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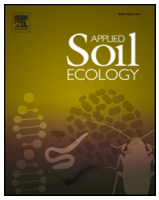

Influence of land use on the microbiological properties of urban soils

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ABSTRACT

Advancing towards sustainable cities needs a better understanding of all components of urban ecosystems, including soils, but the biological component of urban soils remains poorly studied compared to natural and agricultural soils. With the objective of advancing knowledge in this aspect, we studied the microbiological properties in 61 soils of the city of Santiago de Compostela (NW Spain), under several land uses (urban grassland, urban forest, urban garden, and periurban arable land). We determined thirteen extracellular enzymatic activities as a proxy of microbial community functioning, along with measures of microbial activity (basal respiration), microbial abundance (DNA extraction and real-time PCR), and the composition of microbial communities (Illumina MiSeq sequencing on 16S rRNA and ITS marker regions). Results showed a high variability in all of the microbiological variables among soils, reflecting the typical spatial heterogeneity of urban soils. Respiration and enzymatic activities were highly correlated to soil organic matter contents but not affected by land use within the city, with the exception of alkaline phosphomonoesterase activity, which was higher in urban garden soils and correlated positively to pH and availability of phosphorus and nitrogen. Unlike fungal abundance, we recorded a higher bacterial abundance in the urban grasslands than in the other land uses. While the composition of bacterial communities was structured in a more homogeneous, land use-dependent manner (33% variance, p_{Adonis} = 0.001), the respective fungal communities were more heterogeneous and less influenced by group-dependent characteristics (18%, p_{Adonis} = 0.001). Soil pH had a larger influence on the bacterial community composition (28% variance) compared to the fungal composition (8.5% variance). Overall, these findings provide evidence that the typically high soil heterogeneity of urban ecosystems is the main driving force for the urban soil microbiome, with soil organic matter determining largely microbial activity.

1. Introduction

The worldwide intensification of urbanization during the last decades represents a number of important environmental challenges to urban areas, including significant impacts on land and soils. Urban sprawl and infrastructures occupy and/or destroy fertile soils, in many cases sealing or compacting them, modifying them by excavation or mixture with other materials, or submitting them to diffuse and point pollution from industry, traffic, combustion or waste disposal (Bechet et al., 2019). As a consequence, soils in cities present very specific characteristics that make them different from soils in non-urban areas and require specific approaches to their study. Urban soils can comprise of highly disturbed and manipulated materials altered through mixing, filling, transportation, and other perturbations caused by construction-related activities (Levin et al., 2017). Besides, a wide variety of contaminants can accumulate in this type of soils due to the presence of several sources of pollution near or within the cities (Li et al., 2018). Altogether this often results in soils with high amounts of artefacts and coarse fractions, high levels of compaction and/or sealing, poor physical conditions, low contents in organic matter, and low fertility (Morel et al., 2015; Levin et al., 2017; Prokof'eva et al., 2020).

In any case, generalizations cannot be confidently made in this regard, since a very important general feature of urban soils is their high heterogeneity and lack of spatial logic compared to natural environments, as well as a wide range of activities over time and a frequent and often arbitrary change of use (Norra and Stuben, 2003; Burghardt et al.,

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2015; Ajmone-Marsan et al., 2016; Levin et al., 2017). In this sense, soils remain as the least known element of urban ecosystems, still considered as a secondary compartment beyond vegetation with regard to their functions, and they are rarely considered as an essential resource in this transition to sustainable cities (Blanchart et al., 2018; Lal et al., 2021). Although the study of urban soils has been increasing in the last decades, research is in general more focused on pollution than on any other aspect. One of the least known aspects of urban soils is their microbiological component, as the accumulation of knowledge about soil microbiology until now has been concentrated on natural or agricultural environments with few investigations carried out on urban soils (Bond-Lamberty and Thomson, 2010; Demina et al., 2018; Guilland et al., 2018). Taking into account properties of urban soils such as low fertility, poor physical condition and possible presence of high pollutant concentrations, low microbial activity and diversity could be expected in many cases (Schmidt et al., 2017). The inherent heterogeneity of urban ecosystems, the diverse impact of human activities and the lack of spatial logic of urban soils also suggest that those environments' microbiomes could differ from those in natural and agricultural soils. Up to now there are studies that reported differences in soil bacterial diversity between urban and non-urban ecosystems (Huot et al., 2017; Wang et al., 2017; Parajuli et al., 2018), while others did not (Reese et al., 2016). Despite these discrepancies in microbial diversity, these studies provide evidence of microbial community compositional shifts as a result of urbanization. In this regard, the existing studies show that the high heterogeneity of urban ecosystems and urban soils produces a high variability of microbial activity and abundance with respect to natural and agricultural soils (Ivashchenko et al., 2014; Igalavithana et al., 2017; Demina et al., 2018; Beroigui et al., 2020).

