Revealing the taxonomy and functional potential of urban soil microbiomes

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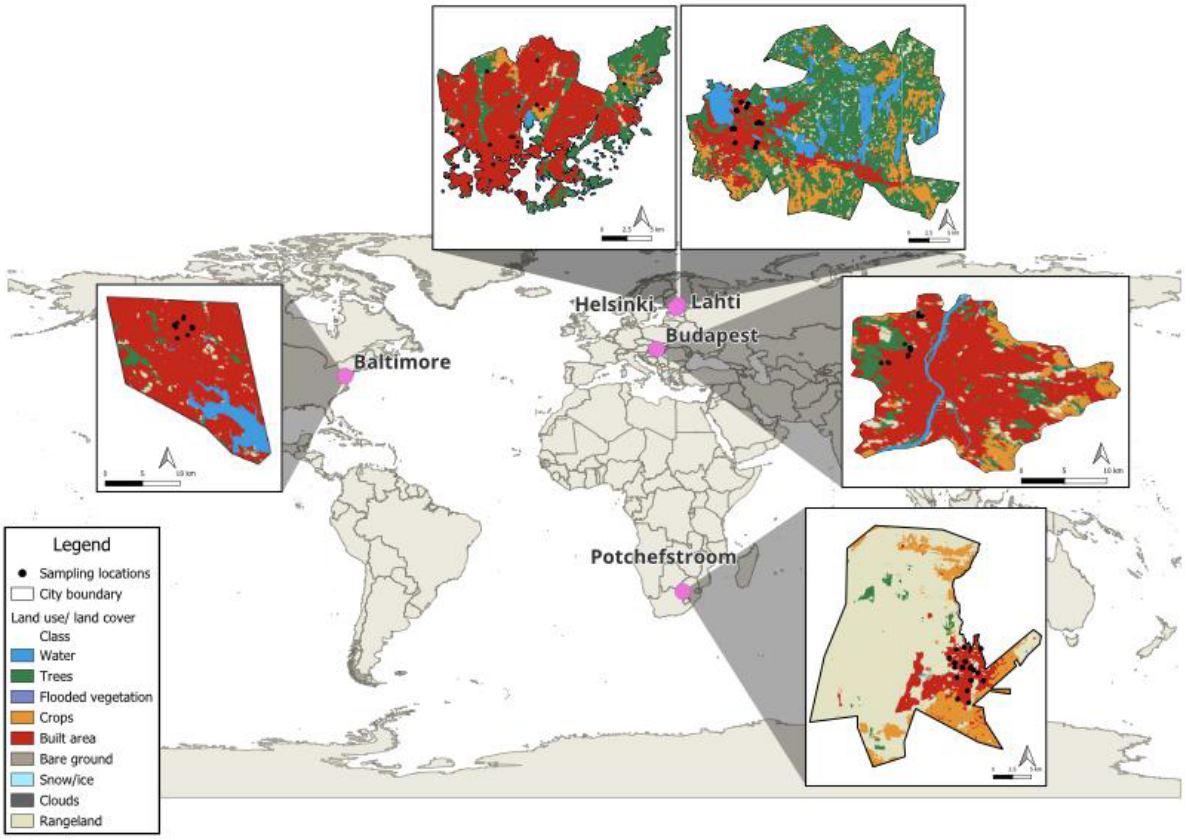
Sentence summary.

Taxonomic and functional potential of global urban soil microbiomes are partially decoupled and reveal ubiquitous potential for contaminant cycling.

Feature image.



Abstract.

Urbanization is a leading cause of global biodiversity loss, but its impact on soil microorganisms and biogeochemistry remains uncertain. To address this gap, we investigate urban soil microbiome composition using metagenomic sequences from the Global Urban Soil Environment Ecology Network (GLUSEEN). We seek to reveal (1) changes in taxonomic composition and functional potential and (2) ubiquitous (core) taxa and metabolisms across the urban soil microbiome. We hypothesize that soil taxonomic and functional diversity are correlated due to the unique selective pressures of the urban ecosystem, and that the potential for carbon, nutrient, and pollutant cycling is a key feature of the urban soil microbiome. We find that Baltimore soil microbiomes are distinct from all other cities, displaying the highest taxonomic and lowest functional diversity. Only 66.3% of taxonomic and functional composition is correlated across all cities, highlighting differing functional potential within similar soil microbial taxa. Metabolic processes involving carbon and nitrogen cycling are abundant, as well as xenobiotic degradation. Core urban microbiota include many common soil bacteria and methane- and nitrogen-cycling archaea. Understanding the predominant characteristics of microbial taxonomy and functional potential in urban soils will aid in understanding feedback between growing metropolitan areas and processes driving climate change.

Introduction

Human activity in urban centers is shaping the way organisms evolve and interact with each other, impacting both urbanized and surrounding ecosystems (Pickett *et al.* 2011). Although only 1% of total global land cover is built, 70% of the world's population is predicted to reside in these urban areas by 2050 (United Nations 2018; Ritchie, Samborska and Roser 2024). While studies have focused on how urbanization is redefining ecosystems, our knowledge of the urban soil microbiome still remains limited. These microbiomes play a critical role in global biogeochemistry (Dubey *et al.* 2019; Naylor *et al.* 2020). Robust understanding of the community structure and the functional potential is therefore crucial in predicting the functional and the ecological roles of microbes in the urban soil ecosystem.

Rapidly increasing urbanization and associated changes in land-use are responsible for biodiversity loss of macro-organisms (i.e. animal and plant communities) and for increased pollution, both of which may subsequently impact urban biogeochemistry and soil microbiome function (Pauchard *et al.* 2006; Nagendra *et al.* 2014). Land clearing for agriculture and urban expansion, for instance, have resulted in fragmentation of forest biomes across the world (Reinmann *et al.* 2020). This practice also leads to increased pollutants, like nitrate and ammonium, which in turn, change soil biogeochemical properties and microbiome function (Wang *et al.* 2017). Additionally, industrial activities can lead to heavy metal and organic contamination in urban soils, in particular polycyclic aromatic hydrocarbons (PAHs), that are toxic to humans and are added to soil through processes like burning fossil fuels and waste incineration (Fernández-Luqueño *et al.* 2011; Delegan *et al.* 2022). Finally, urban land use and management practices like fertilization, irrigation, and impervious surfaces often increase nutrient inputs into soils via runoff (Fenn *et al.* 2003). Nitrogen runoff in particular can enhance microbial denitrification processes that release nitrous oxide – a potent greenhouse gas (Kettering *et al.* 2013; Toor *et al.* 2017).

In parallel, soil microorganisms (i.e., bacteria, archaea, and fungi) are major conductors of global carbon and nutrient cycles. Soil biogeochemical properties that are impacted by urbanization like moisture, temperature, pH, and carbon and nutrient concentrations have a strong effect on microbial communities (Rousk *et al.* 2010; Brockett, Prescott and Grayston 2012; Karhu *et al.* 2014; Graham *et al.* 2016; Widdig *et al.* 2020). Moreover, microbial diversity is also dependent on interspecies and plant interactions (Wilpiszeski *et al.* 2019; Sharma *et al.* 2020; McClure *et al.* 2022). Changes of these properties within urban soils have been shown to increase the relative abundance of genes involved in greenhouse gas emissions like methanogenesis and denitrification (Delgado-Baquerizo, Eldridge and Yu-Rong 2021). More generally, urban soil microbiomes have been shown to exhibit homogenization of bacterial communities with high functional diversity across multiple cities, as compared to surrounding natural ecosystems (Gill *et al.* 2020; Delgado-Baquerizo, Eldridge and Yu-Rong 2021).

The extent to which the urban environment drives soil microbiomes towards a common state (i.e., microbiome composition and function; urban convergence hypothesis) versus supporting a robust, diverse microbial community (i.e., the urban mosaic) remains debated (Pouyat *et al.* 2015). Within the dataset re-analyzed here, for example, it was reported (Pouyat *et al.* 2015) that soil biogeochemical properties like organic carbon and total nitrogen content converged due to anthropogenic factors. Other studies have also seen the effect of urban convergence on soil properties, such as soil particle size and soil carbon content (Herrmann, Schifman and Shuster 2020). However, due to the plethora of land covers and uses contained within different metropolitan areas, it remains to be explored whether or not urbanization has a uniform effect that will drive microbial communities towards greater biodiversity loss or greater taxonomic and functional potential. Standardized spatial observations distributed within and across cities are key to resolving these questions.

The emergence of cultivation-independent next-generation sequencing methods has enhanced exploration of uncultured microbial diversity (i.e. microbial dark matter). Historical investigations of soil microorganisms relied on cultivated bacteria and archaea that only make up 0.001-0.1% of the total estimated prokaryotic species and/or amplicon sequencing that does not capture functional diversity (Lewis *et al.* 2020). The expansion of prokaryotic taxonomy based on new sequencing approaches has revealed novel bacteria and archaea participating in nutrient cycles that may have significant implication in our understanding of biogeochemical processes in soil (Parks *et al.* 2020). These recent advances in soil metagenomics allow unprecedented resolution into the urban microbiome. Though only cultivation of microorganisms can bring certainty towards biogeochemical processes, metagenomics allows inferences towards functional potential. Through this approach, we can discover specific taxonomic groups to target for cultivation to further gain insight into the soil microbiome.

