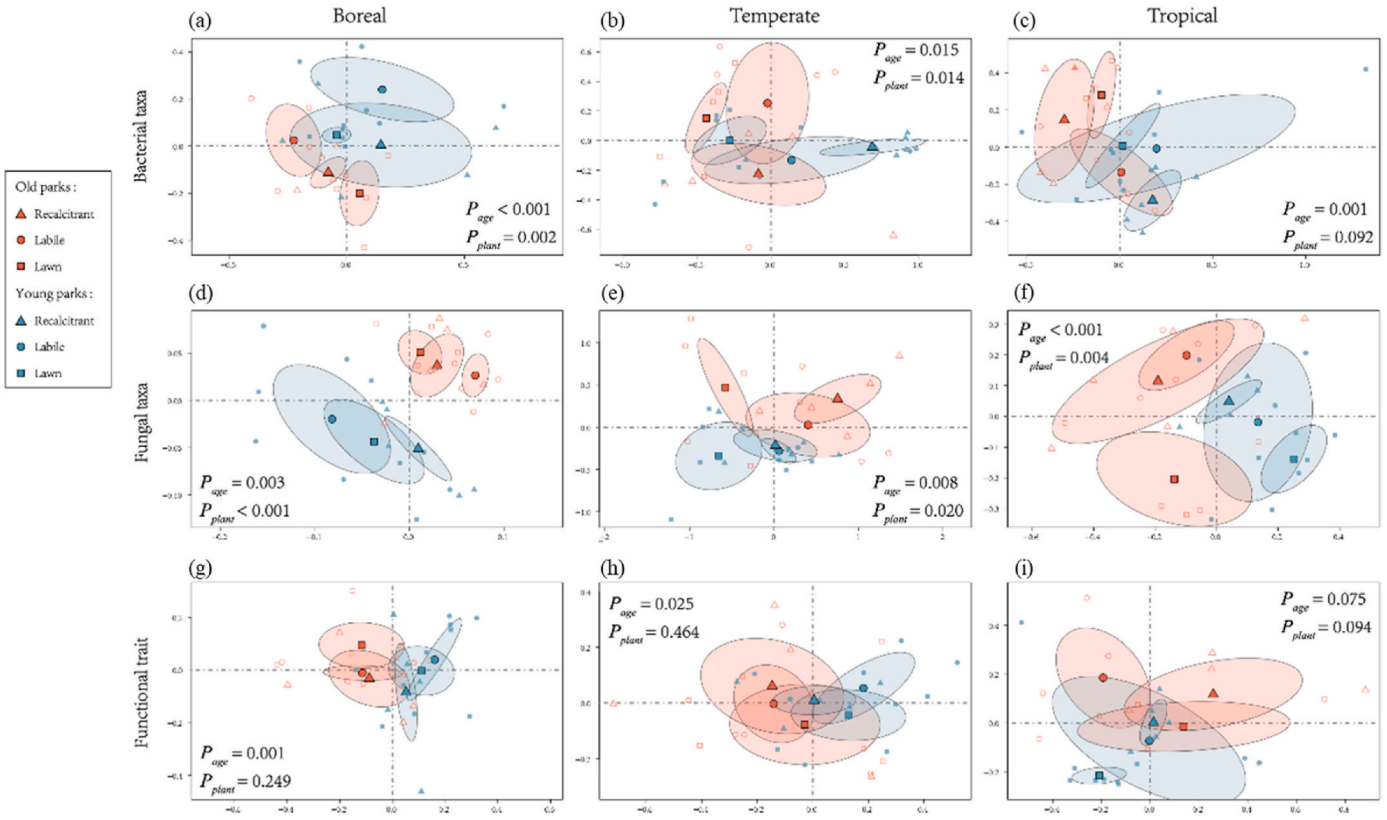

Fig. 4. NMDS ordinations show the composition of the bacterial (a–c) and fungal (d–f) communities, and microbial traits (g–i) among vegetation types in young vs. old parks across biomes. The stress values of each figure are listed in Table S4. Ellipses represent 95% confidence intervals. Statistical inference and estimates of significance were calculated using the *envfit* function in the vegan R package.

surprisingly unresponsive to vegetation type across biomes. Similarly, the relative abundances of the functional genes responded only weakly to vegetation type. This suggests that the carbon source may not account for the change of functional diversity, implying a more complicated, indirect relationship between vegetation type and ecosystem function. In contrast, the biome effect was clear and functional gene abundances mainly decreased towards the tropics, with the notable exception of C degradation that increased. The greater relative abundance of ascomycetes in tropical park soils may suggest higher cellulolytic potential in warmer regions (Sauvadet et al., 2016; Voriskova and Baldrian 2013), whereas the lesser functional trait richness in warmer regions may stem from a lower temperature sensitivity of other soil processes, such as N mineralization and nitrification (Auyeung et al., 2013; Kirschbaum 1995).

