Title: Soil biodiversity supports the delivery of multiple ecosystem functions in urban greenspaces

Authors: Kunkun Fan[1,2], Haiyan Chu[1,2]*, David J. Eldridge[3], Juan J. Gaitan[4,5,6], Yu-Rong Liu[7], Blessing Sokoya[8], Jun-Tao Wang[9], Hang-Wei Hu[10], Ji-Zheng He[10], Wei Sun[11], Haiying Cui[11], Fernando D. Alfaro[12], Sebastian Abades[12], Felipe Bastida[13], Marta Díaz-López[13], Adebola R. Bamigboye[14], Miguel Berdugo[15,16], José L. Blanco-Pastor[17], Tine Grebenc[18], Jorge Duran[19], Javier G. Illán[20], Thulani P. Makhalanyane[21], Arpan Mukherjee[22], Tina U. Nahberger[18], Gabriel F. Peñaloza-Bojacá[23], César Plaza[24], Jay Prakash Verma[22], Ana Rey[25], Alexandra Rodríguez[19], Christina Siebe[26], Alberto L. Teixido[27], Pankaj Trivedi[28], Ling Wang[11], Jianyong Wang[11], Tianxue Yang[11], Xin-Quan Zhou[7], Xiaobing Zhou[29], Eli Zaady[30], Leho Tedersoo[31], Manuel Delgado-Baquerizo[32,33]*.

Affiliations:
[1]State Key Laboratory of Soil and Sustainable Agriculture, Institute of Soil Science, Chinese Academy of Sciences, 71 East Beijing Road, Nanjing 210008, China.
[2]University of Chinese Academy of Sciences, Beijing 100049, China.
[3]Centre for Ecosystem Science, School of Biological, Earth and Environmental Sciences, University of New South Wales, Sydney, New South Wales 2052, Australia.
[4]National Institute of Agricultural Technology (INTA), Institute of Soil Science. Hurlingham 1686, Argentina.
[5]National University of Luján, Department of Technology. Luján 6700, Argentina.
[6]National Research Council of Argentina (CONICET). Buenos Aires 1425, Argentina.
[7]College of Resources and Environment, Huazhong Agricultural University, Wuhan 430070, China.
[8]Cooperative Institute for Research in Environmental Sciences, University of Colorado, Boulder, Colorado, USA.
[9]Hawkesbury Institute for the Environment, Western Sydney University, Penrith, New South Wales 2751, Australia.
[10]School of Agriculture and Food, Faculty of Veterinary and Agricultural Sciences, The University of Melbourne, Parkville, Victoria, 3010, Australia.
[11]Institute of Grassland Science, Northeast Normal University, Changchun 130024, China.
[12]GEMA Center for Genomics, Ecology and Environment, Faculty of Interdisciplinary Studies, Universidad Mayor, Santiago, Chile.
[13]CEBAS-CSIC, Murcia, 30100, Spain.
[14]Natural History Museum (Botany Unit), Obafemi Awolowo University, Ile-Ife, Nigeria.
[15]Institut de Biologia Evolutiva (UPF-CSIC), 08003 Barcelona, Spain.
[16]Institute of Integrative Biology, Department of Environment Systems Science, ETH Zurich, Univeritätstrasse.

[17]Department of Plant Biology and Ecology, University of Seville, Avda. Reina Mercedes 6, ES-41012 Seville, Spain.
[18]Department of Forest Physiology and Genetics, Slovenian Forestry Institute, Ljubljana, Slovenia.
[19]Centre for Functional Ecology, Department of Life Sciences, University of Coimbra, 3000-456 Coimbra, Portugal.
[20]Department of Entomology, Washington State University, Pullman, WA 99164, USA.
[21]Centre for Microbial Ecology and Genomics, Department of Biochemistry, Genetics and Microbiology, University of Pretoria, Pretoria 0028, South Africa.
[22]Institute of Environment and Sustainable Development Banaras Hindu University Varanasi 221005 (U.P.), India.
[23]Laboratório de Sistemática Vegetal, Departamento de Botânica, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Av. Antônio Carlos, 6627, Pampulha, Belo Horizonte, 31270-901 MG, Brazil.
[24]Instituto de Ciencias Agrarias, Consejo Superior de Investigaciones Científicas, 28006 Madrid, Spain.
[25]Department of Biogeography and Global Change, National Museum of Natural History (MNCN), Spanish National Research Council (CSIC) C/ Serrano 115bis, 28006 MADRID, Spain.
[26]Instituto de Geología, Universidad Nacional Autónoma de México, Ciudad Universitaria, México D.F. CP 04510, México.
[27]Departamento de Botância e Ecologia, Instituto de Biociências, Universidade Federal de Mato Grosso, Av. Fernando Corrêa, 2367, Boa Esperança, Cuiabá, 78060- 900 MT, Brazil.
[28]Microbiome Network and Department of Agricultural Biology, Colorado State University, Fort Collins, CO 80523, USA.
[29]State Key Laboratory of Desert and Oasis Ecology, Xinjiang Institute of Ecology and Geography, Chinese Academy of Sciences, Urumqi 830011, China.
[30]Department of Natural Resources, Agricultural Research Organization, Institute of Plant Sciences, Gilat Research Center, Mobile Post Negev, 8531100, Israel.
[31]Department of Mycology and Microbiology, University of Tartu, Ravila 14a, 50411 Tartu, Estonia.
[32]Instituto de Recursos Naturales y Agrobiología de Sevilla (IRNAS), CSIC, Av. Reina Mercedes 10, E-41012, Sevilla, Spain.
[33]Unidad Asociada CSIC-UPO (BioFun). Universidad Pablo de Olavide, 41013 Sevilla, Spain.