Here, we use metagenomic sequences from soils collected by the Global Urban Soil Environment Ecology Network (GLUSEEN; Pouyat et al., 2017) to investigate the taxonomic and functional diversity of the urban soil microbiome. We hypothesize that across cities, changes in taxonomic and functional diversity are correlated due to strong selective pressures associated with nutrients and contaminants in the urban environment and subsequently is driving convergence of microorganisms. Specifically, we hypothesize that microbial taxa and genes related to carbon, nutrient, and pollutant loading have high abundance and are key features of the urban microbiome. Previous work with this dataset, by Epp Schmidt et al. (2019, 2017), has shown some convergence of archaeal and bacterial communities, with archaeal convergence being driven by ammonia-oxidizers coupled with convergence of ammonia oxidizing genes. Additionally, Epp Schmidt et al. (2019) reported a high abundance of nickel transport genes which is associated with increased abundance of methanogens (Epp Schmidt *et al.* 2019). However, Epp Schmidt et al., (2019, 2017) did not explore aspects of urban

microbiomes related to contaminants and other carbon and nitrogen cycling processes. We further extend this work by reporting concomitant changes in taxonomy and functional diversity within and across cities; extracting the core urban microbiome across all cities; and uncovering a newly reported set of widespread soil genomic potential for the tolerance and/or cycling of urban pollutants.

Methods

Dataset Description

We used publicly available metagenomic sequencing data from the Global Urban Soil Environment Ecology Network (GLUSEEN) to investigate the urban soil microbiome from 66 samples across five globally-distributed cities: USA: Baltimore (n=11), Finland: Helsinki (n=12), Finland: Lahti (n=14), Hungary: Budapest (n=14), and South Africa: Potchefstroom (n =15) (Figure 1; (Yarwood and Epp Schmidt)). After excluding reference sites, soils spanned three levels of urbanization that encompass a gradient of common anthropogenic impacts, remnant: within city limits, turf: managed land, and ruderal: high degree of physical disturbance as described by Pouyat et al. (2015). Soil collection and metagenomic sequencing were methods described by Epp Schmidt et al., 2017. Briefly, soils were collected prior to 2017 from 0-10 cm depth, and shotgun metagenomic sequencing was performed on Illumina HiSeq 3000 using standardized methods. Samples used for this publication are available at MG-RAST (https://www.mg-rast.org/mgmain.html?mgpage=project&project=mgp16346).

Taxonomic and Functional Inference of Metagenomes

Sequences from each soil were retrieved from Metagenomic Rapid Annotations using Subsystems Technology (MG-RAST) and processed using the Joint Genome Institute (JGI) Integrated Microbial Genomes and Microbiomes (IMG/M) standard workflows (Clum *et al.*

2021). Briefly, reads meeting the QC standards were assembled into contigs using BBDuk version 38.79, bbcms version 38.44 and metaSPADES version 3.13.0 (Nurk *et al.* 2017; Bushnell 2023). Read-based taxonomy was assigned to assembled contigs using the genome-based taxonomy database, and assembled contigs were further annotated against the Kyoto Encyclopedia of Genes and Genomes (i.e., KEGG Orthology, KO; (Kanehisa et al., 2019, 2000, 2023)) as part of standard JGI workflows (Parks *et al.* 2018; Chaumeil *et al.* 2020). After sequence processing, we summarized taxa and KO for each soil sample into matrices by counting sequences in each soil assigned to every unique taxon (order-level) or KO. Counts in each matrix were normalized using a relative log expression (RLE; Pereira et al., 2018) implemented in the R package 'DESeq2' (Love, Huber and Anders 2014). Code is available on figshare (https://figshare.com/s/d788b28a869481c25689).

Statistical Analysis

To generate maps of land use for each city, we used QGIS 3.32.3-Lima, overlapped with land use data for 2017 obtained from Environmental Systems Research Institute, ESRI (Figure.1; Kontgis et al., 2017; 2021). Land use analyses were performed in QGIS equipped with a semi-automatic classification plug-in (Congedo, 2021; "Welcome to the QGIS Python API documentation project," 2023.).

All other statistical analyses were implemented in R (version 4.3.1) with 'vegan', 'usedist', and 'ComplexUpset' packages and visualized using the 'ggplot2' package (Wickham 2016; Bittinger 2020; Lex *et al.* 2020; Oksanen *et al.* 2022; Krassowski). We examined how taxonomic diversity varied within and across cities by calculating alpha (richness, Shannon Diversity Index, and Pielou evenness) and beta diversity (Bray-curtis dissimilarity matrices paired with ordination plots). We compared alpha diversity across cities using Tukey's honestly significant difference (HSD) post hoc test. Variation in microbiome composition across soils was

visualized using non-metric multidimensional scaling (NMDS) and statistical differences were assessed with a permutational multivariate analysis of variance (PERMANOVA), for microbial taxonomy (i.e., microbial orders) and functional potential (i.e., KO) independently (Anderson 2017). Then, we individually compared how taxonomic and functional composition changed with geographic distance, using the Haversine dissimilarity matrix within and across cities, using Mantel tests. KOs were mapped to pathways using the 'KEGGREST' package in R (Tenenbaum and Maintainer 2023). Stacked bar charts were generated in 'ggplot' to visualize the top 10 most abundant pathways, as well as the top 10 most abundant bacterial and archaeal phyla by summed normalized abundance across all soils in a given city ("phyloT: a phylogenetic tree generator" n.d.). For visualizations of read-based taxonomy, we generated phylogenetic trees using phyloT v2 (phyloT□: a phylogenetic tree generator), an online tree generator based on the Genome Taxonomy Database. Then, we visualized the tree in R using the packages, 'ggtree' (Yu *et al.* 2017), 'treeio' (Wang *et al.* 2020), and 'ggnewscale' (Campitelli 2020). We generated heatmaps using the 'pheatmap' package (Kolde 2019).

Results

Microbial Diversity of Urban Soils

The taxonomic diversity of Baltimore was higher (within-sample diversity) and distinct (across-samples diversity) compared to other cities. Taxonomic richness (order-level) in Baltimore soils (261.455, Table S1) was nearly two-fold higher than all other cities (120.571-172.200, P<0.001, Table S2). Shannon diversity followed a similar trend in Baltimore (**H'**= 3.770, Figure 2, Table S3) vs. other cities (**H'** range: 0.801-1.872, p-value < 0.001, Figure 2, Table S4). When assessing dissimilarity in microbial community composition across cities, Baltimore was also distinct from other cities (PERMANOVA, P = 0.01, Figure 3, Table S5), where 33% of the taxonomic diversity variance was attributed to location. This was associated

with proportionally fewer members of *Psuedomondata* and *Bacilliota* than other common bacterial phyla in Baltimore (Figure 2).

With respect to functional diversity (i.e., normalized KO), Baltimore soils again separated from soils in the other cities. Baltimore soils had the lowest average functional richness compared to Budapest and Potchefstroom (mean = 2,189.636 vs. 3440.714 and 3325.067 unique KO per sample, P= 0.021 and 0.04, respectively, Table S6, S14) and the lowest average Shannon diversity when compared to all other metropolitan areas (**H'**= 7.110, **H'** range= 7.669-7.816, P<0, Figure 2, Table S7, S8). Overall, 34.5% of the variance of functional diversity across all cities was due to geographic location (p=0.01, Figure 3, Table S9). Across all soils, KEGG pathways with the most mapped genes were the carbohydrate metabolism and amino acid metabolism pathways (map01110; Figure 2). Interestingly, xenobiotic degradation was found in abundance of ~3% in all cities and was highest at 4.319% in Baltimore (Figure 2, Table S10).

We then tested the relationship between the diversity and geographic position to assess the extent to which taxonomic and functional attributes of urban microbial communities changed through space (Figure 3). Both taxonomic and functional dissimilarity increased with distance across all soils (33%, 34.5%, P= 0.01). However, when looking at each city individually, neither taxonomic nor functional diversity varied significantly with distance for any city (Table S11, S12). Dissimilarity in taxonomic and functional composition was coupled across all cities (r= 0.663, P= 0.01, Table S13), and within Budapest, Helsinki, and Potchefstroom (r=0.515, P=0.01, r=0.665, P= 0.01, r=0.543, P=0.01, respectively) with the notable exception of Baltimore and Lahti where functional potential did not seem to vary with taxonomy.

Core Urban Microbial Taxa and Metabolic Potential

226 microbial orders were common to soil microbiomes across all cities (Figure 4). The most abundant shared orders of bacteria included *Enterobacterales*, *Burkholderiales*, *Bacillales*, *Lactobacillales*, *Rhizobiales*, and *Caulobacterales*. *Nitrososphaerales* was by far the most abundant shared archaeal order, followed by other nitrogen- and methane-cycling (*Nitrosopumilales*, *Candidatus* Nitrosocaldales, *Methanosarcinales*, *Methanomicrobiales*, *Methanobacteriales*) and salt-tolerant archaea (*Halobacteriales*, *Haloferacales*). We also note that soil DNA viruses were ubiquitous and were more abundant than the 17th ranked microbial order (bacterial or archaeal). Interestingly, Baltimore had the most unique microbial orders compared to all other cities, and Helsinki was the only other city that had unique microbial orders (Figure 4). Unique soil taxa in Baltimore included *Fervidicoccales*, *Anaeroplasmatales*, unclassified *Elusimicrobia*, and unclassified *Thermotogae* (Figure 4).

