Soil Organisms 98 (SI) · 2026

Online First
DOI 10.25674/473

A call for collaboration on urban soil biodiversity monitoring through eDNA methods – the Global Urban Soil Biodiversity (Global-USB) Project

Xin Sun[1,2,3]*, Jake M. Robinson[4,5], Stefan Geisen[6], Anton Potapov[7,8,9], Sardar Khan[10], Venuste Nsengimana[11], Ngwa M. Ngwabie[12], Alexei V. Tiunov[13], Haifeng Yao[1,2,3]*, Linxiu Zhang[14,15], Martin F. Breed[4,5] and Nico Eisenhauer[8,16]

[1] State Key Laboratory of Regions and Urban Ecology, Ningbo Observation and Research Station, Institute of Urban Environment, Chinese Academy of Sciences, Xiamen 361021, China
[2] Zhejiang Key Laboratory of Urban Environment Processes and Pollution Control, CAS Haixi Industrial Technology Innovation Center in Beilun, Ningbo 315830, China
[3] University of Chinese Academy of Sciences, Beijing 100049, China
[4] The Aerobiome Innovation and Research Hub, College of Science and Engineering, Flinders University, Bedford Park, SA, 5042, Australia
[5] College of Science and Engineering, Flinders University, Bedford Park, SA, 5042, Australia
[6] Laboratory of Nematology, Wageningen University and Research, Wageningen, the Netherlands
[7] Senckenberg Museum of Natural History Görlitz, Görlitz 02826, Germany
[8] German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig, Leipzig 04103, Germany
[9] International Institute Zittau, TUD Dresden University of Technology, Zittau 02763, Germany
[10] Department of Environmental Sciences, University of Peshawar, Peshawar, 25120, Pakistan
[11] Centre of Excellence in Biodiversity and Natural Resource Management, College of Science and Technology, University of Rwanda, Kigali, Rwanda
[12] Department of Agricultural and Environmental Engineering, College of Technology, The University of Bamenda, N. W. Region, Bambili, Cameroon
[13] A.N. Severtsov Institute of Ecology and Evolution, Russian Academy of Sciences, Moscow, Russia
[14] United Nations Environment Programme-International Ecosystem Management Partnership, Beijing 100101, China
[15] Institute of Geographic Sciences and Natural Resources Research, Chinese Academy of Sciences, Beijing 100101, China
[16] Institute of Biology, Leipzig University, Puschstraße 4, 04103 Leipzig, Germany
* Corresponding author, email: xsun@iue.ac.cn, hfyao@iue.ac.cn

Received 11 May 2025 | Accepted 29 August 2025 | Published online 3 March 2026

Abstract

Urban soil biodiversity sustains critical ecosystem functions such as nutrient cycling and plant growth, while also supporting human health through pathogen suppression, soil remediation, and human immune system training. Yet, the distribution of urban soil biodiversity and its dependence on the influencing city-specific factors, such as economic development (e.g., GDP, Human Development Index), population density, and effects of climate change remain poorly understood. To bridge this research gap, we propose a global initiative applying environmental DNA (eDNA) metabarcoding to classify, map and better understand urban soil

SENCKENBERG
NATURE RESEARCH

ISSN 1864-6417 · **eISSN** 2509-9523
www.soil-organisms.org

biodiversity and uncover its environmental drivers. We introduce a sampling and analysis protocol that facilitates the integration of global urban soil biodiversity data using eDNA analysis (acronym: Global-USB). We aim to build and grow a global network of researchers who contribute and analyze urban soil biodiversity data in a standardized way. This initiative will assess the impacts of urban land use and development on soil biota to gain insights into subsequent feedback effects on ecosystem functions and human well-being. By creating new and integrating established global datasets, this effort aims to generate actionable scientific insights to inform sustainable urban planning and policy, contributing to cities that support both ecological integrity and human well-being.

Keywords Urban soils | environmental DNA metabarcoding | urbanization | driving factors

1 Introduction

The urban population continues to grow, with environments dominated by residential, commercial, and industrial land expanding rapidly (Haase et al., 2018). According to the United Nations' World Urbanization Prospects, the global urban population is projected to increase by 2.5 billion by 2050, with nearly 90% of this growth concentrated in Asia and Africa (United Nations, 2019). Simultaneously, under the dual pressures of global climate change and human activities, ecological issues such as frequent extreme weather events, worsening environmental pollution, and biodiversity loss are becoming increasingly prominent (Seto et al., 2012).