***Corresponding authors:**
Manuel Delgado-Baquerizo[32,33]*
Haiyan Chu[1,2]*
Email: m.delgadobaquerizo@gmail.com; hychu@issas.ac.cn

Abstract

While the contribution of biodiversity to supporting multiple ecosystem functions is well-established in natural ecosystems, the relationship of the above and belowground diversity with ecosystem multifunctionality remains virtually unknown in urban greenspaces. Here, we conducted a standardized survey of urban greenspaces from 56 municipalities across six continents, aiming to investigate the relationships of plant and soil biodiversity (diversity of bacteria, fungi, protists, and invertebrates, and metagenomics-based functional diversity) with 18 surrogates of ecosystem functions from nine ecosystem services. We found that soil biodiversity across biomes was significantly and positively correlated with multiple dimensions of ecosystem functions, and contributed to key ecosystem services such as microbial-driven carbon pools, organic matter decomposition, plant productivity, nutrient cycling, water regulation, plant–soil mutualism, plant pathogen control, and antibiotic resistance regulation. Plant diversity only indirectly influenced multifunctionality in urban greenspaces via changes in soil conditions that were associated with soil biodiversity. These findings were maintained after controlling for climate, spatial context, soil properties, vegetation, and management practices. This study provides solid evidence that conserving soil biodiversity in urban greenspaces is key to support multiple dimensions of ecosystem functioning, which is critical for the sustainability of urban ecosystems and human well-being.

Main

Urban greenspaces, such as urban forests and grass lawns, are fundamental for sustaining healthy and vibrant human populations, and, in many cases, represent the only point of contact citizens have with nature. Yet, urban forests and lawns also play critical roles in supporting biodiversity and ecosystem services that are at the core of the Sustainable Development Goals[1]. For instance, healthy and sustainable urban greenspaces support multiple dimensions of ecosystem functioning, including recreation, urban heating, and pollution regulation (less noise and contamination), climate change mitigation (soil carbon sequestration and regulation of greenhouse gas emissions), water regulation, and pathogen control[2-5]. Urban greenspaces also support less obvious ecosystem services for citizens, such as soil nutrient cycling, plant–soil mutualisms, and plant productivity. These less apparent services are critically important not only for carbon sequestration and pathogen control, but also provide habitat for biodiversity, the foundation of all life on Earth. A better understanding of the environmental factors and management practices associated with these ecosystem functions and services is critical to ensure the sustainability of urban greenspaces. This is likely to become more crucial with an increase in the global human population and rising concerns of climate change.

In natural ecosystems, above- and below-ground biodiversity plays essential roles in promoting multiple ecosystem functions and services simultaneously (hereafter ecosystem multifunctionality)[6-9]. Global surveys and experiments demonstrate that soil biodiversity can drive the multifunctionality of natural environments[10-13]. Much less is known, however, about the relationship of above- and below-ground biodiversity with ecosystem multifunctionality in urban greenspaces. Soils in urban greenspaces are home to a diverse community of microbes[3,14], including bacteria, fungi, protists, and invertebrates co-occurring in the soil multitrophic food-webs. Moreover, a local study in Berlin, Germany, suggested that plant diversity indirectly promote soil multifunctionality in city parks via changes in the biodiversity of soil fauna[15]. Yet, the extent to which biodiversity of different soil organisms is associated with multiple dimensions of ecosystem functioning in urban greenspaces remains virtually unknown; the linkage between the diversity of soil microbial traits and multifunctionality is far less studied, and has never been investigated in urban greenspaces; particularly across broad climatic gradients. Apart from its scientific relevance, a greater understanding of urban environments can provide critical knowledge that helps us to manage them across markedly different biotic and environmental gradients, often with widely different management practices.

Here we conducted a standardized field survey to investigate the relationship of plant and soil biodiversity (taxonomic and functional information based on amplicon sequencing and metagenomics) with multiple ecosystem functions [18 surrogates of ecosystem functions associated with nine ecosystem services: microbial-driven carbon (C) pools, water regulation, nutrient cycling, plant–soil mutualism, organic matter (OM) decomposition, plant productivity, pathogen control, antibiotic resistance gene (ARG) control, and multifunctionality] in urban greenspaces of 56 municipalities across six continents (Fig. 1 and Extended Data Fig. 1; Supplementary Table 1 and 2). In each urban greenspace, we established three transects and collected topsoil composite samples across a representative area of 900 m^2 (Supplementary Fig. 1). Perennial plant diversity (richness; the number of perennial plant species) was measured in the field. Soil taxonomic (bacteria, fungi, protists, and invertebrates) and functional (diversity of functional genes; hereafter microbial traits) traits were determined using next-generation sequencing techniques. Ecosystem functioning was described considering multiple aspects of ecosystem functions, measured in the field and laboratory, including ecosystem multifunctionality (weighted EMF)[13], individual functions, the number of functions working over a given functional threshold, and multiple dimensions of ecosystem function evaluated using ecological network theory. Further, we collected information on urban management practices, soil properties, and climate to investigate the direct and indirect influence of environmental conditions on biodiversity and function in urban greenspaces.

We hypothesized that (i) soil biodiversity (i.e., richness of bacteria, fungi, protists and invertebrates, and functional genes), resident in soils, supports multiple aspects of ecosystem functions in urban greenspaces. Each group of soil organisms might support different aspects of ecosystem functions. While the diversity of larger organisms is especially important for supporting a high number of functions working at high levels of functioning (> 75% threshold), the diversity of smaller organisms such as bacteria and fungi is important for explaining a high number of functions working at low levels of functioning (< 25% threshold). Larger organisms (e.g., invertebrates) control the entry of processed organic matter from litter to the soil system[13], whereas smaller organisms play critical roles in nutrient cycling and later mineralization processes. (ii) Soil biodiversity would be more important than plant diversity in driving multifunctionality in urban greenspaces. Unlike in natural environments, plants are typically introduced to urban greenspaces many times a year, and subject to direct and frequent disturbance from city park management practices. Thus, the contribution of plants to ecosystem functions in urban greenspaces would be restricted and dynamic, which might alter the often-reported positive relationship between aboveground diversity and multifunctionality in natural environments[16,17]. Plant diversity might still play vital roles in directly benefiting soil biodiversity by forming symbiotic systems (e.g., mycorrhizal plant–arbuscular mycorrhizal fungi, mycorrhizal plant–ectomycorrhizal fungi[18], plant–diazotrophs) or indirectly influencing soil biodiversity by altering soil conditions.