Consequently, knowledge on urban soil microbiology cannot be simply transposed and considerable efforts must be made to shed light onto this matter (Schwartz et al., 2015; Guilland et al., 2018; Delgado-Baquerizo et al., 2021). Studying soils in an urban-rural gradient is the approach most commonly employed in the literature to evaluate the effects of urbanization (Guilland et al., 2018). Other studies have tried to understand the heterogeneity of urban soil microbiological properties by studying the effect of factors such as proximity to industries and highways (Papa et al., 2010), settlement age (Eldridge et al., 2021) or functional zoning, including industrial, residential or public (Wang et al., 2011; Ivashchenko et al., 2014, 2019). In this sense, Guilland et al. (2018) pointed out the necessity of comparative studies between different land uses in an intra-urban context, such as grass-covered areas, street trees, urban wasteland, sports grounds, urban gardens, green roofs, among others. Here, we followed this rationale aiming to contribute knowledge of the microbiological component of urban soils and to understand how land use shaped them. For this, we selected different land-use categories (urban grasslands, urban forest areas, urban gardens, and periurban croplands) in the city of Santiago de Compostela (northwestern Spain), aiming to (i) characterize soil microbial abundances and microbial activity from urban greenspaces; (ii) determine the composition and diversity of bacterial and fungal communities in the urban soils; and (iii) identify the environmental factors influencing microbial activity and community composition in urban ecosystems.

The soils of the city of Santiago are currently the object of an exhaustive study that includes aspects such as morphology, classification, fertility, and pollution (Herbón et al., 2021; Paradelo et al., 2021, 2022), a comprehensive approach to the evaluation of urban soils that has not been undertaken to date in Spain. The study of the biological component of these soils allows to complement the existing knowledge of physical and chemical aspects. In this regard, we expect that: (i) the typical heterogeneity of urban ecosystems will be reflected in the microbial abundance, activity, and microbiome composition across the surveyed urban soils; (ii) microbial abundance and activity in the surveyed urban soils will not greatly differ from that in non-urban soils in line with their comparable high organic matter contents and low pollution status (Paradelo et al., 2021); (iii) those factors shaping the microbial community will differ between bacteria and fungi, since bacteria are known to respond more quickly to disturbances (Glassman et al., 2018) and to occupy smaller spatial scales than fungi (Reese et al., 2016).

2. Materials and methods

2.1. Study area

The municipality of Santiago de Compostela, located in the northwest of the Iberian Peninsula, has an area of 222 km^2 and counts 97,000 inhabitants. The climate is warm and wet and, according to the Köppen−Geiger Climate Classification, the city is located in the temperate oceanic climate (Cfb) zone (Kottek et al., 2006). The mean annual air temperature is 13.0 °C; August is the warmest month (mean air temperature 19 °C) and January is the coldest one (mean air temperature: 8 °C). The average annual precipitation is 1787 mm. The relatively low values for potential evapotranspiration (<300 mm in summer and 50–100 mm in winter) result in a positive water balance (600–800 mm) (Martínez Cortizas and Pérez Alberti, 1999).

In the center of Santiago de Compostela there are over 25 green areas, including grasslands, forest areas, and urban allotment gardens. These areas range from 1 to 40 ha and they are all located very closely, distributed within a small 6-km^{-2} area in the city center (Fig. 1), where no industries or highways exist nearby. Owing to the very short distances between them, these three different land uses coexist at some places. For the present study, a total of 61 urban soils were selected randomly (Fig. 1, Table S1). As grasslands are the most common surfaces in urban parks in Santiago, most soils ($n = 35$) correspond to this type of land use, followed by urban forest areas ($n = 14$) and urban and periurban agriculture soils ($n = 12$), including the city's eight urban allotment garden areas.

2.2. Soil sampling

Three sets of composite samples (0–10 cm), composed by mixing three subsamples, were taken with an Edelmann auger and sieved immediately in the field through a 5-mm mesh at each sampling point. The first set of samples was kept at 4 °C until analyses and used to determine basal respiration and enzymatic activities. The second set of samples were kept at −20 °C and used for DNA extraction, which was subjected to real-time PCR and Illumina Miseq sequencing analyses. The third set of samples was air dried in the laboratory, sieved by 2 mm and used to subsequently determine pH, texture, total C and N, and available N and P contents. Table S1 presents the main physicochemical properties of the soils in this study. Overall, the soils are acid (only one soil with pH over 7), rich in organic matter (total organic carbon contents ranging from 6 to 110 g kg^{-1} and total N ranging from 0.6 to 6.0 g kg^{-1}), and characterized by loam or sandy-loam textures. Additional information about the morphology, chemical composition, fertility and pollution of the soils of the city of Santiago de Compostela can be found in previous works (Herbón et al., 2021; Paradelo et al., 2021, 2022).