Further, 3,667 unique KOs were shared across all cities (Figure 4). The most abundant ubiquitous KOs encoded proteins known to be involved in transport and environmental information processing and/or in xenobiotic degradation. These included K07347 (outer membrane usher protein), K02014 (iron complex outer membrane receptor protein), K02035 (peptide/nickel transport system substrate-binding protein), K01992 (ABC-2 type transport system permease protein), K01990 (ABC-2 type transport system ATP-binding protein), K00626 (acetyl-CoA C-acetyltransferase [ACAT]), K00799 (glutathione S-transferase [Gst]).

Lahti had the most unique microbial KO's at 973, followed by Baltimore (259), Potchefstroom (121), Budapest (116), and Helsinki (84). Unique KO's to Lahti included K12515 (tight adherence protein G [*tadG*]), K14556 (small nucleolar RNA-associated protein 12 [*DIP2*]), K17939 (GDP-perosamine N-acetyltransferase; [*perB*]), K00423 (L-ascorbate oxidase), and K18354 (vancomycin resistance protein [*vanK*]); while unique KO's to Baltimore included K16124 (tyrocidine synthetase III [*tycC*]), K18074 (terephthalate 1,2-dioxygenase oxygenase

alpha subunit [*tphA2*]), K15243 (2,4,5-trichlorophenoxyacetic acid oxygenase; [*tftA*]), and K09141 (uncharacterized protein).

Because xenobiotic degradation pathways were observed to be prevalent across cities and are relatively rare in pristine soils, we further investigated the distribution of KO categorized under xenobiotic degradation. Genes involved in transformation of catechol and/or protocatechuate were present in almost all urban soils (map00362; Figure 5). Transformation of catechol yields pyruvate, acetyl-CoA, and succinyl-CoA that are involved in the TCA cycle, providing a link between contaminant degradation and central metabolisms (energy generation) of microorganisms. Our observations therefore indicate a potential for complete transformation of aromatic pollutants for microbial energy generation.

Two versions of catechol degradation pathways are currently known. The first of these two is a six-step pathway involving catechol ortho-cleavage enzymes [CatABC]; (K03381, K01856, and K03464), 3-oxoadipate enol-lactonase [PcaD]; (K01055), 3-oxoadipate CoA-transferase [PcaIJ]; (K01032) and acetyl-CoA acyltransferase [FadA]; (K00632) (Eulberg et al., 1998; Shi et al., 2021). A complete ortho-cleavage pathway was detected in 6 out of 12 soil from Helsinki and was complete or near-complete (i.e., 80% or higher of the total number of genes in the pathway) was found in Lahti (5/14), Budapest (5/14), and Potchefstroom (4/15). A near-complete ortho-cleavage pathway was detected in a single soil sample from Baltimore.

The catechol ortho-cleavage pathway is preceded by the transformation of protocatechuate, conducted by protocatechuate 3,4-dioxygenase [PcaGH]; (K00448 and K00449), 3-carboxy-cis,cis-muconate cycloisomerase [PcaB]; (K01857) and 4-carboxymuconolactone decarboxylase [PcaC]; (K01607) generates 3-oxoadipate enol-lactone. A complete or a near-complete set of genes encoding the first three steps of the

protocatechuate transformation pathway (i.e., PcaGH, PcaB and PcaC) was present in 38 of 66 soils, distributed across cities.

The second catechol degradation pathway (meta-cleavage) consists of four steps, catalyzed by catechol 2,3-dioxygenase [CatE or DmpB]; (K07104 or K00446), 2-hydroxymuconate-semialdehyde hydrolase [DmpD]; (K10216), 2-keto-4-pentenoate hydratase [MhpD]; (K02554), and 4-hydroxy 2-oxovalerate aldolase [MhpE]; (K01666) (Harayama *et al.* 1989). Nearly all genes encoding the meta-cleavage pathway, including CatE or DmpB, MhpD and MhpE were present in about half of all urban soils (33 of 66 soils). While DmpD was only detected in 10 of 66 soils, it is not essential for catechol degradation.

Additionally, we investigated metabolic pathways associated with nitrogen cycling considering the prevalence of nitrogen pollution in cities due to fertilizer usage and other anthropogenic processes. Many core metabolic processes relating to nitrogen were ubiquitous across urban soils (Figure 5), and genes relating to dissimilatory nitrate reduction in particular were widespread across municipalities (Cheng *et al.* 2022). Specifically, nitrate reductase [NarGHI] was present in 41 of 66 soils, while nitrite reductase [NirBD], was found in 54 of 66 soils.

Discussion

Urban centers are increasing in density and are shaping biogeochemistry in our environment, in turn, shaping soil microbial taxonomy and functional diversity (Christel *et al.* 2023). Soil microbial functions are an essential component of global climate change, as microorganisms regulate soil C storage and respiration (Gougoulias, Clark and Shaw 2014). Here, we hypothesized that the soil microbiomes would converge in taxonomic and functional diversity within and across cities due to strong selective pressures of anthropogenic processes. In testing the urban convergence hypothesis, we reveal key features of the urban microbiome,

including microbial taxa and genes related to carbon, nutrient, and pollutant loading. Understanding novel functions and differing community compositions in urban centers will help elucidate lasting impacts of urbanization on the soil microbiome and consequently the ecosystem at large.

Taxonomy and Functional Potential of Urban Soil Microbiomes

There is a great array of functional potential in the urban microbiome compared to taxonomic diversity, as evidenced by higher KO diversity and richness versus taxonomic diversity and richness across all cities. The sizable functional potential lends support for the urban mosaic hypothesis, highlighting increased diversity in urban soils (Scholier *et al.* 2023) and variation in microbial composition across urban land uses (Christel *et al.* 2023). For example, Mo et al. and Scholier et al. (2024; 2023) reported increased alpha diversity in anthropogenic areas compared to natural areas. In contrast, taxonomic convergence across cities provides evidence towards the urban convergence hypothesis, where physical characteristics of the urban environment drive microbial similarities. This is consistent with findings in urban greenspaces where despite greater functional diversity, homogenous microbial communities were present (Delgado-Baquerizo, Eldridge and Yu-Rong 2021; Mo *et al.* 2024). While microbial community structure has a large effect on functional potential, environmental variables have been reported to be highly explanatory to changes in function (Graham *et al.* 2016). Furthermore, horizontal gene transfer could play a large role in inconsistencies in correlations between taxonomy and functional potential (Martiny, Treseder and Pusch 2013).

Interestingly, Baltimore appears to have a distinct soil microbiome relative to all other urban centers. It has the highest taxonomic diversity and the least functional diversity compared with all other cities, which all have similar diversity. The unique taxa and pathways found in Baltimore are associated with degradation of urban contaminants. There were proportionally

fewer members of *Psuedomondata* and *Bacilliota* than other common bacterial phyla in Baltimore soils, as compared to other metropolitan areas; and Baltimore soils contained microbial members of four taxonomic orders not found in any other city (*Fervidicoccales*, *Anaeroplasmatales*, unclassified *Elusimicrobia*, and unclassified *Thermotogae*). These unique microbial orders contain the potential for alternative energy generating metabolisms using iron, nitrogen, and other anaerobic terminal electron acceptors (Bhandari and Gupta 2014; Méheust *et al.* 2020). *Thermotogae*, in particular, contains the genus *Mesatoga* which has been found to be associated with polychlorinated biphenyls (PCBs) in Baltimore Harbor (Bhandari and Gupta 2014). *Mesatoga* has also been associated with the degradation of PAHs and chlorophenols that are also common urban contaminants (Hoffman *et al.* 1984; Czaplicka 2004; Brown and Peake 2006; Bhandari and Gupta 2014; Gallego *et al.* 2018). Collectively, these compounds are of key interest to urban ecosystems because of their detrimental impacts on human health and long residence times (Wang *et al.* 2013; Kumar *et al.* 2020). The unique microbial taxa in Baltimore that are associated with these compounds could be indicative of a shifting soil microbiome in response to urban pollutants.

The metabolic potential of Baltimore soils (i.e., unique KO's, K16124, K12405, K18074, K15243, and K09141) is consistent with taxonomic observations. Most notably, K18074 (terephthalate 1,2-dioxygenase oxygenase component alpha subunit [*tpha2*]) is associated with a protein involved in PAH degradation (Fukuhara *et al.* 2008; Liu *et al.* 2022), and K15243 (2,4,5- trichlorophenoxyacetic acid oxygenase [*tftA*]) encodes a protein involved in chlorocyclohexane and chlorobenzene degradation, which both fall under the xenobiotic degradation pathway (Vyas and Murthy 2015; Muñoz-García *et al.* 2019). Xenobiotic degradation is not commonly found in the soil microbiome of pristine ecosystems but has been identified previously in urban soils (Sharma, Hansda and Chand 2023). Anthropogenic activities such industrial productivity and fossil fuel combustion are major sources of organic

contaminants (An *et al.* 2013; Gieg, Fowler and Berdugo-Clavijo 2014; Tan *et al.* 2015), which may ultimately drive the distinct functional potential we observed in the urban microbiome of Baltimore's soils. Additionally, K16124 (tyrocidine synthetase III [*tycC*]) and K12405 ((3R)-3-hydroxyacyl-CoA dehydrogenase [HSD17B4]) encode processes related to polyketides, which are essential to root microbiomes (Dror *et al.* 2020; Dror, Jurkevitch and Cytryn 2023). The unique presence of these genes in Baltimore may suggest potential novel metabolites in urban soils that could be crucial towards plant health (Dror *et al.* 2020). With ever increasing urbanization, the relationship between contaminants and the urban soil microbiome merits deeper investigation through more targeted investigations.