Against this backdrop, urban soils with associated biota play critical roles as interfaces connecting natural ecosystems and human society (Guilland et al., 2018). Urban soils, including their biota, are essential for maintaining a dynamic biological equilibrium and providing vital ecosystem services, such as carbon sequestration, pollutant purification, nutrient cycling, and climate regulation (Bardgett & van der Putten, 2014; Delgado-Baquerizo et al., 2020). Moreover, urban soils contribute to human health by suppressing pathogens, remediating soils, and via exposure to microbes and their products (Fan et al., 2023; Sun et al., 2023). Understanding and preserving urban soil biodiversity is therefore crucial for building sustainable and resilient city environments that supports both ecological integrity and human well-being.

Recent research has significantly advanced our understanding of urban soil biodiversity. Global studies reveal that urban green spaces harbor higher soil microbial diversity but exhibit strong community homogenization compared to surrounding natural ecosystems (Delgado-Baquerizo et al., 2021). Continental-scale studies in China support these patterns and raised new dynamics, including land-use-dependent variation and decoupled parasite-metazoan host biodiversity relationships in urban environments (Gong et al., 2023; Li et al., 2023; Sun et al., 2025). This homogenization is largely driven by similarities in urban planning, development practices, environmental conditions, and cultural preferences that create comparable habitats across cities (Groffman et al., 2017). However, at the scale of a single city, some soil communities may retain higher local soil biodiversity with weaker homogenization effects (Whitehead et al., 2022; Bock et al., 2024). Such variability underscores the importance of examining the impact of urbanization on biodiversity across different spatial scales. Despite these advances, significant gaps remain in our understanding of how urbanization gradients affect biodiversity patterns across different ecosystem types (e.g., woodlands vs. grasslands), and the consequences of these biodiversity changes for ecosystem services. Additionally, due to the limited geographic scope of existing samples, the influence of urban factors, such as economic development, population density, and local policies, on these patterns remains poorly understood.

Environmental DNA (eDNA) methods have emerged as an efficient tool for monitoring biodiversity, especially in freshwater systems (Rees et al., 2014; Wang et al., 2021). Previous studies utilizing eDNA methods have also demonstrated their feasibility and effectiveness in monitoring soil biodiversity, and this paves the way for their application in large-scale urban soil biodiversity assessments (Delgado-Baquerizo et al., 2021; Mills et al., 2017; Yao et al., 2023). This approach holds great promises for advancing our understanding of urban soil ecosystems and developing strategies to protect and enhance their critical functions. Here, we introduce a sampling and analysis protocol that facilitates the integration of global urban soil biodiversity data using eDNA analysis (acronym: **Global-USB**). We aim to build and grow a global network of researchers who contribute and analyze data in a standardized way.

2 Project Overview

The Urban Soil Biodiversity Project is a pioneering international initiative supported by the National Natural Science Foundation of China (NSFC) and the United Nations Environment Programme (UNEP), spanning from 2024 to 2028. This collaborative effort brings together leading scientific research teams from Australia, Europe, Africa, and Asia to investigate the distribution patterns and environmental drivers of urban soil biodiversity across multiple cities worldwide. The central focus of the project is to understand how urbanization influences the relationship between soil biodiversity and the driving factors.

As of April 2025, the team has collected soil samples from over 20 cities in China, 1 city in Russia, 5 cities in Germany, 2 cities in Switzerland, and 4 cities in Rwanda. Over the next two years (2026–2027), the project aims to expand its scope to include additional cities across diverse climate zones and developing regions (e.g., Australia, Africa, Latin America), ensuring a comprehensive global perspective. This initiative seeks to generate critical insights into urban soil ecosystems, informing sustainable urban planning and policy to support both ecological and human health.

3 A standardized urban soil biodiversity monitoring using eDNA methods

3.1 Sampling Methodology

In each city, soil samples are collected from two natural ecosystems and two urbanized counterparts, representing the main ecosystem types in the region. The natural ecosystems consist of undisturbed or semi-natural vegetation, typically located in peri-urban areas. In contrast, urban sites are situated within city limits and experience higher levels of human activity compared to natural sites. For example, in Chinese and German cities, four land-use types are selected: (1) peri-urban forests, (2) natural or semi-natural grasslands, (3) urban woodlands, and (4) urban grasslands. For each city and land-use type, four sampling plots are established,