4.3. Park age effect (hypothesis 4)

Community composition. The soil microbial community and functional trait compositions were structured by park age across biomes, corroborating earlier findings in a boreal system (Hui et al., 2017a). This strong park age effect likely results from the length of time that plants have interacted with the soils (Kotze et al., 2021; Setälä et al., 2016) through above- and belowground OM inputs. At maximum, plants in the old parks had ten times longer to modify soils. Thus, it is not surprising that microbial communities also responded to park age, *i.e.,* the length of plant-soil interaction.

Community metrics of the microbiota. Unlike community composition, park age had a small effect on bacterial, fungal and functional trait richness, trait abundances and the abundances of functional genes across climatic zones. This is in partial support of Hui et al. (2017a) in the boreal zone, where bacteria did not respond to park age, whereas fungal richness tended to be less in older parks. These results support our previous work in these cities (Kotze et al., 2021), where vegetation type effects on soil properties were independent of park age. Even though soil OM, fertility, and microbial activity can increase with age of urban lawns (Cheng et al., 2008), our results suggest that, in the absence of aboveground litter inputs from trees (as a result of continuous litter removal by raking), the time that different plants had modified soils (*i.e.*, park age) has little impact on the diversity and abundance of soil microbes in highly managed urban parks. In essence, human disturbance in young and old urban parks may prevent plant–soil interactions from reaching a steady state even decades after park construction, resulting in similar soil properties (Kotze et al., 2021) and soil communities under different vegetation types regardless of park age.

Park age also had a small/no effect on the relative abundance of functional potential in these systems. Even if the microbial communities were compositionally different due to soil properties (such as soil carbon and pH) that had changed by the length of time different types of plants had modified them (Yin et al., 2021), their functions were not, suggesting functional redundancy and resistance to urban disturbances. It is important to note that we specifically examined differences in the topmost soil (mineral and/or humus-mineral layers) underneath plants producing either recalcitrant or labile litter within the organic horizon but excluding litter. Process rates among the biomes, habitats and different plants may have varied but remained undetected, as the GeoChip gene arrays only represents the metagenomic potential and the presence of the probed functional genes, not the activity of the microbial communities. This highlights the need to complement these data with measures of activity for a more holistic understanding of microbial functionality. The inherent limitations of GeoChip, such as its inability to capture novel gene variants or microbial taxa absent on the gene array, also underscores the potential for underestimating microbial diversity and function (He et al., 2015; Robinson et al., 2018). Furthermore, microbial community turnover in our study may be insufficient to