Results

Soil biodiversity drive urban greenspace ecosystem functions

In soil samples from urban greenspaces across broad climatic gradients, we found that soil multidiversity (standardized average of the diversity of soil bacteria, fungi, protists, and invertebrates) and the diversity of individual soil organisms were positively and significantly correlated with multiple and individual ecosystem functions (Fig. 2). Soil multidiversity was particularly correlated with key services such as microbial-driven C pools (i.e., mineral-associated carbon and labile carbon content), OM decomposition, plant–soil mutualism, and plant productivity. Moreover, the diversity of key individual soil organisms supported different aspects of ecosystem functions and services (Fig. 2C). For example, the biodiversity (richness; the number of phylotypes) of microfauna (Arachnida, Collembola, and Nematodes) was significantly and positively correlated with microbial-driven C pools, OM decomposition, and nutrient cycling; and the biodiversity of nematodes and tardigrades was significantly associated with plant–soil mutualism and plant productivity. On the other hand, the biodiversity of bacteria and some protists (e.g., Oomycota, Ciliophora, and Dinoflagellata) was significantly correlated with plant–soil mutualism and nutrient cycling, while the biodiversity of other protists (e.g., Cercozoa, Chlorophyta, Ciliophora, Ochrophyta, and Rhodophyta) were specifically associated with pathogen control. The biodiversity of fungal groups such as fungal decomposers and root endophytes was significantly correlated with microbial-driven C pools, water regulation, OM decomposition, nutrient cycling, and plant productivity (Fig. 2C). We further found that the plant diversity showed no correlations with multifunctionality (Supplementary Fig. 2) or individual ecosystem functions (Fig. 2C). These results, and those of soil biodiversity, were maintained when the analyses were repeated within urban forests and non-forest (i.e., lawns and gardens) greenspaces (Supplementary Fig. 3).

The biodiversity of soil common taxa (i.e., the top 10% of soil taxa in terms of relative abundance and co-occurring in > 25% locations) of bacteria, fungi, protists, and invertebrates were more consistently associated with the delivery of multiple ecosystem functions in urban greenspaces compared with that of rare taxa (i.e., the bottom 90% of soil taxa in terms of relative abundance and co-occurring in < 25% locations) (Fig. 2C; Supplementary Fig. 4 and 5; Supplementary Table 3 and 4). Even so, the biodiversity of soil rare invertebrates and rare fungi with relatively larger body size tended to contribute to more specific functions when compared with the diversity of rare bacteria and rare protists (Fig. 2C; Supplementary Fig. 4 and 5; Supplementary Table 3 and 4).

Soil biodiversity and multi-threshold ecosystem functioning

We then investigated the relationships of soil biodiversity and plant diversity with the number of functions being delivered over an ecosystem functional threshold. Such knowledge is critical to better understanding whether soil biodiversity is important for (a) supporting a high number of functions working at high levels of functioning, (b) maintaining basal levels of functioning (i.e., high number of functions working at low level of functioning), or (c) both. Soil multidiversity was positively associated with the number of functions above multiple thresholds, whereas plant diversity showed no significant correlations with ecosystem function (Fig. 3A). The diversity of bacteria, fungi, and protists supported a high number of functions working over a low/medium threshold (< 50% of their maximum rates/availabilities), yet the diversity of large soil invertebrates was important for supporting a high number of functions at high thresholds (> 75% of their maximum rates/availabilities) (Fig. 3B).

Soil biodiversity and multi-dimension of ecosystem functions

To better understand the relationship between biodiversity and multiple dimensions of ecosystem functions, we used a approach, based on ecological network theory, to identify clusters of ecosystem functions that highly correlated with each other within a network of ecosystem functions. Three independent dimensions of ecosystem functions were identified. Dimension #1 included net plant productivity, phosphorus mineralization, available nitrate, starch degradation, chitin degradation, hemicellulose degradation, infiltration potential, and ARG control. Dimension #2 comprised available phosphorus, soil respiration, plant–soil mutualism, lignin-induced respiration, and glucose-induced respiration. Dimension #3 consisted of mineral-associated carbon, pathogen control, labile carbon content, and available ammonium (Fig. 4A). Our findings indicated that soil biodiversity was highly positively and significantly correlated with multiple dimensions of ecosystem functioning (Fig. 4B), though no significant correlation was found between plant diversity and multiple dimensions of ecosystem functions (Extended Data Fig. 2). Soil multidiversity and the biodiversity of soil invertebrates were particularly important and showed significantly positive correlations with all the dimensions of ecosystem functioning. The biodiversity of soil fungi was significantly correlated with Dimensions #1 and #3 of ecological functions, while the biodiversity of bacteria and protists had significant correlations with Dimension #2 of ecological functions (Fig. 4B).

Soil biodiversity, plant diversity, and ecosystem functions

Given that data were collected across a broad environmental gradient, the results were further investigated after accounting for multiple fundamental environmental factors such as climate, soil properties, management practices, and vegetation. Variation partitioning analysis (VPA) was first performed to quantify the unique variation of ecosystem function explained by soil and plant biodiversity. Soil biodiversity was a significant ecological predictor explaining a unique portion of variation (i.e., not accounted by other factors) in ecosystem multifunctionality, the multiple dimensions of ecosystems functioning, and multiple individual ecosystem functions, not accounted by climate, vegetation, and soil properties (Fig. 5A). In agreement with the above-explained results, plant diversity had a limited capacity to explain multifunctionality in urban greenspaces (Fig. 5A). We also found that, as expected, abiotic properties (e.g., soil variables, climate, and space) together played a predominant role in explaining multiple ecosystem functions (Fig. 5A; Supplementary Table 5).

Structural equation modeling (SEM) was then used to further investigate the direct and indirect relationships of environment and soil biodiversity in explaining ecosystem multifunctionality. A direct association between soil multidiversity and multifunctionality was detected even after accounting for the effects of all other environmental factors simultaneously. Again, plant diversity had a limited contribution to supporting ecosystem functions in urban greenspaces, and those contributions associated with multifunctionality were likely to be indirectly driven by changes in soil conditions (e.g., concentrations of total nitrogen) that influence soil biodiversity (Fig. 5B). Furtherly, we identified an important role of spatial location, soil properties, and management practices in explaining soil biodiversity and multifunctionality (Fig. 5B; Supplementary Table 6 and 7). For example, management practices in urban greenspaces including mowing were positively associated with multifunctionality, while fertilization and irrigation managements were indirectly associated with multifunctionality by suppressing soil biodiversity (Fig. 5B; Extended Data Fig. 3).