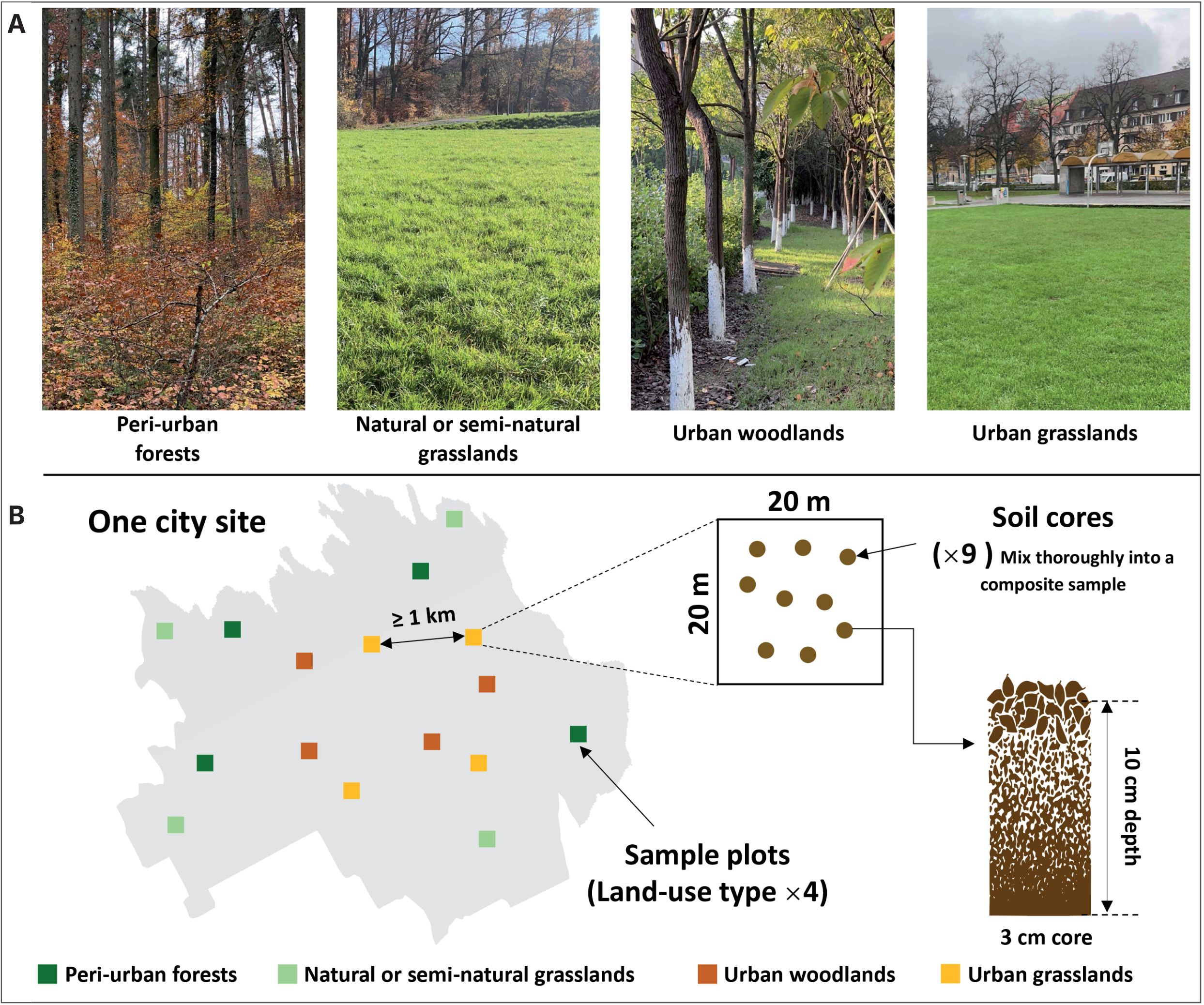

Figure 1. The soil sampling design. (**A**) The example photographs of the four land-use types (peri-urban forests, natural or semi-natural grasslands, urban woodlands, and urban grasslands), and (**B**) example of soil sampling design for individual land-use type and location/city. Four replicate plots will be sampled for each land-use type at each location/city; in each plot, nine soil cores will be taken and combined into one composite sample.

spaced at least 1,000 m apart to minimize spatial dependence. At each sampling plot, a 20 m × 20 m area is randomly established and positioned 20–50 m away from the edge to minimize potential edge effects. Within each plot, nine soil cores are collected to a depth of 10 cm using a 3 cm diameter auger (with litter). These cores are thoroughly mixed to form a single composite sample per plot. All samples should be cooled in the field and immediately frozen in the lab at -20°C to preserve their integrity until further analysis (Fig. 1). Between plots, the soil corer should be thoroughly cleaned or sterilized with 75% ethanol solution.