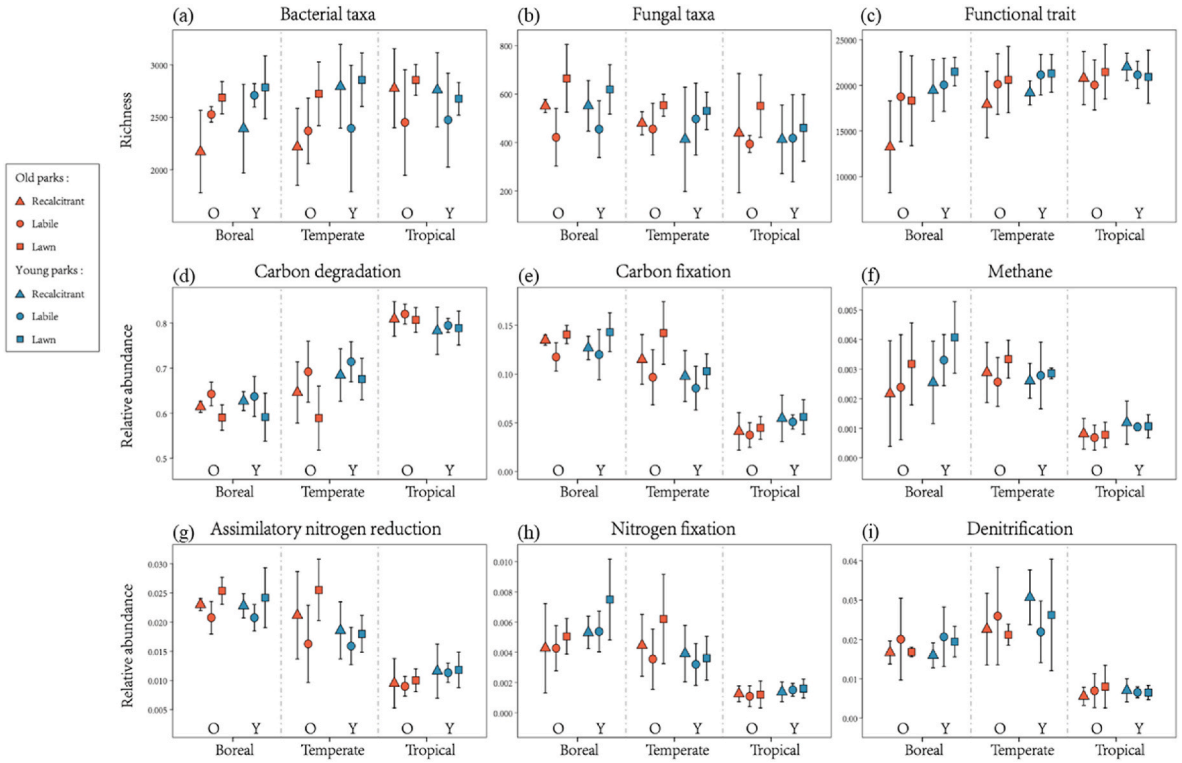

Fig. 5. The effects of vegetation type on microbial communities between urban parks (young vs old parks). Bacterial (a), fungal (b), and functional trait richness (c), and the relative abundances of six functional traits (d–i). Other diversity indices (Shannon-diversity and evenness), taxon/trait abundance, and representative polyphosphate degradation and sulfide oxidation functional gene groups can be found in Figs. S3 and S4. GLMM predicted values are listed in Extended Table 2.

observe changes in functional potentials, a system attribute that may primarily be regulated by climate.

5. Conclusions

The effects of urbanisation and the influence of vegetation type on the urban soil microbiota have not been tackled across biomes. Our results highlight that urbanisation and vegetation type are important in structuring soil communities across biomes, as is suggested to be the case in other, non-urban ecosystems (Cornwell et al., 2008; Pouyat et al., 2015; Wardle et al., 2004). Our data suggest functional redundancies among the soil microbiota across biomes and habitats, suggesting that anthropogenic disturbances associated with urbanisation may not compromise ecosystem service provisioning in urban greenspace soils. These data also indicate that vegetation type within urban parks strongly affects microbial communities, providing further support for the ecological theory that plant functional traits regulate the outcomes of ecosystem processes across regions (Grime 2006; Wardle et al., 2004).

Author contributions

Heikki Setala: Conceptualization, Funding acquisition, Supervision, Writing – review & editing. Nan Hui: Conceptualization, Funding acquisition, Writing – original draft, Writing – review & editing. Ian Yesilonis: Investigation, Methodology. David Wardle: Investigation, Writing – review & editing. Katalin Szlavecz: Investigation, Validation. Richard Pouyat: Investigation, Supervision. Changyi Lu: Investigation, Software. D. Johan Kotze: Conceptualization, Methodology, Writing – review & editing. Ari Jumpponen: Methodology, Validation, Visualization. Lantian Su: Software, Writing – original draft. Bangxiao Zheng: Methodology, Writing – original draft

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.

Data availability

No data was used for the research described in the article.

Acknowledgments

This research was financially supported by the National Key R&D Program of China (no. 2023YFE0112000), the Science and Technology Commission of Shanghai Municipality (no. 22230713300) and the National Science Foundation of China (no. 32371843), which were originated from the PARKTRAITS project (Academy of Finland, no. 1315987). We acknowledge Dr. Subhadip Ghosh, Dr. Benjamin Lee, Dr. Shawn Lum, Yinhong Hu, Elliott Stratham, Mohamed Yusof, John Allen, Janne Auranen, Peter Immonen and Allan Delesantro who have helped us in the field, as well as Dr. Xinxin Liu who performed the qPCR assays.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.soilbio.2024.109352.

References

Abrego, N., Crosier, B., Somervuo, P., Ivanova, N., Abrahamyan, A., Abdi, A., Hamalainen, K., Junninen, K., Maunula, M., Purhonen, J., Ovaskainen, O., 2020. Fungal communities decline with urbanization-more in air than in soil. ISME Journal 14, 2806–2815. https://doi.org/10.1038/s41396-020-0732-1.

Allen, J.A., Setala, H., Kotze, D.J., 2020. Dog urine has acute impacts on soil chemistry in urban greenspaces. Frontiers in Ecology and Evolution 8. https://doi.org/10.3389/fevo.2020.615979.