Spatial variation in functional potential and taxonomic composition across the urban soil microbiome

Similar to previous work on this dataset, we found that urban microbiome composition and functional diversity seemed to align with the predictions of the urban convergence hypothesis (Epp Schmidt *et al.* 2019). For each individual city, neither taxonomic nor functional diversity varied significantly through space (Table S11, S12). Across cities, taxonomic and functional dissimilarty showed 33% and 34.5% correlation with spatial distances, respectively. This indicates that although microbial diversity does not vary across local scales, overarching drivers such as soil mineralogy or climate also play a role in structuring urban microbiomes at a global scale. Indeed, the mixture of diverse land uses within cities may obscure patterns based on space alone, but large differences in the environment may also influence global patterns in the urban microbiome (Christel *et al.* 2023).

Microbial taxonomic composition and functional potential were partially decoupled, as they were only 66.3% correlated at the global scale and only Budapest, Helsinki, and Potchefstroom were correlated (51.2%, 66.5%, 54.3%, respectively) at the local scale. Microbial

functional potential is expected to be somewhat correlated to taxonomic composition due to phylogenetic conservation of microbial traits, which can lead to similar responses to environmental factors like substrate availability and climatic variables (Martiny, Treseder and Pusch 2013). However, if ecological selection operates on microbial genomic elements that are exchanged between organisms or if selection operates on unrelated genes and organisms, shifts in microbial taxonomy may not be coordinated with changes in genomic potential (Knelman and Nemergut 2014; Bier *et al.* 2015; Graham *et al.* 2016; Graham and Knelman 2023). We posit that the diversity of factors such as available substrates, carbon sources, and/or pollutants in the urban environment may create a mosaic of genes present despite taxonomic convergence. Given the usage of microbial taxa as biomarkers of ecosystem health (Dubey *et al.* 2019) and as engineering solutions (Bala *et al.* 2022), the disconnect between taxonomy and potential function observed here for urban soils is an important consideration in sustainable management practices.

Ubiquitous (core) taxa and metabolisms in global cities

Bacterial orders that were ubiquitous across cities are typified by microorganisms usually associated with plant health, rhizosphere functions, and antibiotic and/or contaminant production and consumption. For example, *Enterobacterales* reside in root systems of plants and some members have been found to maintain plant health under abiotic stress (Andrés-Barrao *et al.* 2021). *Bacillus*, a common microorganism in the *Bacillales* order, is also ubiquitous in soils and promotes plant health through nutrient cycling and pathogen protection (Garbeva, van Veen and van Elsas 2003; Liu *et al.* 2019). With regards to antibiotic and contaminant metabolism, some members in the *Enterobacterales* order (e.g., *Enterobacteria*) harbor increased antibiotic resistance genes in urban centers, while others are human pathogens (e.g., *Salmonella*, *Escherichia*, *Shigella*). *Burkholderiales* have also been shown to participate in pentachlorophenol degradation, a chemical used as a pesticide that persists in soil and has

been coupled to nitrogen, sulfate, and iron reduction (Tong *et al.* 2015; Kang *et al.* 2018; Fresia *et al.* 2019). While we cannot determine if the ubiquity of these organisms in the urban environment is coupled with increased trace metals, pollutants, and harsh conditions, their high plasticity and potential to pick up genes (Brunder and Karch 2000; Justice *et al.* 2008; Liu *et al.* 2019), may make ubiquitous microbes a key focus for future research as we determine causes of decoupling taxonomy and functional potential.

The most abundant archaeal orders were associated with nitrogen and methane cycling and possessed salt tolerance traits that are collectively of interest in the urban environment due to increased greenhouse gas emissions, atmospheric N deposition, and pollutants (Fenn *et al.* 2003; Wang *et al.* 2013; Asabere *et al.* 2018; Strokal *et al.* 2021). This is consistent with the finding that archaeal convergence is driven by ammonia-oxidizers (Epp Schmidt *et al.* 2019). Additionally, we found a high abundance of nickel transport genes (K02035, Figure 4) that have been associated with methanogens (Epp Schmidt *et al.* 2019). Future work may seek to understand heavy metal contamination coupling to methane cycling (Epp Schmidt *et al.* 2019; Dang *et al.* 2021). Finally, we also highlight the high abundance of soil DNA viruses which are increasingly thought to influence soil biogeochemistry, relative to other microorganisms (Neri *et al.* 2022; Roux and Emerson 2022; Graham *et al.* 2023; Jansson and Wu 2023). Collectively, these results provide insight into the microorganisms that are likely to mediate the biogeochemical cycling of key nutrients, greenhouse gasses, and pollutants in urban soils.

Microbial metabolism of soil contaminants linked to energy generation pathways.

Catechol is an intermediate in aromatic degradation and the presence of catechol catabolism genes indicates the likelihood of urban pollutants (Harwood and Parales 1996; Ding, Schmeling and Fuchs 2008). Catechol catabolism provides substrates or precursors of the TCA cycle, such as succinyl-CoA and acetyl-CoA, underscoring the metabolic potential for urban

pollutants to be transformed for energy conservation. The degradation of PAHs is of key interest in the case for bioremediation, as these urban pollutants continue to persist in the urban environment and are highly toxic to humans (Pathak *et al.* 2009). The detection of genes involved in nitrogen reduction shows the potential for both greenhouse gas (e.g., nitrous oxide) emissions and contamination of aquatic ecosystems, which is cause of concern due to excess nitrogen in urban environments (Conley *et al.* 2009; Menegat, Ledo and Tirado 2022; Dang *et al.* 2023; Wang *et al.* 2024). While many ubiquitous and highly abundant metabolic functions unsurprisingly encoded outer membrane and transport proteins (K07347, K02014, K01992, and K01990), genes involved in the dissimilatory nitrate reduction pathway (K00370, K00371, K00374, K00362 and K00363) and the beta-ketoadipate pathway (K00448, K00449, K01857, K01607, K03381, K01856, K03464, K01055, K01031, K01032, K00632) were also present in high abundance.

Conclusion

Future studies on the urban soil microbiota are essential as urbanization and the human population continue to expand. While we hypothesized that changes in urban soil microbiome taxonomic and functional diversity would be correlated to each other, our results only partially supported this hypothesis, suggesting that environmental influences in urban soils exert different selective pressures on microbial taxa vs. the functional potential they contain. Baltimore soils, in particular, had a distinct soil microbiome, and continuous efforts are needed to see how the soil microbiome changes over time as a result of urbanization. We also highlight xenobiotic degradation as a ubiquitous metabolic pathway of key interest, as microbes have been used in bioremediation of pollutants, such as PAHs, and is also indicative of other contaminants due to urbanization. Solutions to combat the detrimental impacts of urbanization, like urban greenspaces, are important areas of study for further understanding the role that soil microbiomes could play in maintaining healthy urban ecosystems (Pataki *et al.* 2011). Because

soil is the largest terrestrial reservoir for carbon storage and changes in land use associated with urbanization influence soil health, it is also key to continue monitoring soil microbiomes and their role in greenhouse gas emissions (Georgiou *et al.* 2022). This is particularly important in urban spaces where run-off may drive increased cycling of nitrogen and organic contaminants (Fenn *et al.* 2003; Kettering *et al.* 2013; Wang *et al.* 2017). Overall, our synthesis reveals unique and ubiquitous features of the urban soil microbiome that provide a basis for future studies on services the urban soil microbiome may provide insight in managing the effects urbanization has on soil and human health.

Funding.

This material is based upon work supported by the U.S. Department of Energy, Office of Science, Biological and Environmental Research program Early Career award to EBG.

Acknowledgements.

The work was performed by Pacific Northwest National Laboratory, operated by Battelle Memorial Institute for the U.S. Department of Energy under Contract DE-AC05-76RL01830. We thank the Global Urban Soil Environment Ecology Network for providing publicly available data upon which this publication is based. We also thank Caroline G. Simonsen, William L. Peterson, Saige G. Smith, and James Kourum for guidance in drafting this publication.

Conflict of Interest.

The authors report no conflicts of interest.

Figures.