All sampling sites must report their latitude and longitude coordinates accurately recorded using GPS devices (WGS84 coordinate system recommended, with precision to 6 decimal places). These geolocation data will serve as the fundamental reference for subsequent climate characteristic analysis by the core team (e.g., WorldClim database retrieval) and socioeconomic factor extraction (including but not limited to spatial statistical analyses of population density, urban area and urbanization rate of residents).

3.2 Analyses of soil and soil biota

3.2.1 Essential

Soil biota: Each composite soil sample (fresh and unsieved) will be thoroughly mixed, and 10 g will be homogenized. Genomic DNA will be extracted from 0.5 g of soil using the FastDNA® Spin Kit for Soil (MP Biomedicals, Santa Ana, USA), following the manufacturer's protocol. The quality and quantity of the extracted DNA will be evaluated using 1% agarose gel electrophoresis and spectrophotometric analysis with the NanoDrop ND-2000 (Thermo Fisher Scientific, Wilmington, USA), respectively. Manufacturers can be adjusted in consultation with the core team if compliance issues arise.

High-throughput sequencing will be employed to analyze soil biota by targeting specific genetic regions:

- Bacteria: The V4-V5 region of the 16S rRNA gene, amplified using the primer sets 515F (5'-GTGCCAGCMGCCGCGG-3') and 907R (5'-CCGTCAATTCMTTTRAGTTT-3') (Biddle et al., 2008).
- Fungi: The internal transcribed spacer 1 (ITS1) region, amplified using the primer sets ITS1F (5'-CTTGGTCATTTAGAGGAAGTAA-3') and ITS2R (5'-GCTGCGTTCTTCATCGATGC-3') (Adams et al., 2013; White et al., 1990).
- Protists and Metazoa: The V4 region of the 18S rRNA gene, amplified using the primer sets F-TAReuk454FWD1 (5'-CCAGCASCYGCGGTAATTCC-3') and R-TAReukREV3 (5'-ACTTTCGTTCTTGATYRA-3') (Stoeck et al., 2010).

3.2.2 Desirable

Plant taxa at the site will be documented through written descriptions and/or photographs.

Soil properties will be assessed through a range of physical and chemical analyses. Texture analysis will be performed to determine the proportion of clay, silt and sand within the samples. Nutrient stoichiometry will be measured by quantifying total concentrations of carbon (C), nitrogen (N), phosphorus (P) and sulfur (S), allowing for the calculation of elemental ratios that reflect soil nutrient balance. Additional assessments will include total organic carbon (TOC), soil pH, and moisture content, measured gravimetrically by drying fresh soil samples at 105°C until constant weight.

To assess nutrient availability, concentrations of ammonium nitrogen (NH_4^+-N), nitrate nitrogen (NO_3^--N), and available phosphorus will be measured. Soil extractions will be performed using standard KCl (for nitrogen forms) and Olsen (for phosphorus) methods, followed by spectrophotometric quantification.

Microbial activity will be assessed using glucose-induced respiration, where a known quantity of glucose is added to fresh soil, and subsequent CO_2 evolution is measured over a fixed incubation period as an indicator of microbial metabolic potential.

Soil texture (clay, silt, and sand content) will be analyzed using a laser diffraction particle size analyzer, while bulk density will be measured via the core method.

Where applicable, we will also quantify concentrations of key heavy metals (e.g., As, Cd, Cr, Pb, and Hg) to assess potential contamination. These will be extracted via acid digestion and analyzed using inductively coupled plasma mass spectrometry (ICP-MS), providing insight into soil pollution levels and associated ecological risks (For data template, please see supplementary material).

4 Mode of collaboration

We welcome diverse forms of cooperation, including participation in sampling, data sharing, and providing local support for fieldwork. All contributions are valued and acknowledged, as long as they fit the scope of the project and can be implemented accordingly. The main contributors will be invited to contribute as co-authors to resulting products, if their data is used. Data will be shared within this collaborative network, but its use should be governed by the level of contribution to the initiative.

4.1 Soil collection and delivery to designated hubs

If local collaborators are unable to conduct the required analyses, soil samples collected following the standardized protocols can be sent to designated hubs. Currently, these hubs are located in:

- China: Institute of Urban Environment, Chinese Academy of Sciences.
- Germany: German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig.