Andreote, F.D., Silva, M., 2017. Microbial communities associated with plants: learning from nature to apply it in agriculture. Current Opinion in Microbiology 37, 29–34. https://doi.org/10.1016/j.mib.2017.03.011.

Auyeung, D.S.N., Suseela, V., Dukes, J.S., 2013. Warming and drought reduce temperature sensitivity of nitrogen transformations. Global Change Biology 19, 662–676. https://doi.org/10.1111/gcb.12063.

Bahram, M., Hildebrand, F., Forslund, S.K., Anderson, J.L., Soudzilovskaia, N.A., Bodegom, P.M., Bengtsson-Palme, J., Anslan, S., Coelho, L.P., Harend, H., Huerta-Cepas, J., Medema, M.H., Maltz, M.R., Mundra, S., Olsson, P.A., Pent, M., Polme, S., Sunagawa, S., Ryberg, M., Tedersoo, L., Bork, P., 2018. Structure and function of the global topsoil microbiome. Nature 560, 233–237. https://doi.org/10.1038/s41586-018-0386-6.

Baldrian, P., Kolarik, M., Stursova, M., Kopecky, J., Valaskova, V., Vetrovsky, T., Zifcakova, L., Snajdr, J., Ridl, J., Vlcek, C., Voriskova, J., 2012. Active and total microbial communities in forest soil are largely different and highly stratified during decomposition. The ISME Journal 6, 248–258. https://doi.org/10.1038/ismej.2011.95.

Bardgett, R.D., Wardle, D.A., 2010. Aboveground-belowground Linkages: Biotic Interactions, Ecosystem Processes, and Global Change. Oxford University Press.

Bates, D., Mächler, M., Bolker, B., Walker, S., 2015. Fitting linear mixed-effects models using lme4. Journal of Statistical Software 67, 1–48. https://doi.org/10.18637/jss.v067.i01.

Buzzard, V., Michaletz, S.T., Deng, Y., He, Z.L., Ning, D.L., Shen, L.N., Tu, Q.C., Van Nostrand, J.D., Voordeckers, J.W., Wang, J.J., Weiser, M.D., Kaspari, M., Waide, R. B., Zhou, J.Z., Enquist, B.J., 2019. Continental scale structuring of forest and soil diversity via functional traits (vol 3, pg 1298, 2019). Nature Ecology & Evolution 3, 1607. https://doi.org/10.1038/s41559-019-1014-z, 1607.

Carson, C.M., Jumpponen, A., Blair, J.M., Zeglin, L.H., 2019. Soil fungal community changes in reponse to long-term fire cessation and N fertilization in tallgrass prairie. Fungal Ecology 41, 45–55.

Chen, Y.J., Martinez, A., Cleavenger, S., Rudolph, J., Barberan, A., 2021. Changes in soil microbial communities across an urbanization gradient: a local-scale temporal study in the arid southwestern USA. Microorganisms 9. https://doi.org/10.3390/microorganisms9071470.

Cheng, Z., Richmond, D., Salminen, S., Grewal, P., 2008. Ecology of urban lawns under three common management programs. Urban Ecosystems 11, 177–195.

Cornwell, W.K., Cornelissen, J.H.C., Amatangelo, K., Dorrepaal, E., Eviner, V.T., Godoy, O., Hobbie, S.E., Hoorens, B., Kurokawa, H., Perez-Harguindeguy, N., Quested, H.M., Santiago, L.S., Wardle, D.A., Wright, I.J., Aerts, R., Allison, S.D., van Bodegom, P., Brovkin, V., Chatain, A., Callaghan, T.V., Diaz, S., Garnier, E., Gurvich, D.E., Kazakou, E., Klein, J.A., Read, J., Reich, P.B., Soudzilovskaia, N.A., Vaieretti, M.V., Westoby, M., 2008. Plant species traits are the predominant control on litter decomposition rates within biomes worldwide. Ecology Letters 11, 1065–1071. https://doi.org/10.1111/j.1461-0248.2008.01219.x.

Crowther, T.W., van den Hoogen, J., Wan, J., Mayes, M.A., Keiser, A.D., Mo, L., Averill, C., Maynard, D.S., 2019. The global soil community and its influence on biogeochemistry. Science 365, 772. https://doi.org/10.1126/science.aav0550.

Davidson, E.A., Janssens, I.A., 2006. Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. Nature 440, 165–173. https://doi.org/10.1038/nature04514.

Delgado-Baquerizo, M., Eldridge, D.J., Liu, Y.-R., Sokoya, B., Wang, J.-T., Hu, H.-W., He, J.-Z., Bastida, F., Moreno, J.L., Bamigboye, A.R., 2021. Global homogenization of the structure and function in the soil microbiome of urban greenspaces. Science Advances 7, eabg5809.