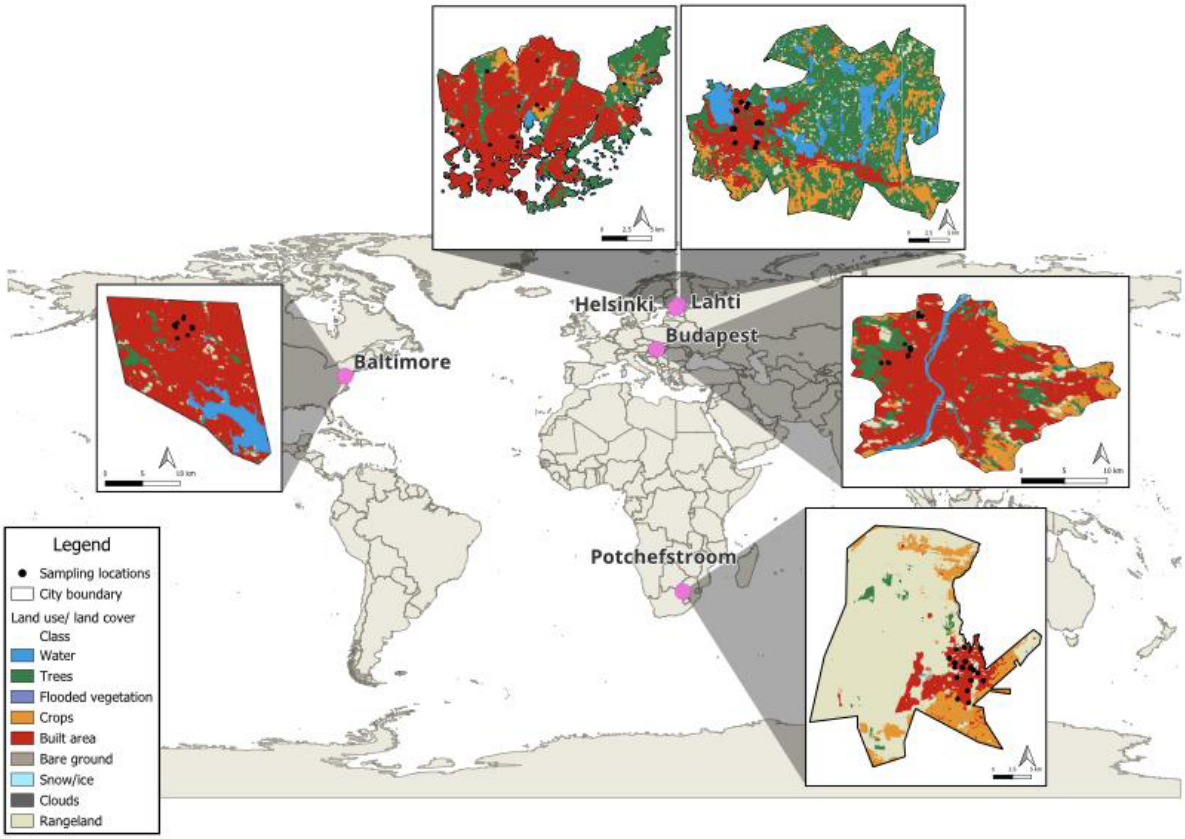


Figure 1. Geographic distribution and land use/land cover of urban soil microbiomes. All soils are from locations with a built area land use/land cover classification according to ESRI (Kontgis *et al*., 2017; 2021). Cities are colored by land use/land cover categories, and sampling locations are denoted by black dots.

Alt text: A world map with enhanced sections for each city of interest displaying overlayed land use category by color and exact sampling locations as black dots.

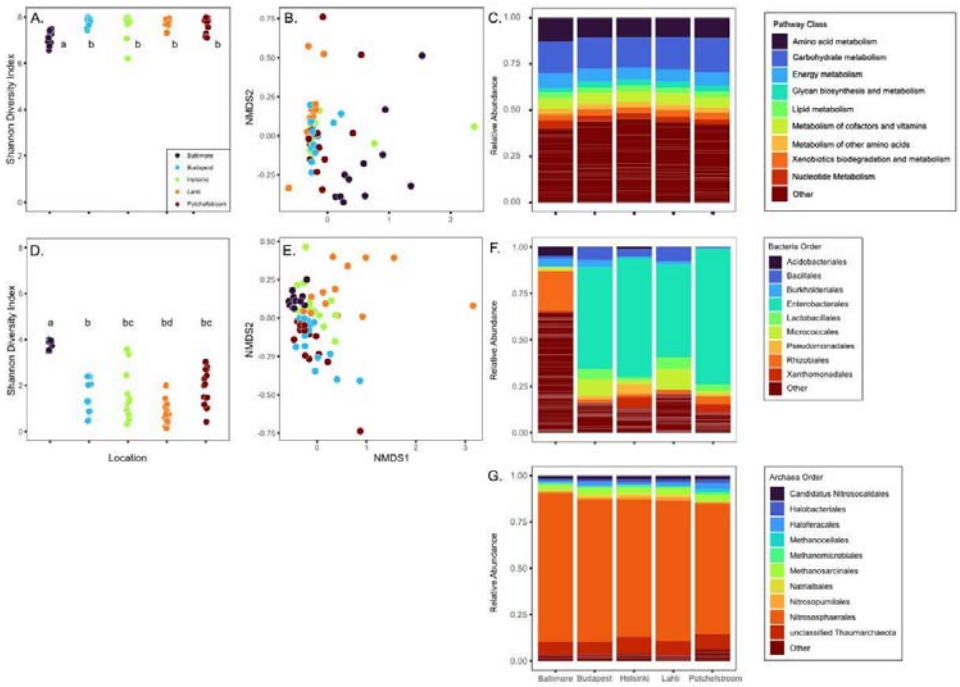


Figure 2. Alpha and beta diversity of microbial taxonomy and KO. Shannon Diversity Index and Non-metric MultiDimensional Scaling (NMDS) for each sampling location for KO (A-B) and taxonomic order (D-E), colored by city. The most abundant (C) metabolic pathways, (F) bacterial orders, and (G) archaeal orders are shown with stacked bar plots.

Alt text: Panel A and D show plots of Shannon Diversity; Panel A has KO on the y-axis and sampling location on the x-axis while Panel D has taxonomic order on the y-axis and sampling location on the x-axis. Panels B and E show NMDS plots; Panel B shows a NMDS of KO and Panel E shows a NMDS of taxonomy. Panel E shows a barchart of pathway classes, Panel F shows a barchart of bacteria, and Panel G shows a barchart of archaea.

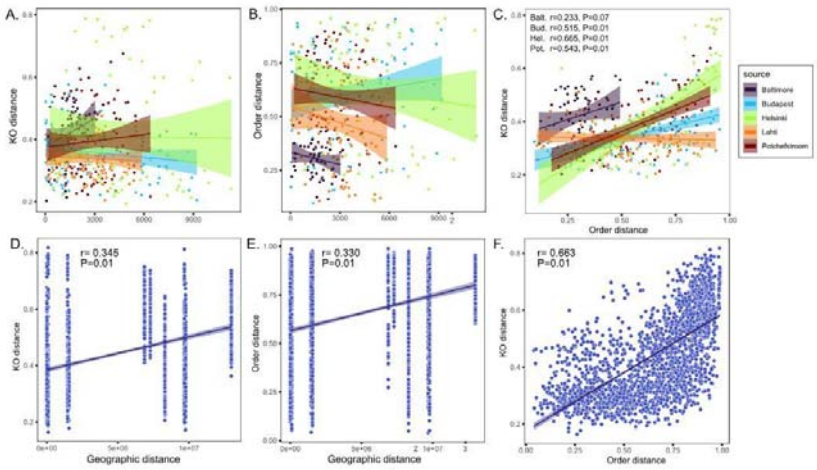


Figure 3. Correlations between urban soil microbiome taxonomy, functional potential, and geographic distance. (A-C) show correlations within individual cities, denoted by color. (A) and (B) show dissimilarity in the composition of KO's and microbial order, respectively, vs. geographic distance. (C) shows KO vs. taxonomic dissimilarity. (D) and (E) show dissimilarity in the composition of KO's and microbial order, respectively, vs. geographic distance across all cities. For all plots, geographic dissimilarity is calculated using Haversine distances, and KO and taxonomic dissimilarity are calculated with Bray-Curtis dissimilarity. Taxonomy is grouped at the order-level for all analyses.

Alt text: (A-C) compare urban soil microbiome taxonomy, functional potential, and/or geographic distance within each city (color). (D-E) compare urban soil microbiome taxonomy, functional potential, and/or geographic distance across all cities combined. Statistics are from Mantel tests.