We encourage the establishment of additional national hubs to facilitate broader participation. International soil transportation should follow the agreements to the Convention on Biological Diversity (CBD) and Nagoya Protocol. Once the analyses are completed at the hubs, the resulting data will be shared with the respective local collaborators.

4.2 Data delivery and sharing

Where feasible, we encourage local collaborators to perform soil measurements following the outlined protocols and share the data directly. Additionally, raw data from previously conducted experimental studies is also welcome. Shared data should be sent directly via email to the corresponding author.

4.3 Local sampling support provision

If local collaborators are unable to conduct the sampling or perform the required analyses independently, additional support may be provided. This includes assistance with logistics, such as renting a vehicle and hiring a driver. In such cases, core team members will travel to the respective cities and conduct the sampling in collaboration with the local partners, ensuring adherence to the standardized protocols. Requests should be addressed to the core team members at the Urban Soil Ecology Lab, Institute of Urban Environment, Chinese Academy of Sciences.

5 Building a global community of researchers

Regular online meetings will be held to share the progress of the network and to allow for broad participation. Moreover, to facilitate in-person exchange, potential sessions will also be organized at international meetings (e.g., Global Soil Biodiversity Conferences).

6 Future prospects

The Global Urban Soil Biodiversity (Global-USB) Project will provide a comprehensive understanding of how urbanization impacts the distribution of soil biodiversity and the driving factors. In particular, the worldwide collaboration and standardization will shed light on the effects of economic and climatic factors, as well as the impact of public awareness and policy frameworks in different countries, on urban soil biodiversity and ecosystem functions. This initiative will generate critical evidence to guide the protection, management, and restoration of soil biodiversity in urban areas. By doing so, it will support urban greening efforts, ensuring that cities can deliver functional ecosystems and essential services to their inhabitants. Ultimately, this will contribute to fostering healthier, more resilient, and sustainable urban environments for people across the globe.

Acknowledgements

X.S. acknowledges support from the National Natural Science Foundation of China (no. 32361143523), the National Key Research and Development Program of China (no. 2023YFF1304600), the International Partnership Program of Chinese Academy of Sciences (no. 322GJHZ2022028FN) and the support of the Alexander von Humboldt Foundation. N.E. acknowledges support from iDiv (German Research Foundation, DFG, grant no. FZT 118, 202548816) and the DFG (grant no. Ei 862/29-1).

References

Adams, R. I., Miletto, M., Taylor, J. W., & Bruns, T. D. (2013). Dispersal in microbes: fungi in indoor air are dominated by outdoor air and show dispersal limitation at short distances. *The ISME Journal*, *7*(7), 1262-1273. https://doi.org/10.1038/ismej.2013.28

Bardgett, R. D., & Van Der Putten, W. H. (2014). Belowground biodiversity and ecosystem functioning. *Nature,* 515(7528), 505-511. https://doi.org/10.1038/nature13855

Biddle, J. F., Fitz-Gibbon, S., Schuster, S. C., Brenchley, J. E., & House, C. H. (2008). Metagenomic signatures of the Peru Margin subseafloor biosphere show a genetically distinct environment. *Proceedings of the National Academy of Sciences,* 105(30), 10583-10588. https://doi.org/10.1073/pnas.0709942105

Bock, H. W. et al. (2024). Soil animal communities demonstrate simplification without homogenization along an urban gradient. *Ecological Applications*, 34, e3039. https://doi.org/10.1002/eap.3039.

Delgado-Baquerizo, M., Eldridge, D. J., Liu, Y. R., Sokoya, B., Wang, J. T., Hu, H. W., ... & Fierer, N. (2021). Global homogenization of the structure and function in the soil microbiome of urban greenspaces. *Science Advances,* 7(28), eabg5809. https://doi.org/10.1126/sciadv.abg5809

Delgado-Baquerizo, M., Reich, P. B., Trivedi, C., Eldridge, D. J., Abades, S., Alfaro, F. D., ... & Singh, B. K. (2020). Multiple elements of soil biodiversity drive ecosystem functions across biomes. *Nature Ecology & Evolution,* 4(2), 210-220. https://doi.org/10.1038/s41559-019-1084-y

Fan, K., Chu, H., Eldridge, D. J., Gaitan, J. J., Liu, Y. R., Sokoya, B., ... & Delgado-Baquerizo, M. (2023). Soil biodiversity supports the delivery of multiple ecosystem functions in urban greenspaces. *Nature Ecology & Evolution,* 7(1), 113-126. https://doi.org/10.1038/s41559-022-01935-4