Garcia-Palacios, P., Maestre, F.T., Kattge, J., Wall, D.H., 2013. Climate and litter quality differently modulate the effects of soil fauna on litter decomposition across biomes. Ecology Letters 16, 1045–1053. https://doi.org/10.1111/ele.12137.

Grime, J.P., 2006. Plant Strategies, Vegetation Processes, and Ecosystem Properties. John Wiley & Sons.

Grimm, N.B., Faeth, S.H., Golubiewski, N.E., Redman, C.L., Wu, J.G., Bai, X.M., Briggs, J. M., 2008. Global change and the ecology of cities. Science 319, 756–760. https://doi.org/10.1126/science.1150195.

Habtewold, J.Z., Helgason, B.L., Yanni, S.F., Janzen, H.H., Ellert, B.H., Gregorich, E.G., 2020. Litter composition has stronger influence on the structure of soil fungal than bacterial communities. European Journal of Soil Biology 98. https://doi.org/10.1016/j.ejsobi.2020.103190.

Heděnec, P., Jiménez, J.J., Moradi, J., Domene, X., Hackenberger, D., Barot, S., Frossard, A., Oktaba, L., Filser, J., Kindlann, P., Frouz, J., 2022. Global distribution of soil fauna functional groups and their estimated litter consumption across biomes. Scientific Reports 12, 17362.

He, Z., Van Nostrand, J.D., Zhou, J., 2015. Chip-based metagenomic technologies for analyzing microbial community functional structure and activities. In: Nelson, K.E. (Ed.), Encyclopedia of Metagenomics. Spring, Boston, MA. https://doi.org/10.1007/978-1-4899-7478-5_760.

Hui, N., Jumpponen, A., Francini, G., Kotze, D.J., Liu, X.X., Romantschuk, M., Strommer, R., Setala, H., 2017a. Soil microbial communities are shaped by vegetation type and park age in cities under cold climate. Environmental Microbiology 19, 1281–1295. https://doi.org/10.1111/1462-2920.13660.

Hui, N., Liu, X., Kotze, D.J., Jumpponen, A., Francini, G., Setälä, H., 2017b. Ectomycorrhizal fungal communities in urban parks are similar to those in natural forests but shaped by vegetation and park age. Applied and Environmental Microbiology 83, e01797-01717.

Huo, Y., Bai, Y.H., Qu, J.H., 2017. Unravelling riverine microbial communities under wastewater treatment plant effluent discharge in large urban areas. Applied Microbiology and Biotechnology 101, 6755–6764. https://doi.org/10.1007/s00253-017-8384-4.

Jansson, J.K., Hofmockel, K.S., 2020. Soil microbiomes and climate change. Nature Reviews Microbiology 18, 35–46.

Jesus, E.D., Marsh, T.L., Tiedje, J.M., Moreira, F.M.D., 2009. Changes in land use alter the structure of bacterial communities in Western Amazon soils (vol 3, pg 1004, 2009). ISME Journal 3, 1222. https://doi.org/10.1038/ismej.2009.98, 1222.

Kirschbaum, M.U., 1995. The temperature dependence of soil organic matter decomposition, and the effect of global warming on soil organic C storage. Soil Biology and Biochemistry 27, 753–760.

Klassen, J.L., 2018. Defining microbiome function. Nature Microbiology 3, 864–869.

Kotze, D.J., Ghosh, S., Hui, N., Jumpponen, A., Lee, B., Lu, C.Y., Lum, S., Pouyat, R., Szlavecz, K., Wardle, D.A., Yesilonis, I., Zheng, B.X., Setala, H., 2021. Urbanization minimizes the effects of plant traits on soil provisioned ecosystem services across climatic regions. Global Change Biology 27, 4139–4153. https://doi.org/10.1111/gcb.15717.

Kotze, D.J., Setälä, H., 2021. Urbanisation differently affects decomposition rates of recalcitrant woody material and labile leaf litter. Urban Ecosystems 25, 65–74. https://doi.org/10.1007/s11252-021-01125-3.

Koziol, L., Bever, J.D., 2017. The missing link in grassland restoration: arbuscular mycorrhizal fungi inoculation increases plant diversity and accelerates succession. Journal of Applied Ecology 54, 1301–1309. https://doi.org/10.1111/1365-2664.12843.

Liu, L., Barberan, A., Gao, C., Zhang, Z.C., Wang, M., Wurzburger, N., Wang, X., Zhang, R., Li, J.X., Zhang, J., 2022. Impact of urbanization on soil microbial diversity and composition in the megacity of Shanghai. Land Degradation & Development 33, 282–293. https://doi.org/10.1002/ldr.4145.