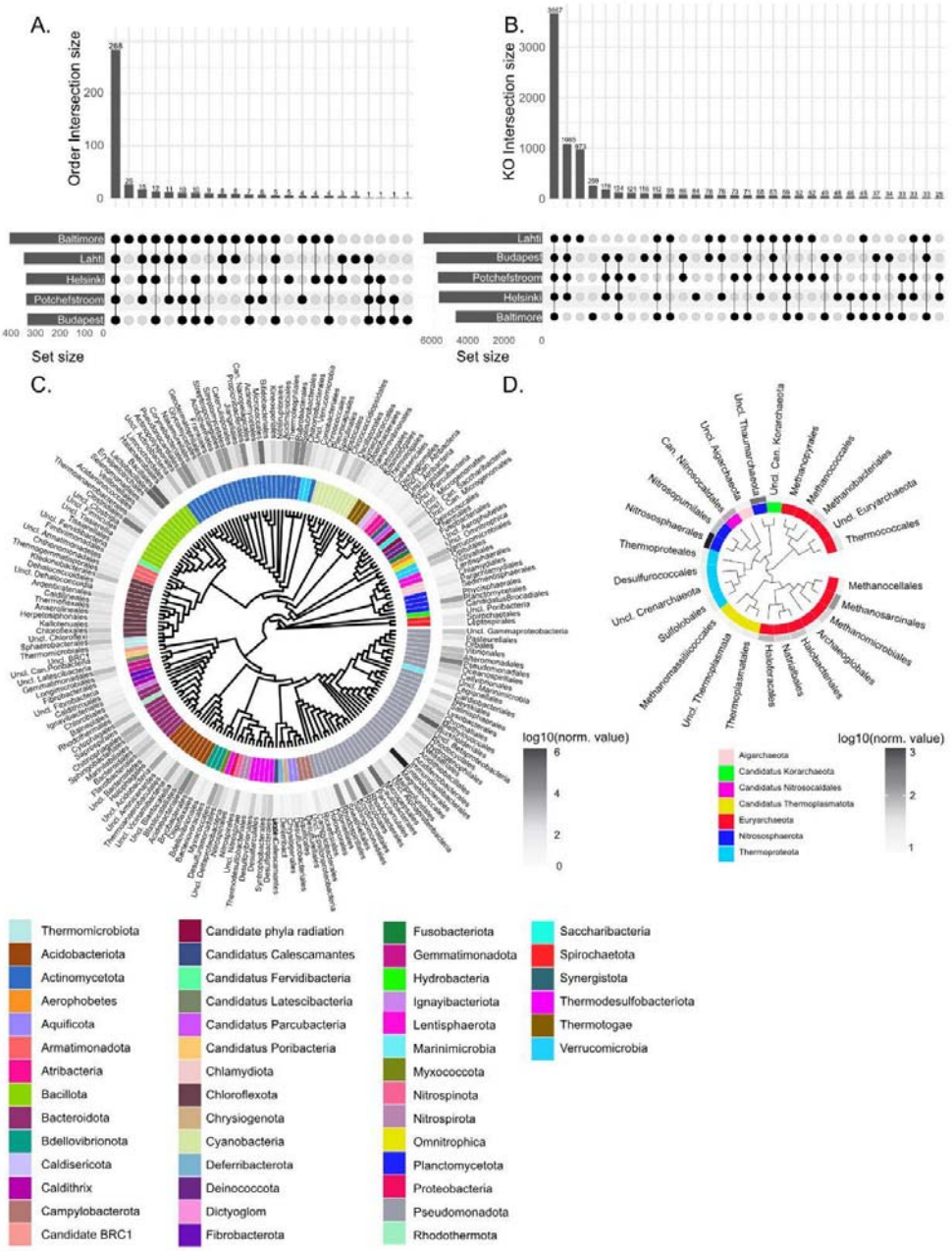


Figure 4. Core Taxonomy and Functional Potential of the Urban Soil Microbiome. Upset plots show the number of shared and unique (A) taxonomies and (B) potential functions across cities. Phylogenetic trees of (C) bacteria and (D) archaeal orders shared across all cities. The phylum associated with each order is denoted by color, and normalized abundances are shown in grayscale.

Alt text: Panel A and B show upset plots of taxonomy and function respectively, with number of shared artifacts shown above the bar and indicated on the y-axis. The x-axis shows which intersection corresponds to the y-axis; those highlighted in black are what is the intersection being observed. The sample set size on the right shows observed unique artifacts per city. Panel C and D show phylogenetic trees of shared bacteria and archaea, respectively, with the log10 of the normalized value depicted in grayscale.

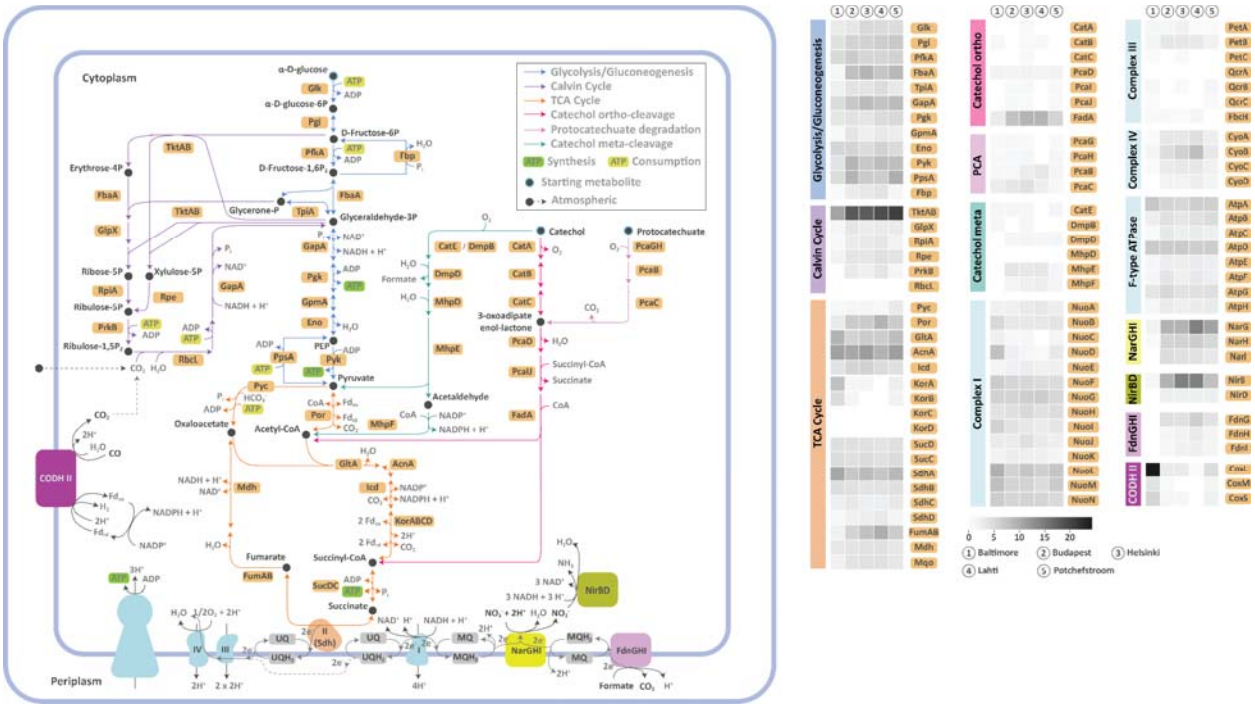


Figure 5. Selected metabolic pathways characteristics of urban soil microbiomes. Selected pathways were manually reconstructed from KEGG pathways and are color-coded as indicated in the legend. The median normalized gene abundance for each city is shown in the heatmap (grayscale).

Alt text: Cell diagram with metabolic pathways detected with abundances of specific genes shown through grayscale heatmaps on the right, organized by pathway.

References.

An D, Caffrey SM, Soh J *et al.* Metagenomics of Hydrocarbon Resource Environments Indicates Aerobic Taxa and Genes to be Unexpectedly Common. *Environ Sci Technol* 2013;**47**:10708–17.

Anderson MJ. Permutational Multivariate Analysis of Variance (PERMANOVA). *Wiley StatsRef: Statistics Reference Online*. John Wiley & Sons, Ltd, 2017, 1–15.

Andrés-Barrao C, Alzubaidy H, Jalal R *et al.* Coordinated bacterial and plant sulfur metabolism in Enterobacter sp. SA187–induced plant salt stress tolerance. *Proc Natl Acad Sci U S A* 2021;**118**:e2107417118.

Asabere SB, Zeppenfeld T, Nketia KA *et al.* Urbanization Leads to Increases in pH, Carbonate, and Soil Organic Matter Stocks of Arable Soils of Kumasi, Ghana (West Africa). *Front Environ Sci* 2018;**6**, DOI: 10.3389/fenvs.2018.00119.

Bala S, Garg D, Thirumalesh BV *et al.* Recent Strategies for Bioremediation of Emerging Pollutants: A Review for a Green and Sustainable Environment. *Toxics* 2022;**10**:484.

Bhandari V, Gupta RS. The Phylum Thermotogae. In: Rosenberg E, DeLong EF, Lory S, et al. (eds.). *The Prokaryotes: Other Major Lineages of Bacteria and The Archaea*. Berlin, Heidelberg: Springer, 2014, 989–1015.

Bier RL, Bernhardt ES, Boot CM *et al.* Linking microbial community structure and microbial processes: an empirical and conceptual overview. *FEMS Microbiol Ecol* 2015;**91**:fiv113.

Bittinger K. usedist: Distance Matrix Utilities. 2020.

Brockett B, Prescott C, Grayston S. Soil Moisture is the Major Factor Influencing Microbial Community Structure and Enzyme Activities Across Seven Biogeoclimatic Zones in Western Canada. *Soil Biol Biochem* 2012;**44**:9–20.

Brown JN, Peake BM. Sources of heavy metals and polycyclic aromatic hydrocarbons in urban stormwater runoff. *Sci Total Environ* 2006;**359**:145–55.

Brunder W, Karch H. Genome plasticity in Enterobacteriaceae. *Int J Med Microbiol IJMM* 2000;**290**:153–65.

Bushnell B. BBMap. 2023.

Campitelli E. ggnewscale: Multiple Fill and Colour Scales in “ggplot2”. 2020.