Diversity of soil microbial traits and ecosystem functioning

To further understand the importance of soil functional biodiversity in supporting ecosystem services in urban greenspaces, we investigated the contribution of the diversity of soil microbial traits in explaining ecosystem multifunctionality. Shotgun metagenomic sequencing was performed on a subset of 27 sites representing the entire gradient of climatic and vegetation conditions in this survey (Extended Data Fig. 1). The analyses revealed a significant correlation between the diversity of soil microbial traits and ecosystem multifunctionality (Fig. 6A and 6B), and these correlations remained significant alongside the increasing gene coverage (Fig. 6A). We then focused on the diversity of specific functional gene categories known to be associated with soil biodiversity, and potentially important for supporting ecosystem function, although gene–function evidence is still lacking. The diversity of soil microbial traits related to methane, nitrogen, phosphate, and sulfur metabolism was positively correlated with multifunctionality (Fig. 6C; Supplementary Table 8), whereas those related to infectious diseases, biosynthesis of vancomycin group antibiotics, drug resistance, and antimicrobial resistance were negatively correlated with the multifunctionality and several individual ecosystem functions (Fig. 6D; Supplementary Table 9). Specifically, the diversity of soil microbial traits related to methane metabolism was negatively correlated with microbial-driven C pools, whereas the diversity of genes closely associated with nitrogen, phosphorus, and sulfur metabolism was positively correlated with ecosystem services of nutrient cycling (Fig. 6E). No correlation was found between environmental variables and the diversity of gene groups related to human diseases and antibiotic resistance (Fig. 6E).

Discussion

The importance of soil biodiversity for supporting ecosystem multifunctionality has been previously reported in natural ecosystems[13,17]; however, such relationships were largely undescribed in urban greenspaces. Our findings provide insights into the fundamental importance of soil biodiversity in sustaining ecosystem multifunctionality of urban greenspaces across contrasting climates and vegetation types, with implications for the management of city parks and grass lawns. This study suggests that soil biodiversity across biomes (diversity of bacteria, fungi, protists, and invertebrates, and that of functional traits based on metagenomics) is positively correlated with multiple dimensions of ecosystem functions in urban greenspaces, urban greenspaces with greater soil biodiversity support higher levels of key groups of functions such as microbial-driven C pools, OM decomposition, plant productivity, nutrient cycling, water regulation, plant–soil mutualism, plant pathogen control, and antibiotic resistance regulation. Specifically, the biodiversity of soil common taxa can be particularly important for ecosystem multifunctionality in urban greenspaces compared with that of rare taxa. The biodiversity of soil invertebrates was especially vital for supporting a high number of functions working at high levels of functioning in urban greenspaces. Importantly, the results were consistent even after accounting for multiple environmental factors such as climate, vegetation, soil properties, and management practices. Thus, conserving soil biodiversity is key to sustaining the multiple ecosystem functions provided by urban greenspaces.

Consistent with the first hypothesis, soil biodiversity across biomes and functional traits were positively and significantly correlated with multiple and individual ecosystem functions in urban greenspaces across broad climatic gradients. The diversity of key individual soil organisms (the diversity of soil bacteria, fungi, protists, and invertebrates) and functional genes supported different aspects of ecosystem functions and services. Consequently, the biodiversity of soil organisms at multiple trophic levels[19] and high functional gene coverage might be needed to explain multiple aspects of ecosystem functions and services supported by urban greenspaces. Specifically, using the machine learning-based random forest model, we further detected 77 key-stone soil taxa and 159 microbial traits that were accurately predictive of multifunctionality in urban greenspaces. The combination of 77 key-stone soil biota (Supplementary Table 10; Extended Data Fig. 4) belonging to Nematode, Cercozoa, Amoebozoa, Ochrophyta, Ciliophora, Mortierellomycetes, Sordariomycetes, and rapidly growing Proteobacteria[20-22] are crucial bioindicators of soil processes, and co-occurring in soil multitrophic food-webs[23-26]. The relative abundances of key functional genes, such as methane monooxygenase subunit A encoding (*pmoA*) gene of greenhouse methane gas emissions[27], ferredoxin-nitrate reductase encoding (*narB*) gene of nitrogen cycling[28], alkaline phosphatase D encoding (*phoD*) gene of phosphorus mineralization[29], and sulfate adenylyltransferase subunit 2 encoding (*cysD*) gene of sulfur cycling[25], were positively associated with multifunctionality in urban greenspaces (Supplementary Table 11; Extended Data Fig. 5).

The biodiversity of soil invertebrates (both common and rare invertebrates) was particularly important for ecosystem multifunctionality, and showed significantly positive correlations with more specific functions, such as microbial-driven C pools, OM decomposition, nutrient cycling, plant–soil mutualism and plant productivity, and especially vital for supporting a high number of functions working at high levels of functioning. These results are in agreement with those found previously in natural ecosystems worldwide[13], and suggest that the influence of larger soil invertebrates (Arachnida, Collembola, Nematodes, and Tardigrades) at high trophic levels [e.g., degrading large amounts of animal and plant litter[30], and further controlling the inputs of resources to the system] is essential for maintaining high levels of functioning in city parks and gardens across contrasting environmental conditions. Invertebrates are also known to play prominent engineering roles in terrestrial ecosystems, and their relatively larger body size, compared with microbes, and their relative mobility make them critical engineers in urban soils[31]. Further, the biodiversity of soil common taxa was shown to be particularly important for ecosystem multifunctionality, a result which is commonly found when ecosystem functioning is determined by plant communities (e.g., Grime's mass-ratio hypothesis)[32]. Soil common taxa that account for most biomass with high frequency of occurrence in urban greenspaces could competitively and efficiently utilize an array of resources, and occupy the highly dynamic and diverse environment[33] in urban greenspaces. However, several studies also showed that soil rare microbial taxa are the major drivers of ecosystem multifunctionality in highly managed agricultural ecosystems[34,35].

We further identified three independent dimensions of ecosystem functions highly correlated with each other based on ecological network theory. A similar approach has been used in the past to determine the dimensions of ecosystem stability[36]. Each cluster within the network of ecosystem function (dimension of functions) represents a group of independent functions within the same functional dimension. Thus, unlike for principal components analyses (PCA), this network approach can summarize the entire variation of ecosystem function. The biodiversity of soil invertebrates played important roles in supporting all the dimensions of ecosystem functioning. The biodiversity of soil fungi was particularly correlated with Dimensions #1 and #3 of ecological functions covering microbial-driven C pools, nutrient cycling[37], refractory organic carbon (chitin and hemicellulose) decomposition[38], water regulation, plant productivity, pathogen control, and ARG control, which are closely associated with environmental risks, and plant and human health[39]. The biodiversity of bacteria and protists were significantly correlated with Dimension #2 of ecological functions including labile carbon (glucose and lignin) decomposition[40] and plant–soil mutualism. Our work further highlighted the importance of a diverse soil biota in supporting distinct dimensions of ecosystem functioning in urban greenspaces.