Lu, C.Y., Kotze, D.J., Setälä, H.M., 2021. Evergreen trees stimulate carbon accumulation in urban soils via high root production and slow litter decomposition. Science of the Total Environment 774. https://doi.org/10.1016/j.scitotenv.2021.145129.

Meier, C.L., Bowman, W.D., 2008. Links between plant litter chemistry, species diversity, and below-ground ecosystem function. Proceedings of the National Academy of Sciences of the United States of America 105, 19780–19785. https://doi.org/10.1073/pnas.0805600105.

Merilä, P., Malmivaara-Lämsä, M., Spetz, P., Stark, S., Vierikko, K., Derome, J., Fritze, H., 2010. Soil organic matter quality as a link between microbial community structure and vegetation composition along a successional gradient in a boreal forest. Applied Soil Ecology 46, 259–267. https://doi.org/10.1016/j.apsoil.2010.08.003.

Mgelwa, A.S., Hu, Y.L., Xu, W.B., Ge, Z.Q., Yu, T.W., 2019. Soil carbon and nitrogen availability are key determinants of soil microbial biomass and respiration in forests along urbanized rivers of southern China. Urban Forestry and Urban Greening 43. https://doi.org/10.1016/j.ufug.2019.05.013.

Mohanram, S., Kumar, P., 2019. Rhizosphere microbiome: revisiting the synergy of plant-microbe interactions. Annals of Microbiology 69, 307–320. https://doi.org/10.1007/s13213-019-01448-9.

Muscarella, R., Uriarte, M., 2016. Do community-weighted mean functional traits reflect optimal strategies? Proceedings of the Royal Society B: Biological Sciences 283. https://doi.org/10.1098/rspb.2015.2434.

Naylo, A., Pereira, S.L.A., Benidirel, L., El Khalil, H., Castro, P.M.L., Ouvrard, S., Schwartz, C., Boularbah, A., 2019. Trace and major element contents, microbial communities, and enzymatic activities of urban soils of Marrakech city along an anthropization gradient. Journal of Soils and Sediments 19, 2153–2165. https://doi.org/10.1007/s11368-018-2221-y.

Nielsen, U.N., Wall, D.H., 2015. Soil Biodiversity and the Environment.

Norton, B.A., Bending, G.D., Clark, R., Corstanje, R., Dunnett, N., Evans, K.L., Grafius, D. R., Gravestock, E., Grice, S.M., Harris, J.A., Hilton, S., Hoyle, H., Lim, E., Mercer, T. G., Pawlett, M., Pescott, O.L., Richards, J.P., Southon, G.E., Warren, P.H., 2019. Urban meadows as an alternative to short mown grassland: effects of composition and height on biodiversity. Ecological Applications 29. https://doi.org/10.1002/eap.1946.

Pouyat, R.V., Szlavecz, K., Yesilonis, I.D., Groffman, P.M., Schwarz, K., 2010. Chemical, physical, and biological characteristics of urban soils. Urban ecosystem ecology 55, 119–152.

Pouyat, R.V., Yesilonis, I.D., Dombos, M., Szlavecz, K., Setala, H., Cilliers, S., Hornung, E., Kotze, D.J., Yarwood, S., 2015. A global comparison of surface soil characteristics across five cities: a test of the urban ecosystem convergence hypothesis. Soil Science 180, 136–145. https://doi.org/10.1097/ss.0000000000000125.

Prada-Salcedo, L.D., Prada-Salcedo, J.P., Heintz-Buschar, A., Buscot, F., Goldmann, K., 2022. Effects of tree composition and soil depth on structure and functionality of belowground microbial communities in temperate European forests. Frontiers in Microbiology 13, 920618. https://doi.org/10.3389/fmicb.2022.920618.

Pugnaire, F.I., Morillo, J.A., Penuelas, J., Reich, P.B., Bardgett, R.D., Gaxiola, A., Wardle, D.A., van der Putten, W.H., 2019. Climate change effects on plant-soil feedbacks and consequences for biodiversity and functioning of terrestrial ecosystems. Science Advances 5. https://doi.org/10.1126/sciadv.aaz1834.

Ramirez, K.S., Leff, J.W., Barberan, A., Bates, S.T., Betley, J., Crowther, T.W., Kelly, E.F., Oldfield, E.E., Shaw, E.A., Steenbock, C., Bradford, M.A., Wall, D.H., Fierer, N., 2014. Biogeographic patterns in below-ground diversity in New York City's Central Park are similar to those observed globally. Proceedings of the Royal Society B: Biological Sciences 281. https://doi.org/10.1098/rspb.2014.1988.