Gong, X., Sun, X., Thakur, M. P., Qiao, Z., Yao, H., Liu, M., ... & Zhu, Y. G. (2023). Climate and edaphic factors drive soil nematode diversity and community composition in urban ecosystems. *Soil Biology and Biochemistry,* 180, 109010. https://doi.org/10.1016/j.soilbio.2023.109010

Guilland, C., Maron, P. A., Damas, O., & Ranjard, L. (2018). Biodiversity of urban soils for sustainable cities. *Environmental Chemistry Letters,* 16, 1267-1282. https://doi.org/10.1007/s10311-018-0751-6

Groffman, P. M. et al. (2017). Ecological homogenization of residential macrosystems. *Nature Ecology & Evolution*, 1, 0191. https://doi.org/10.1038/s41559-017-0191.

Haase, D., Güneralp, B., Dahiya, B., Bai, X., & Elmqvist, T. (2018). The Urban Planet: Knowledge Towards Sustainable Cities. *Global Urbanization*, *19*, 326-339.

Li, Z. P., Geisen, S., Shangguan, H. Y., Tiunov, A. V., Scheu, S., Zhu, Y. G., & Sun, X. (2023). Land use decouples parasite–metazoan host biodiversity associations in soils across subtropical and temperate zones in China. *Global Ecology and Biogeography,* 32(12), 2164-2176. https://doi.org/10.1111/geb.13758

Mills, J. G., Weinstein, P., Gellie, N. J., Weyrich, L. S., Lowe, A. J., & Breed, M. F. (2017). Urban habitat restoration provides a human health benefit through microbiome rewilding: the Microbiome Rewilding Hypothesis. *Restoration Ecology,* 25(6), 866-872. https://doi.org/10.1111/rec.12610

Rees, H. C., Maddison, B. C., Middleditch, D. J., Patmore, J. R., & Gough, K. C. (2014). The detection of aquatic animal species using environmental DNA–a review of eDNA as a survey tool in ecology. *Journal of Applied Ecology,* 51(5), 1450-1459. https://doi.org/10.1111/1365-2664.12306

Seto, K. C., Güneralp, B., & Hutyra, L. R. (2012). Global forecasts of urban expansion to 2030 and direct impacts on biodiversity and carbon pools. *Proceedings of the National Academy of Sciences,* 109(40), 16083-16088. https://doi.org/10.1073/pnas.1211658109

Stoeck, T., Bass, D., Nebel, M., Christen, R., Jones, M. D., Breiner, H. W., & Richards, T. A. (2010). Multiple marker parallel tag environmental DNA sequencing reveals a highly complex eukaryotic community in marine anoxic water. *Molecular Ecology*, 19, 21-31. https://doi.org/10.1111/j.1365-294X.2009.04480.x

Sun, X., Liddicoat, C., Tiunov, A., Wang, B., Zhang, Y., Lu, C., ... & Zhu, Y. G. (2023). Harnessing soil biodiversity to promote human health in cities. *npj Urban sustainability,* 3(1), 5. https://doi.org/10.1038/s42949-023-00086-0

Sun, X., Robinson, J.M., Delgado-Baquerizo, M., ... & Zhu, Y. G. (2025) Unforeseen high continental-scale soil microbiome homogenization in urban greenspaces. *Nature Cities*. https://doi.org/10.1038/s44284-025-00294-y

United Nations. Department of Economic and Social Affairs. (2019). World Urbanization Prospects: The 2018 Revision. United Nations, Department of International Economic and Social Affairs.

Wang, Y., Chen, K., Gao, J., Wang, M., Dong, J., Xie, Y., ... & Wang, B. (2021). Environmental DNA of preservative ethanol performed better than water samples in detecting macroinvertebrate diversity using metabarcoding. *Diversity and Distributions,* 27(10), 1989-2002. https://doi.org/10.1111/ddi.13284

White, T. J., Bruns, T., Lee, S. J. W. T., & Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *PCR protocols: a guide to methods and applications,* 18(1), 315-322.

Whitehead, J., Roy, J., Hempel, S., & Rillig, M. C. (2022). Soil microbial communities shift along an urban gradient in Berlin, Germany. *Frontiers in Microbiology*, 13, 972052. https://doi.org/10.3389/fmicb.2022.972052

Yao, H., Li, Z., Geisen, S., Qiao, Z., Breed, M. F., & Sun, X. (2023). Degree of urbanization and vegetation type shape soil biodiversity in city parks. *Science of the Total Environment,* 899, 166437. https://doi.org/10.1016/j.scitotenv.2023.166437