Both variation partitioning modeling and structural equation modeling further showed that the direct associations between soil biodiversity and ecosystem functions in urban greenspaces were robust after accounting for multiple fundamental environmental factors such as climate, soil properties, management practices, and vegetation. This provides strong support for the existence of a genuine relationship between soil biodiversity and ecosystem functions in urban greenspaces. Management measures in urban greenspaces play key roles in affecting ecosystem multifunctionality, e.g., mowing was directly positively associated with multifunctionality, fertilization and irrigation managements were indirectly associated with multifunctionality by suppressing soil biodiversity. Regular mowing was not only predominantly for aesthetic reasons but also for horticultural complexity, litter dynamics, soil organic carbon enrichment, and soil biodiversity[41]. Therefore, the management (e.g., precision fertilization, timely irrigation, and regular mowing) of urban greenspaces is an important regulator of soil biodiversity-multifunctionality relationships, providing a forward guidance for urban greenspace intervention mode. Taken together, our analyses provide further support for the linkage between soil biodiversity and functions in urban greenspaces.

Plant diversity had a limited capacity to influence ecosystem functions in urban greenspaces. One likely explanation is that the impact of direct management practices on plant communities in urban greenspaces limits the positive influence of plant diversity on ecosystem functioning often reported in natural ecosystems. This is in agreement with a local-scale study suggesting that plant diversity indirectly influenced multifunctionality by changes in soil fauna associated with plant cover–diversity positive feedback[15]. Plants in urban greenspaces are also often non-indigenous species, have come from elsewhere, often a different continent, and have been selected for their horticultural value rather than their capacity to improve surface soils. These specials will be unlikely to have co-evolved with the soils and their microbial communities, or the climatic and environmental conditions (e.g., pollution, salinity, soil texture, water deficit) at a site[42], reducing their positive influence on ecosystem functions[15]. However, we would like to highlight that plant diversity is likely to be indispensable for other non-measured ecosystem services such as air purification, cooling, relaxation, and beautification, other than the basic ecosystem functions in natural ecosystems, and therefore, a fundamental component of urban greenspaces.

In summary, the results provide solid evidence that taxonomic and functional soil biodiversity is tied to the delivery of multiple ecosystem functions in urban greenspaces. This includes multifunctionality, multiple individual functions, number of functions working over multiple thresholds, multiple dimensions of ecosystem functions, and key ecosystem services such as microbial-driven C pools, organic matter decomposition, plant productivity, nutrient cycling, water regulation, plant–soil mutualism, plant pathogen control, and antibiotic resistance regulation. Our results were consistent after accounting for climate, soil properties, vegetation, and management practices. Unexpectedly, plant diversity had a limited role in explaining the ecosystem functioning of city parks. Importantly, this study provides insights into the importance of conserving soil biodiversity for supporting the functioning of urban greenspaces, with implications for the sustainability of city parks and gardens under the ongoing urbanization and global change processes, and for the well-being of the many billions of citizens depending on these ecosystems.

Methods

Study sites

A standardized field survey was conducted in urban greenspaces (urban parks and large residential gardens) of 56 municipalities in 17 countries across six continents between 2017 and 2019 (Fig. 1; Supplementary Table 1). These municipalities were selected to cover a wide range of environmental conditions (mean annual temperature and precipitation ranged from 3.1°C to 26.4°C and 210 to 1577 mm, respectively). At each location, we surveyed a characteristic 30 m by 30 m plot (in a city park or shared garden; Supplementary Table 1) using three parallel transects of equal length, spaced 15 m down the part[2], and collected information on the plant richness (number of perennial plant species) and plant cover of each location based on these three 30-m transects[2], and further annotated management information including irrigation, fertilization, and mowing. Other information collected *in situ* included locations (e.g., distance from the equator) (Supplementary Table 1).

Soil sampling

To account for spatial heterogeneity in the plots, we collected three composite soil samples (from five soil cores, top 5-cm depth) under the most common environments (vascular plants and open areas between plant canopies covered by bare soils and non-vascular plants)[2] found at each plot (Supplementary Fig. 1). We focused on surface soils because (1) city parks and gardens can have shallow soils due to extensive surface preparation and disturbance, and (2) the uppermost layer is typically the most biologically active in terms of soil biodiversity, carbon storage, nutrient cycling, plant activity, microbial biomass, and atmospheric carbon exchange. A total of 168 composite soil samples (three composite samples per plot) from 56 urban greenspaces were analyzed in this study. The climatic variable aridity index was extracted from the Global Aridity Index (Global-Aridity_ET0) datasets[43].

Soil physicochemical analyses

We measured soil pH, total nitrogen (N), soil organic carbon (SOC), total phosphorus (P), and soil texture (percentage of sand) for all samples. Soil pH was determined as described previously[13], with a pH meter, in a 1:2.5 mass: volume soil and water suspension. Total soil nitrogen was analyzed in 168 composite samples (three composite soil samples per plot) using an Elemental Analyser (C/N Flash EA 112 Series-Leco TruSpec). Soil organic carbon was determined in all composite samples by colorimetry after oxidation with a mixture of potassium dichromate and sulfuric acid. Total phosphorus was determined in all composite samples, after nitric-perchloric acid digestion, using an ICP-OES spectrometer (ICAP 6500 DUO; Thermo-Scientific, Waltham, MA, USA). Soil texture (percentage of sand) was determined in a composite soil sample per plot according to Kettler *et al*[44]. Total nitrogen and soil organic carbon were highly correlated (Spearman coefficient = 0.96; P < 0.001), suffering multicollinearity which was not good for multivariable analyses. Consequently, we only included soil pH, total nitrogen, C: N, total phosphorus, and sand content in the statistical models.