Reese, A.T., Savage, A., Youngsteadt, E., McGuire, K.L., Koling, A., Watkins, O., Frank, S. D., Dunn, R.R., 2016. Urban stress is associated with variation in microbial species composition—but not richness—in Manhattan. ISME Journal 10, 751–760.

Rita, W., Swantara, I., Asih, I.A., Sinarsih, N., 2018. Antibacterial activity of Samanea saman leaf ethanol extract against Escherichia coli and Staphylococcus aureus and its total flavonoid and phenolic contents. Journal of Chemistry 12, 120–126.

Robinson, C., Wallace, D., Hyun, J.H., Polimene, L., Benner, R., Zhang, Y., Cai, R., Zhang, R., Jiao, N., 2018. An implementation strategy to quantify the marine microbial carbon pump and its sensitivity to global change. National Science Review 5, 474–480. https://doi.org/10.1093/nsr/nwy070.

Santalahti, M., Sun, H., Jumpponen, A., Pennanen, T., Heinonsalo, J., 2016. Vertial and seasonal dynamics of fungal communities in boreal Scots pine forest soil. FEMS Microbiology Ecology 92, fiw170.

Sauvadet, M., Chauvat, M., Cluzeau, D., Maron, P.A., Villenave, C., Bertrand, I., 2016. The dynamics of soil micro-food web structure and functions vary according to litter quality. Soil Biology and Biochemistry 95, 262–274. https://doi.org/10.1016/j.soilbio.2016.01.003.

Schmidt, D.J.E., Pouyat, R., Szlavecz, K., Setala, H., Kotze, D.J., Yesilonis, I., Cilliers, S., Hornung, E., Dombos, M., Yarwood, S.A., 2017. Urbanization erodes ectomycorrhizal fungal diversity and may cause microbial communities to converge. Nature Ecology & Evolution 1. https://doi.org/10.1038/s41559-017-0123.

Schneider, T., Keiblinger, K.M., Schmid, E., Sterflinger-Gleixner, K., Ellersdorfer, G., Roschitzki, B., Richter, A., Eberl, L., Zechmeister-Boltenstern, S., Riedel, K., 2012. Who is who in litter decomposition? Metaproteomics reveals major microbial players and their biogeochemical functions. ISME Journal 6, 1749–1762.

Schops, R., Goldmann, K., Herz, K., Lentendu, G., Schoning, I., Bruelheide, H., Wubet, T., Buscot, F., 2018. Land-use intensity rather than plant functional identity shapes bacterial and fungal rhizosphere communities. Frontiers in Microbiology 9. https://doi.org/10.3389/fmicb.2018.02711.

Setälä, H., McLean, M.A., 2004. Decomposition rate of organic substrates in relation to the species diversity of soil saprophytic fungi. Oecologia 139, 98–107. https://doi.org/10.1007/s00442-003-1478-y.

Setälä, H.M., Francini, G., Allen, J.A., Hui, N., Jumpponen, A., Kotze, D.J., 2016. Vegetation type and age drive changes in soil properties, nitrogen, and carbon sequestration in urban parks under cold climate. Frontiers in Ecology and Evolution 4. https://doi.org/10.3389/fevo.2016.00093.

Shade, A., Peter, H., Allison, S.D., Baho, D.L., Berga, M., Burgmann, H., Huber, D.H., Langenheder, S., Lennon, J.T., Martiny, J.B.H., Matulich, K.L., Schmidt, T.M., Handelsman, J., 2012. Fundamentals of microbial community resistance and resilience. Frontiers in Microbiology 3. https://doi.org/10.3389/fmicb.2012.00417.

Shi, L.L., Dossa, G.G.O., Paudel, E., Zang, H.D., Xu, J.C., Harrison, R.D., 2019. Changes in fungal communities across a forest disturbance gradient. Applied and Environmental Microbiology 85. https://doi.org/10.1128/aem.00080-19.

Šnajdr, J., Cajthaml, T., Valášková, V., Merhautová, V., Petránková, M., Spetz, P., Leppänen, K., Baldrian, P., 2011. Transformation of Quercus petraea litter: successive changes in litter chemistry are reflected in differential enzyme activity and changes in the microbial community composition. FEMS Microbiology Ecology 75, 291–303.

Solly, E.F., Schoning, I., Boch, S., Kandeler, E., Marhan, S., Michalzik, B., Muller, J., Zscheischler, J., Trumbore, S.E., Schrumpf, M., 2014. Factors controlling decomposition rates of fine root litter in temperate forests and grasslands. Plant and Soil 382, 203–218. https://doi.org/10.1007/s11104-014-2151-4.