Chaumeil P-A, Mussig AJ, Hugenholtz P *et al.* GTDB-Tk: a toolkit to classify genomes with the Genome Taxonomy Database. *Bioinformatics* 2020;**36**:1925–7.

Cheng Y, Elrys AS, Merwad A-RM *et al.* Global Patterns and Drivers of Soil Dissimilatory Nitrate Reduction to Ammonium. *Environ Sci Technol* 2022;**56**:3791–800.

Christel A, Dequiedt S, Chemidlin-Prevost-Bouré N *et al.* Urban land uses shape soil microbial abundance and diversity. *Sci Total Environ* 2023;**883**:163455.

Clum A, Huntemann M, Bushnell B *et al.* DOE JGI Metagenome Workflow. *mSystems* 2021;**6**:e00804-20.

Congedo L. Semi-Automatic Classification Plugin: A Python tool for the download and processing of remote sensing images in QGIS. *J Open Source Softw* 2021;**6**:3172.

Conley DJ, Paerl HW, Howarth RW *et al.* Controlling Eutrophication: Nitrogen and Phosphorus. *Science* 2009;**323**:1014–5.

Czaplicka M. Sources and transformations of chlorophenols in the natural environment. *Sci Total Environ* 2004;**322**:21–39.

Dang C-C, Xie G-J, Liu B-F *et al.* Heavy metal reduction coupled to methane oxidation：Mechanisms, recent advances and future perspectives. *J Hazard Mater* 2021;**405**:124076.

Dang Q, Zhao X, Xi B *et al.* The key role of denitrification and dissimilatory nitrate reduction in nitrogen pollution along vertical landfill profiles from metagenomic perspective. *J Environ Manage* 2023;**342**:118300.

Delegan Y, Sushkova S, Minkina T *et al.* Diversity and Metabolic Potential of a PAH-Degrading Bacterial Consortium in Technogenically Contaminated Haplic Chernozem, Southern Russia. *Processes* 2022;**10**:2555.

Delgado-Baquerizo M, Eldridge DJ, Yu-Rong L. Global homogenization of the structure and function in the soil microbiome of urban greenspaces | Science Advances. 2021.

Ding B, Schmeling S, Fuchs G. Anaerobic Metabolism of Catechol by the Denitrifying Bacterium Thauera aromatica—a Result of Promiscuous Enzymes and Regulators? *J Bacteriol* 2008;**190**:1620–30.

Dror B, Jurkevitch E, Cytryn E. Global-scale analysis reveals distinct patterns of non-ribosomal peptide and polyketide synthase encoding genes in root and soil bacterial communities. *Soil Ecol Lett* 2023;**5**:38–45.

Dror B, Wang Z, Brady SF *et al.* Elucidating the Diversity and Potential Function of Nonribosomal Peptide and Polyketide Biosynthetic Gene Clusters in the Root Microbiome. *mSystems* 2020;**5**:10.1128/msystems.00866-20.

Dubey A, Malla MA, Khan F *et al.* Soil microbiome: a key player for conservation of soil health under changing climate. *Biodivers Conserv* 2019;**28**:2405–29.

Epp Schmidt DJ, Kotze DJ, Hornung E *et al.* Metagenomics Reveals Bacterial and Archaeal Adaptation to Urban Land-Use: N Catabolism, Methanogenesis, and Nutrient Acquisition. *Front Microbiol* 2019;**10**.

Epp Schmidt DJ, Pouyat R, Szlavecz K *et al.* Urbanization erodes ectomycorrhizal fungal diversity and may cause microbial communities to converge. *Nat Ecol Evol* 2017;**1**:0123.

Fenn ME, Baron JS, Allen EB *et al.* Ecological Effects of Nitrogen Deposition in the Western United States. *BioScience* 2003;**53**:404–20.

Fernández-Luqueño F, Valenzuela-Encinas C, Marsch R *et al.* Microbial communities to mitigate contamination of PAHs in soil—possibilities and challenges: a review. *Environ Sci Pollut Res* 2011;**18**:12–30.

Fresia P, Antelo V, Salazar C *et al.* Urban metagenomics uncover antibiotic resistance reservoirs in coastal beach and sewage waters. *Microbiome* 2019;**7**:35.

Fukuhara Y, Kasai D, Katayama Y *et al.* Enzymatic Properties of Terephthalate 1,2-Dioxygenase of Comamonas sp. Strain E6. *Biosci Biotechnol Biochem* 2008;**72**:2335–41.

Gallego A, Soulé J, Napolitano H *et al.* Biodegradability of Chlorophenols in Surface Waters from the Urban Area of Buenos Aires. *Bull Environ Contam Toxicol* 2018;**100**, DOI: 10.1007/s00128-018-2300-1.

Garbeva P, van Veen JA, van Elsas JD. Predominant Bacillus spp. in Agricultural Soil under Different Management Regimes Detected via PCR-DGGE. *Microb Ecol* 2003;**45**:302–16.

Georgiou K, Jackson RB, Vindušková O *et al.* Global stocks and capacity of mineral-associated soil organic carbon. *Nat Commun* 2022;**13**:3797.

Gieg LM, Fowler SJ, Berdugo-Clavijo C. Syntrophic biodegradation of hydrocarbon contaminants. *Curr Opin Biotechnol* 2014;**27**:21–9.

Gill AS, Purnell K, Palmer MI *et al.* Microbial Composition and Functional Diversity Differ Across Urban Green Infrastructure Types. *Front Microbiol* 2020;**11**:912.

Gougoulias C, Clark JM, Shaw LJ. The role of soil microbes in the global carbon cycle: tracking the below-ground microbial processing of plant-derived carbon for manipulating carbon dynamics in agricultural systems. *J Sci Food Agric* 2014;**94**:2362–71.

Graham EB, Camargo AP, Wu R *et al.* Global Biogeography of the Soil Virosphere. 2023:2023.11.02.565391.

Graham EB, Knelman JE. Implications of Soil Microbial Community Assembly for Ecosystem Restoration: Patterns, Process, and Potential. *Microb Ecol* 2023;**85**:809–19.

Graham EB, Knelman JE, Schindlbacher A *et al.* Microbes as Engines of Ecosystem Function: When Does Community Structure Enhance Predictions of Ecosystem Processes? *Front*

Microbiol 2016;**7**, DOI: 10.3389/fmicb.2016.00214.
Harayama S, Rekik M, Ngai KL *et al.* Physically associated enzymes produce and metabolize 2-hydroxy-2,4-dienoate, a chemically unstable intermediate formed in catechol metabolism via meta cleavage in Pseudomonas putida. *J Bacteriol* 1989;**171**:6251–8.
Harwood CS, Parales RE. THE β-KETOADIPATE PATHWAY AND THE BIOLOGY OF SELF-IDENTITY. *Annu Rev Microbiol* 1996;**50**:553–90.
Herrmann DL, Schifman LA, Shuster WD. Urbanization drives convergence in soil profile texture and carbon content. *Environ Res Lett* 2020;**15**:114001.
Hoffman EJ, Mills GL, Latimer JS *et al.* Urban runoff as a source of polycyclic aromatic hydrocarbons to coastal waters. *Environ Sci Technol* 1984;**18**:580–7.
Jansson JK, Wu R. Soil viral diversity, ecology and climate change. *Nat Rev Microbiol* 2023;**21**:296–311.
Justice SS, Hunstad DA, Cegelski L *et al.* Morphological plasticity as a bacterial survival strategy. *Nat Rev Microbiol* 2008;**6**:162–8.
Kanehisa M. KEGG: Kyoto Encyclopedia of Genes and Genomes. *Oxf Acad* 2000;**Nucleic Acids Research**:27–30.
Kanehisa M. Toward understanding the origin and evolution of cellular organisms. *Wiley Online Libr* 2019;**Protein Science**:1947–51.
Kanehisa M, Furumichi M, Y. Sato *et al.* KEGG for taxonomy-based analysis of pathways and genomes. *Oxf Acad* 2023;**Nucleic Acids Research**:D587–92.
Kang E, Crouse A, Chevallier L *et al.* Enterobacteria and host resistance to infection. *Mamm Genome Off J Int Mamm Genome Soc* 2018;**29**:558–76.
Karhu K, Auffret MD, Dungait JAJ *et al.* Temperature sensitivity of soil respiration rates enhanced by microbial community response. *Nature* 2014;**513**:81–4.
Kettering J, Ruidisch M, Gaviria C *et al.* Fate of fertilizer 15N in intensive ridge cultivation with plastic mulching under a monsoon climate. *Nutr Cycl Agroecosystems* 2013;**95**:57–72.
Knelman JE, Nemergut DR. Changes in community assembly may shift the relationship between biodiversity and ecosystem function. *Front Microbiol* 2014;**5**, DOI: 10.3389/fmicb.2014.00424.
Kolde R. pheatmap: Pretty Heatmaps. 2019.
Krassowski M. krassowski/complex-upset. Zenodo.
Kumar B, Verma VK, Joshi D *et al.* Polycyclic aromatic hydrocarbons in urban and rural residential soils, levels, composition profiles, source identification and health risk & hazard. *SN Appl Sci* 2020;**2**:2007.
Lewis WH, Tahon G, Geesink P *et al.* Innovations to culturing the uncultured microbial majority | Nature Reviews Microbiology. 2020.
Lex A, Gehlenborg N, Strobelt H *et al. UpSet: Visualization of Intersecting Sets*. IEEE Transactions on Visualization and Computer Graphics (InfoVis '14), 2020.
Liu J, Cui X, Liu Z *et al.* The Diversity and Geographic Distribution of Cultivable Bacillus-Like Bacteria Across Black Soils of Northeast China. *Front Microbiol* 2019;**10**.
Liu J, Liu Y, Dong W *et al.* Shifts in microbial community structure and function in polycyclic aromatic hydrocarbon contaminated soils at petrochemical landfill sites revealed by metagenomics. *Chemosphere* 2022;**293**:133509.
Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol* 2014;**15**:550.
Martiny AC, Treseder K, Pusch G. Phylogenetic conservatism of functional traits in microorganisms. *ISME J* 2013;**7**:830–8.
McClure R, Farris Y, Danczak R *et al.* Interaction Networks Are Driven by Community-Responsive Phenotypes in a Chitin-Degrading Consortium of Soil Microbes. *mSystems* 2022;**7**:e0037222.
Méheust R, Castelle CJ, Matheus Carnevali PB *et al.* Groundwater Elusimicrobia are

metabolically diverse compared to gut microbiome Elusimicrobia and some have a novel nitrogenase paralog. *ISME J* 2020;**14**:2907–22.