Amplicon sequencing and soil biodiversity

Soil DNA was extracted from each of the 168 composite soil samples (three composite soil samples per plot) using the DNeasy PowerSoil Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The diversity of soil bacteria, fungi, protists, and invertebrates was measured via amplicon sequencing using the Illumina MiSeq platform (Illumina Inc., CA, USA) in the University of Colorado Boulder[2]. To characterize the richness (number of phylotypes) of bacteria, protists, and invertebrates, a portion of the prokaryotic 16S and eukaryotic 18S rRNA genes were sequenced using primer pairs of 515F (5'- GTGCCAGCMGCCGCGGTAA-3') /806R (5'-GGACTACHVGGGTWTCTAAT-3')[45] and Euk1391f (5'-GTACACCGCCCGTC-3') /EukBr (5'-TGATCCTTCTGCAGGTTCACCTAC-3')[46], respectively. Bioinformatic processing was performed using DADA2[47]. Phylotypes [i.e., amplicon sequence variants (ASVs)] were identified at the 100% identity level, and rarefied at 5000, 1000, 250 sequences per sample, for bacteria, protists, and invertebrates, respectively[2]. Fungal richness was determined via full-length internal transcribed spacer (ITS) amplicon sequencing using the primers ITS9mun (5'- TGTACACACCGCCCGTCG-3') /ITS4ngsUni (5'-CCTSCSCTTANTDATATGC-3') and the PacBio Sequel II platform in the University of Tartu[48]. Bioinformatic processing was performed as explained above (ASVs at 100% similarity). The fungal ASV abundance table was rarefied at 1000 sequences per sample, and fungal diversity unit was the number of phylotypes (ASVs) (based on plot-level ASV tables, see below), the proportion of taxa unit was percentage.

Assessing ecosystem functions and services

The selection of functions is based on their theoretical link with soil biodiversity (e.g., nutrient cycling, organic matter decomposition etc). A total of 18 surrogates of ecosystem functions associated with nine ecosystem services were measured:

microbial-driven C pools (labile carbon content and mineral-associated carbon), water regulation (infiltration potential and water holding capacity), nutrient cycling (available phosphorus, nitrate, and ammonium), plant–soil mutualism (Arbuscular mycorrhizal fungi [AMF] biomass), organic matter (OM) decomposition (soil respiration, glucose-induced respiration, lignin-induced respiration, and four enzyme activities associated with starch chitin degradation and hemicellulose degradation and P mineralization), plant productivity (net plant productivity; NDVI), pathogen control (inverted proportion of potential fungal plant pathogens), and antibiotic resistance gene (ARG) control (inverted proportion of ARG abundance), and multifunctionality, as described in Delgado-Baquerizo *et al*[13]. Overall, these variables constitute good proxies of ecosystem functions and processes associated with soil biodiversity and the build-up of nutrient pools (carbon, nitrogen, phosphorus), biological productivity, plant health, and environmental security.

Labile carbon content (water extractable carbon) was measured in 168 composite soil samples (three composite soil samples per plot) as described in Bastida *et al*[49]. The content of mineral-associated carbon was determined as described in Lugato *et al*[50] in a composite soil sample per plot. Unlike other C fractions, mineral-associated carbon is more likely to be related to microbial-driven carbon processing in soil, and therefore more probable to represent a surrogate of microbial function. Particulate carbon was excluded from the analyses because this fraction is more likely to represent less processed carbon associated with plant litter entrance. Mineral-associated carbon was determined in a composite soil sample per plot. In this dataset, labile carbon and mineral-associated carbon were significantly correlated with each other (Person coefficient = 0.437, P < 0.001), but did not suffer multicollinearity. Water holding capacity and potential infiltration were measured in a composite soil sample per plot as explained in Delgado-Baquerizo *et al*[51]. Available phosphorus, nitrate, and ammonium were extracted from all composite soils with ion exchange membranes (IEMs) in a mix of 1:15 of soil: distilled water during 24 h, then the content of N and P from these resins was extracted with NaCl 0.7 M for 1 h and determined using the colorimetric methods described in Delgado-Baquerizo *et al*[51]. The biomass of arbuscular mycorrhizal fungi (16:1w5c) was measured using microbial phospholipid fatty acids (PLFAs)[52,53]. Soil (basal) respiration, glucose respiration, and lignin-induced respiration were determined using the MicroResp® technique in a composite soil per plot. Absorbance was measured at 570 nm after the 5 h incubation period (25ºC and 60% water holding capacity)[54]. The activities of β-glucosidase (BG−starch degradation), N-acetylglucosaminidase (NAG−chitin degradation), β-xylosidase (XYL−hemicellulose degradation), and phosphatase (PHOS−P mineralization) were measured in all composite soil samples from 1 g of soil by fluorometry as described in Bell *et al*[55]. Plant productivity was determined using normalized difference vegetation index (NDVI), from Landsat satellite imagery (Landsat 8; mean annual values from 2013–2020; 30 m resolution, same resolution as plots) (https://landsat.gsfc.nasa.gov). The proportion of soil-borne potential fungal plant pathogens was determined in all composite soil samples from the PacBio ITS data (see above) using the FUNGuild database[56]. Pathogen control was determined as −1 x the proposition of soil-borne potential fungal plant pathogens according to Delgado-Baquerizo *et al*[13]. The abundance of ARGs was determined in all composite soil samples based on 285 ARGs as done in Delgado-Baquerizo *et al*[13]. Antibiotic resistance control was determined as −1 x the abundance of total ARGs as described in Delgado-Baquerizo *et al*[13].

Metagenomic sequencing and the diversity of microbial traits

A total of 27 composite soil samples (one composite soil sample per plot) were sequenced using metagenomics. The selection of samples covered a wide range of cities from contrasting climates and populations, and 17 countries from both hemispheres (Extended Data Fig. 1). These composite samples correspond with those collected in open spaces between plant canopies. DNeasy PowerSoil Kit (Qiagen Inc., USA) was used for DNA extraction according to the manufacturer's protocol, and approximately 500 ng of DNA per soil sample was isolated for shotgun metagenomic sequencing[57,58]. Sequencing was performed on an Illumina HiSeq platform (Illumina Inc.) at Majorbio in Shanghai, China. Raw reads PE150 [150–base pair (bp) paired-end reads] were trimmed to remove low-quality reads as follows. First, using SeqPrep software (https://github.com/jstjohn/SeqPrep) to remove the adapter sequences. Second, using the library sickle (https://github.com/najoshi/sickle) to trim reads end. Short reads (< 50 bp) or reads containing N (ambiguous bases) were discarded. The filtered high-quality sequences of the 27 samples were translated to protein sequences using DIAMOND[59], and blast with KEGG Orthology (KO) database (e-value < $1e^{-5}$) using KOBAS 3.0. Then, we further corrected the gene annotation according to GenBank (https://www.ncbi.nlm.nih.gov/genbank/) and RefSeq Database (https://www.ncbi.nlm.nih.gov/refseq/about/prokaryotes/). The proportions of 7031 functional genes were determined from these analyses, and this information was used to analyze patterns in the community composition and diversity of microbial traits. Amplicon and metagenomic sequence data have been previously used to characterize the microbiome of urban greenspaces[3]. Here, this data was used to investigate the relationship between the diversity of soil microbial traits and multifunctionality. We would like to highlight that gene annotations are approximate, and therefore, that extrapolating and linking the diversity soil microbial genes to functions needs to be taken with care, and further investigated in the future to establish more direct linkages.