Steinauer, K., Chatzinotas, A., Eisenhauer, N., 2016. Root exudate cocktails: the link between plant diversity and soil microorganisms? Ecology and Evolution 6, 7387–7396.

Stuble, K.L., Ma, S., Liang, J., Luo, Y., Classen, A.T., Souza, L., 2019. Long-term impacts of warming drive decomposition and accelerate the turnover of labile, not recalcitrant, carbon. Ecosphere 10, e02715.

Szlavecz, K., Yesilonis, I., Pouyat, R. (Eds.), 2017. Soil as a foundation to urban biodiversity. Urban Biodiversity, Edited By Alessandro Ossola and Jari Niemelä. Routledge publisher, London.

Tan, X.L., Kan, L., Su, Z.Y., Liu, X.D., Zhang, L., 2019. The composition and diversity of soil bacterial and fungal communities along an urban-to-rural gradient in south China. Forests 10. https://doi.org/10.3390/f10090797.

Taylor, B.R., Parkinson, D., Parsons, W.F., 1989. Nitrogen and lignin content as predictors of litter decay rates: a microcosm test. Ecology 70, 97–104.

Tedersoo, L., Bahram, M., Toots, M., Diedhiou, A.G., Henkel, T.W., Kjoller, R., Morris, M. H., Nara, K., Nouhra, E., Peay, K.G., Polme, S., Ryberg, M., Smith, M.E., Koljalg, U., 2012. Towards global patterns in the diversity and community structure of ectomycorrhizal fungi. Molecular Ecology 21, 4160–4170. https://doi.org/10.1111/j.1365-294X.2012.05602.x.

Thompson, G.L., Kao-Kniffin, J., 2019. Urban grassland management implications for soil C and N dynamics: a microbial perspective. Frontiers in Ecology and Evolution 7. https://doi.org/10.3389/fevo.2019.00315.

Tkacz, A., Cheema, J., Chandra, G., Grant, A., Poole, P.S., 2015. Stability and succession of the rhizosphere microbiota depends upon plant type and soil composition. ISME Journal 9, 2349–2359. https://doi.org/10.1038/ismej.2015.41.

Uroz, S., Buée, M., Deveau, A., Mieszkin, S., Martin, F., 2016. Ecology of the forest microbiome: hightlights of temperate and boreal ecosystems. Soil Biology and Biochemistry 103, 471–488. https://doi.org/10.1016/j.soilbio.2016.09.006.

van der Heijden, M.G.A., Klironomos, J.N., Ursic, M., Moutoglis, P., Streitwolf-Engel, R., Boller, T., Wiemken, A., Sanders, I.R., 1998. Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. Nature 396, 69–72. https://doi.org/10.1038/23932.

Vivelo, S., Bhatnagar, J.M., 2019. An evolutionary signal to fungal succession during plant litter decay. FEMS Microbiology Ecology 95. https://doi.org/10.1093/femsec/fiz145.

Voriskova, J., Baldrian, P., 2013. Fungal community on decomposing leaf litter undergoes rapid successional changes. ISME Journal 7, 477–486. https://doi.org/10.1038/ismej.2012.116.

Wang, C., Liu, D., Bai, E., 2018. Decreasing soil microbial diversity is associated with decreasing microbial biomass under nitrogen addition. Soil Biology and Biochemistry 120, 126–133. https://doi.org/10.1016/j.soilbio.2018.02.003.

Wardle, D.A., Bardgett, R.D., Klironomos, J.N., Setala, H., van der Putten, W.H., Wall, D. H., 2004. Ecological linkages between aboveground and belowground biota. Science 304, 1629–1633. https://doi.org/10.1126/science.1094875.

Xu, H.D., Yu, M.K., Cheng, X.R., 2021. Abundant fungal and rare bacterial taxa jointly reveal soil nutrient cycling and multifunctionality in uneven-aged mixed plantations. Ecological Indicators 129. https://doi.org/10.1016/j.ecolind.2021.107932.

Yan, B., Lu, Q., He, J., Qi, Y., Fu, G., Xiao, N.W., Li, J.S., 2021. Composition and interaction frequencies in soil bacterial communities change in association with urban park age in Beijing. Pedobiologia 84. https://doi.org/10.1016/j.pedobi.2020.150699.

Yin, X.A., Zhao, L.S., Fang, Q., Ding, G.J., 2021. Differences in soil physicochemical properties in different-aged pinus massoniana plantations in southwest China. Forests 12. https://doi.org/10.3390/f12080987.