Menegat S, Ledo A, Tirado R. Greenhouse gas emissions from global production and use of nitrogen synthetic fertilisers in agriculture. *Sci Rep* 2022;**12**:19777.

Mo L, Zanella A, Squartini A *et al.* Anthropogenic vs. natural habitats: Higher microbial biodiversity pays the trade-off of lower connectivity. *Microbiol Res* 2024;**282**:127651.

Muñoz-García A, Mestanza O, Isaza JP *et al.* Influence of salinity on the degradation of xenobiotic compounds in rhizospheric mangrove soil. *Environ Pollut* 2019;**249**:750–7.

Nagendra H, Sudhira HS, Katti M *et al.* Urbanization and its Impacts on Land Use, Biodiversity and Ecosystems in India. *INTER Discip* 2014;**2**, DOI: 10.22201/ceiich.24485705e.2014.2.46532.

Naylor D, Sadler N, Bhattacharjee A *et al.* Soil Microbiomes Under Climate Change and Implications for Carbon Cycling. *Annu Rev Environ Resour* 2020;**45**:29–59.

Neri U, Wolf YI, Roux S *et al.* Expansion of the global RNA virome reveals diverse clades of bacteriophages. *Cell* 2022;**185**:4023-4037.e18.

Nurk S, Meleshko D, Korobeynikov A *et al.* metaSPAdes: a new versatile metagenomic assembler. *Genome Res* 2017;**27**:824–34.

Oksanen J, Simpson GL, Blanchet FG *et al.* vegan: Community Ecology Package. 2022.

Parks DH, Chuvochina M, Chaumeil P-A *et al.* A complete domain-to-species taxonomy for Bacteria and Archaea | Nature Biotechnology. 2020.

Parks DH, Chuvochina M, Waite DW *et al.* A standardized bacterial taxonomy based on genome phylogeny substantially revises the tree of life | Nature Biotechnology. 2018.

Pataki DE, Carreiro MM, Cherrier J *et al.* Coupling biogeochemical cycles in urban environments: ecosystem services, green solutions, and misconceptions - Pataki - 2011 - Frontiers in Ecology and the Environment - Wiley Online Library. 2011.

Pathak H, Kantharia D, Malpani A *et al.* Naphthalene degradation by Pseudomonas sp. HOB1: In vitro studies and assessment of naphthalene degradation efficiency in simulated microcosms. *J Hazard Mater* 2009;**166**:1466–73.

Pauchard A, Aguayo M, Peña E *et al.* Multiple effects of urbanization on the biodiversity of developing countries: The case of a fast-growing metropolitan area (Concepción, Chile). *Biol Conserv* 2006;**127**:272–81.

Pereira MB, Wallroth M, Jonsson V *et al.* Comparison of normalization methods for the analysis of metagenomic gene abundance data. *BMC Genomics* 2018;**19**:274.

phyloT□: a phylogenetic tree generator.

Pickett STA, Cadenasso ML, Grove JM *et al.* Urban ecological systems: Scientific foundations and a decade of progress. *J Environ Manage* 2011;**92**:331–62.

Pouyat RV, Setälä H, Szlavecz K *et al.* Introducing GLUSEEN: a new open access and experimental network in urban soil ecology. *J Urban Ecol* 2017;**3**:jux002.

Pouyat RV, Yesilonis ID, Dombos M *et al.* A Global Comparison of Surface Soil Characteristics Across Five Cities: A Test of the Urban Ecosystem Convergence Hypothesis. *Soil Sci* 2015;**180**:136.

Reinmann AB, Smith IA, Thompson JR *et al.* Urbanization and fragmentation mediate temperate forest carbon cycle response to climate - IOPscience. 2020.

Ritchie H, Samborska V, Roser M. Urbanization. *Our World Data* 2024.

Rousk J, Bååth E, Brookes PC *et al.* Soil bacterial and fungal communities across a pH gradient in an arable soil. *ISME J* 2010;**4**:1340–51.

Roux S, Emerson JB. Diversity in the soil virosphere: to infinity and beyond? *Trends Microbiol* 2022;**30**:1025–35.

Scholier T, Lavrinienko A, Brila I *et al.* Urban forest soils harbour distinct and more diverse communities of bacteria and fungi compared to less disturbed forest soils. *Mol Ecol* 2023;**32**:504–17.

Sharma M, Sudheer S, Usmani Z *et al.* Deciphering the Omics of Plant-Microbe Interaction: Perspectives and New Insights. *Curr Genomics* 2020;**21**:343–62.

Sharma R, Hansda A, Chand S. Remediation Strategies of Xenobiotics in Urban Soil and Water. In: Singh R, Singh P, Tripathi S, et al. (eds.). *Xenobiotics in Urban Ecosystems: Sources, Distribution and Health Impacts*. Cham: Springer International Publishing, 2023, 227–40.

Strokal M, Bai Z, Franssen W *et al.* Urbanization: an increasing source of multiple pollutants to rivers in the 21st century. *Npj Urban Sustain* 2021;**1**:1–13.

Tan B, Jane Fowler S, Laban NA *et al.* Comparative analysis of metagenomes from three methanogenic hydrocarbon-degrading enrichment cultures with 41 environmental samples. *ISME J* 2015;**9**:2028–45.

Tenenbaum D, Maintainer B. KEGGREST: Client-side REST access to the Kyoto Encyclopedia of Genes and Genomes (KEGG). 2023.

Tong H, Hu M, Li F *et al.* Burkholderiales participating in pentachlorophenol biodegradation in iron-reducing paddy soil as identified by stable isotope probing. *Environ Sci Process Impacts* 2015;**17**:1282–9.

Toor GS, Occhipinti ML, Yang Y-Y *et al.* Managing urban runoff in residential neighborhoods: Nitrogen and phosphorus in lawn irrigation driven runoff. *PLoS ONE* 2017;**12**:e0179151.

United Nations. World Urbanization Prospects - Population Division - United Nations. 2018.

Vyas TK, Murthy SR. Chlorobenzene degradation by Bacillus sp. TAS6CB: A potential candidate to remediate chlorinated hydrocarbon contaminated sites. *J Basic Microbiol* 2015;**55**:382–8.

Wang C, He T, Zhang M *et al.* Review of the mechanisms involved in dissimilatory nitrate reduction to ammonium and the efficacies of these mechanisms in the environment. *Environ Pollut* 2024;**345**:123480.

Wang H, Marshall CW, Cheng M *et al.* Changes in land use driven by urbanization impact nitrogen cycling and the microbial community composition in soils. *Sci Rep* 2017;**7**:44049.

Wang L-G, Lam TT-Y, Xu S *et al.* Treeio: An R Package for Phylogenetic Tree Input and Output with Richly Annotated and Associated Data. *Mol Biol Evol* 2020;**37**:599–603.

Wang X-T, Miao Y, Zhang Y *et al.* Polycyclic aromatic hydrocarbons (PAHs) in urban soils of the megacity Shanghai: Occurrence, source apportionment and potential human health risk. *Sci Total Environ* 2013;**447**:80–9.

Welcome to the QGIS Python API documentation project.

Wickham H. *Ggplot2: Elegant Graphics for Data Analysis*. Springer-Verlag New York, 2016.

Widdig M, Heintz-Buschart A, Schleuss P-M *et al.* Effects of nitrogen and phosphorus addition on microbial community composition and element cycling in a grassland soil. *Soil Biol Biochem* 2020;**151**:108041.

Wilpiszeski RL, Aufrecht JA, Retterer ST *et al.* Soil Aggregate Microbial Communities: Towards Understanding Microbiome Interactions at Biologically Relevant Scales. *Appl Environ Microbiol* 2019;**85**:e00324-19.

[dataset]***** Yarwood SA, Epp Schmidt DJ. MG-RAST.

Yu G, Smith DK, Zhu H *et al.* ggtree: an r package for visualization and annotation of phylogenetic trees with their covariates and other associated data. *Methods Ecol Evol* 2017;**8**:28–36.