Statistical analysis

Plot-level estimations of soil biodiversity and properties

Prior to statistical analyses, within-plot information on all soil properties (e.g., soil pH, total nitrogen, total phosphorus, soil organic carbon, C: N, and soil texture), functions, and soil biodiversity metrics, derived from three composite soil samples per plot with five soil cores each, were averaged to obtain plot-level estimates. By using this approach, plot-level estimates of the proportion and number of phylotypes were obtained for bacteria, fungi, protists, and invertebrates[2] based on 168 composite soil samples at the 56 studied urban sites. This was not needed for those analyses including a single composite soil sample per plot. Analyses on a single composite soil sample per plot are performed like this for logistic reasons (e.g., soil sample availability).

Assessing ecosystem multifunctionality

Ecosystem multifunctionality measures potentially summarize the ability of an ecosystem to deliver multiple functions or services simultaneously, and aim to understand the multidimensional patterns of ecosystem functioning[60]. To obtain weighted ecosystem multifunctionality for each site, we first normalized (log-transform when needed) and standardized each of the 18 functions measured using the 0-1 transformation. These standardized ecosystem functions were then averaged to obtain an ecosystem multifunctionality index. This method is widely used in the ecosystem multifunctionality literature[8,11,16].

Assessing the functions working over multiple thresholds

The multiple threshold approach provides nuanced views of ecosystem multifunctionality that allows for direct comparison between samples and various thresholds. Here, the number of functions beyond a given threshold (25%, 50%, 75%, and 90%) was calculated as described by Byrnes *et al*[6], and as explained by Delgado-Baquerizo *et al*[10], and each threshold represents a functional performance level. Two ecosystems might support the same number of "functions", e.g., nutrient availability, plant productivity, and pathogen control. However, these functions might be expressed at high or low levels of functioning. The thresholds enabled assessing how many functions are actually performing at different levels of functioning. For example, while a high number of functions > 75% threshold indicate that multiple functions of the ecosystem are supporting high levels of functioning (related to the maximum level of function of each measured variable). The relationship between biodiversity and the number of functions over multiple thresholds indicates the capacity of biodiversity to explain a number of functions being delivered over a low (< 25% functional threshold), medium (50% functional threshold), and high (> 75% functional threshold) level of functioning, which provides fingerprints of the influence of biodiversity on multifunctionality[6].

Assessing multiple dimensions of ecosystem functioning

The network of ecological functions is a good representation of the entire variation in the ecosystem functioning, and determines the existence of functional dimensions, with each dimension containing ecosystem functions highly correlated with each other. A co-associated ecological functioning network was constructed to identify the multiple dimensions of ecological functioning. All pairwise Spearman correlations between each function were calculated, and the correlations with negative Spearman coefficient and P-values > 0.01, were removed to focus on the ecological functions that strongly co-occurred and were more likely to influence each other. The main ecological clusters in the network were visualized using Gephi (https://gephi.org/). Multifunctionality of each ecological cluster was calculated by averaging the standardized ecosystem functions that belonged to it.

Relationships between biodiversity and ecosystem functioning

The most dominant phylotypes, those that were both abundant (top 10% of all taxa in terms of relative abundance) and ubiquitous (> 25% locations) across all distributed soils, were defined as soil common taxa. Then, the remaining were considered rare taxa. The Spearman correlations between multiple ecosystem functioning, environmental variables, and the plant richness, soil biodiversity (bacteria, fungi, protist, invertebrate, and microbial traits), common taxa, and rare taxa were calculated by using IBM SPSS

21. Benjamini Hochberg false discovery correction was used to correct false positives in the multiple testing. These Spearman correlations were presented using heatmaps ("pheatmap" R package). Relationships between ecosystem functioning and plant diversity, soil biodiversity were assessed by linear regressions using IBM SPSS 21.

Variation partitioning modeling

VPA was used to quantify the contributions of plant richness, soil biodiversity of the selected groups (bacteria, fungi, protists, and invertebrates), environment [soil properties (soil pH, total nitrogen, C: N, total phosphorus, and sand content) and climate legacies], and space to regulating multifunctionality, multiple dimensions of ecosystem functioning, and individual ecological functions. Specifically, this analysis allowed us to identify the unique and shared portion of the variation in explaining different kinds of ecosystem functioning. The adjusted coefficients of determination in multiple regression/canonical analysis could occasionally take negative values which usually are interpreted as zeros[61]. The "vegan" R package was used to run VPA and calculate *P*-values associated with the unique portions explained by different groups of predictors.

Structural equation modeling

SEM[62] was used to evaluate the direct and indirect effects of soil biodiversity (bacteria, fungi, protists, and invertebrates), vegetations (plant richness and plant cover), space (distance from the equator), climate legacies (aridity), soil properties (soil pH, total nitrogen, C: N, total phosphorus, and sand content), and management practices (irrigation, fertilization, and mowing) on the multifunctionality. Before SEM analyses, we established a priori SEM model, hence its results are not biased by our previous knowledge. In this model, the management practices (irrigation, fertilization, and mowing) were categorical variables with two levels: 1 (a particular management) and 0 (remaining considered management), bootstrapping was used to test the probability that path coefficients differed from zero. Standardized total effects (STEs) of each variable on multifunctionality were calculated to aid interpretation of the SEM. All analyses were performed using IBM SPSS Amos 21 (Chicago, IL: Amos Development Corporation).

Random forest modeling analysis

Random forest modeling was used to regress the normalized ASVs (bacteria, fungi, protists, and invertebrates), functional genes, and ecosystem multifunctionality across the urban greenspaces, and determine the optimal set of ASVs and functional genes related to ecosystem multifunctionality[63]. Ranked lists of ASVs and functional genes in order of random forests reported feature importance scores, were achieved based on the increase in mean-square error of multifunctionality predicted over 999 iterations of the algorithm. Marker ASVs and functional genes were chosen based on the minimum average cross-validation mean-squared errors, which were obtained from five trials of the 10-fold cross-validation based on 1000 decision trees. Random forest regression analyses were performed using the "randomForest" R package.

Reporting Summary

Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

Soil biodiversity, plant diversity, and ecosystem functional data from urban greenspaces are publicly available in Figshare (https://figshare.com/articles/dataset/URBAN_BEF_dataset_vf_original_xlsx/21162493).

Code availability

Code for statistical analyses is available at https://github.com/huahuafan/Global-urban-greenspaces.

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Author Contributions

M.D.B. developed the original idea and designed the research with discussion with H.C. and K.F. M.D.B. coordinated all field and laboratory operations. K.F., M.D.B., and H.C. analyzed data. Field data were collected by M.D.B., D.J.E., Y.R.L., B.S., A.R.B., J.L.B.P., J.G.I., T.P.M., C.S., P.T., E.Z., J.P.V., L.W., J.W., T.G., M.B., G.F.P.B., T.U.N., A.L.T., X.Q.Z., J.D., A. Rodriguez., X.Z., F.A., S.A., C.P., and A. Rey. The manuscript was written by K.F., M.D.B., and H.C., with contributions from all coauthors.

Competing interests

The authors of this manuscript have no conflicts of interest.

Figure Legends

Fig. 1. The locations of the 56 surveyed municipalities in 17 countries across six continents included in this study along with landscapes of their urban greenspaces.

Fig. 2. Soil biodiversity drives multiple ecosystem functions in urban greenspaces. (A) Ordinary least squares linear regression between multidiversity (standardized between 0 and 1) of soil organisms and multifunctionality, n = 56 study sites. (B)

Ordinary least squares linear regression between the diversity of soil bacteria, fungi, protists, and invertebrates and multifunctionality, n = 56 study sites. (C) Heatmap showing the two-sided spearman correlations between soil biodiversity (multidiversity; bacteria, fungi, protists, and invertebrates; rare taxa; common taxa) and ecosystem functions within multiple ecosystem services (microbial-driven C pools, water regulation, nutrient cycling, plant–soil mutualism, OM decomposition, plant productivity, pathogen control, ARG control, multifunctionality). *P* values were adjusted by Benjamini Hochberg false discovery correction, and indicated by asterisks, "*" represents Benjamini Hochberg-adjusted $0.01 < P \leqslant 0.05$; "**" represents Benjamini Hochberg-adjusted $P \leqslant 0.01$; n = 56 study sites.

Fig. 3. Relationships between soil biodiversity and multi-threshold ecosystem functioning. (A) Ordinary least squares linear regressions between soil multidiversity (standardized between 0 and 1), plant diversity, and multi-threshold ecosystem functioning, n = 56 study sites. (B) Ordinary least squares linear regressions between diversity of individual groups of taxa (bacteria, fungi, invertebrates, and protists) and multi-threshold ecosystem functioning. *P* values were indicated by asterisks, "*" represents $0.01 < P \leqslant 0.05$; "**" represents $P \leqslant 0.01$; n = 56 study sites.

Fig. 4. Relationships between soil biodiversity and independent dimensions of ecosystem functioning. (A) Ecological network approach aiming to identify the dimensions of ecosystem function. Each dimension includes functions which are highly correlated with each other. (B) Ordinary least squares linear regressions of soil multidiversity and diversity of bacteria, fungi, protists, and invertebrates (standardized between 0 and 1) with multiple dimensions of ecosystem functioning.

Fig. 5. Contribution of soil biodiversity to ecosystem functions in urban greenspaces. (A) Variation partitioning modeling was used to evaluate the unique and shared portions of variation in ecosystem properties explained by soil biodiversity of the selected groups (bacteria, fungi, protists, and invertebrates), plant richness, environment (soil variables and climate), and space. Biotic factors | shared refers to the percent of shared variation in ecosystem properties explained by soil biodiversity and plant diversity. Abiotic factors | shared refers to the percent of the shared variation in ecosystem properties explained by environment and space. *P*-values associated with the unique portions explained by different groups of predictors are available in Supplementary Table 5 (n = 56 study sites). (B) Using a fitted SEM, we aimed to identify the direct relationship between the multidiversity (combined biodiversity of 4 groups of soil organisms: bacteria, fungi, protists, and invertebrates) and averaging ecosystem multifunctionality. We grouped the different categories of predictors (climate, soil properties, plants, and spatial influence) into the same box in the model for graphical simplicity; however, these boxes do not represent latent variables. Numbers labeling the arrow lines are indicative of the correlations. R^2 denotes the proportion of variance explained. *P* values were indicated by asterisks, "*" represents $0.01 < P \leq 0.05$; "**" represents $P \leq 0.01$. Standardized total effects (STEs) from the SEM, i.e., the sum of direct and indirect effects from each variable on multifunctionality (n = 56 study sites).

Fig. 6. Relationships between the diversity of soil microbial traits and ecosystem multifunctionality. (A) The regression R^2 between multifunctionality and the biodiversity of microbial traits under different gene coverage. (B) Ordinary least squares linear regressions between multifunctionality and all the microbial genes diversity. (C) Ordinary least squares linear regressions between multifunctionality and the biodiversity of genes associated with nutrient cycling (methane metabolism, nitrogen metabolism, phosphate metabolism, sulfur metabolism). (D) Ordinary least squares linear regressions between multifunctionality and the biodiversity of genes associated with human health (infectious diseases, biosynthesis of vancomycin group antibiotics, drug resistance, antimicrobial resistance), n = 27 study sites. (E) Heatmap showing the two-sided spearman correlation between soil functional biodiversity, multiple dimensions of ecological functioning (Dimension #1, Dimension #2, Dimension #3) and ecosystem services (microbial-driven C pools, water regulation, nutrient cycling, plant–soil mutualism, OM decomposition, plant productivity, pathogen control, ARG control, multifunctionality). *P* values were adjusted by Benjamini Hochberg false discovery correction, and indicated by asterisks, "*" represents Benjamini Hochberg-adjusted $0.01 < P \leqslant 0.05$; "**" represents Benjamini Hochberg-adjusted $P \leqslant 0.01$; n = 27 study sites.

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