2.3. Soil analyses

2.3.1. Basal respiration

Soil respiration was determined by static incubation (Guitián and Carballas, 1976; Anderson, 1982). The CO_2 produced during a 10-d period by 25-g soil samples (< 5 mm) incubated at 25 °C at field capacity content was collected in 1 M NaOH solution (10 mL), which was then titrated against 1 M HCl. Three laboratory replicates from each soil sample were incubated.

2.3.2. Enzymatic activities

Thirteen enzyme activities involved in the breakdown or

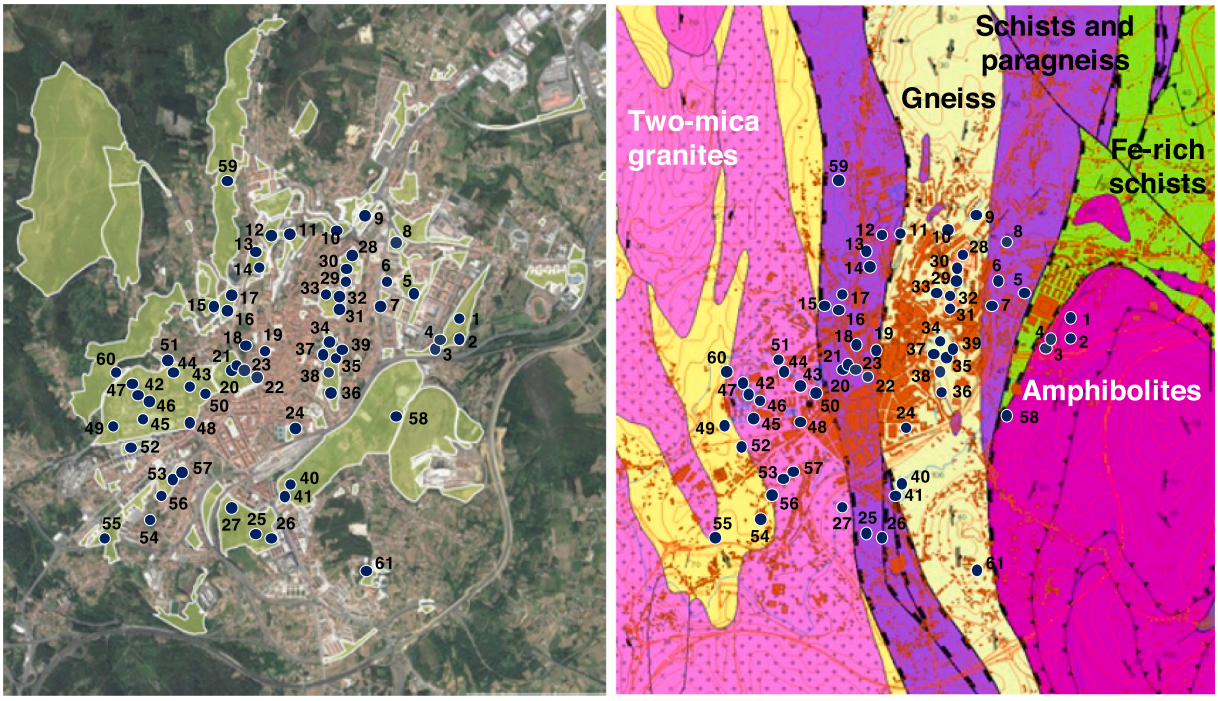

Fig. 1. Sampling locations and geology within the city of Santiago de Compostela (NW Spain).

mineralization of C, N, P, and S into inorganic forms that can be used by plants were determined in the soil samples by applying a hetero-molecular exchange procedure (Cowie et al., 2013) using a lysozyme solution (3%) and bead beating, as described in Bardelli et al. (2017). The enzymes examined were: (i) *C-cycle*: α- and β-glucosidases (*α- and βglu*: starch and cellulose degradation, respectively), cellobiohydrolase (*cell*: cellobiose degradation), xylosidase (*xyl*: xylan degradation), β-galactosidase (*βgal*: galactose degradation), and α-glucuronidase (*uroni*: pectin degradation); (ii) *N-cycle*: serin-like protease (*prot*: protein degradation), chitinase (*chit*: chitin degradation) and leucine-aminopeptidase (*leu*: peptide hydrolysis); (iii) *P-cycle*: alkaline and acid phosphomonoesterases and phytase (*alkP* and *acidP*: organic phosphorous mineralization; *phyt*: phytate degradation); (iv) *S-cycle*: arylsulphatase (*aryS*: organic sulfate esters degradation). Two technical replicates were performed for the enzymatic measurements of each field replicate, and the activities were expressed as nanomoles of 4-methyl-umbelliferyl (MUF) h^{-1} g^{-1} soil (dry weight, dw).

2.3.3. DNA extraction and real-time PCR

DNA was extracted from 0.25 g (fresh weight) of soil samples using DNeasy PowerSoil Kit (Qiagen) according to the manufacturer's protocols. DNA quality was evaluated using BioTek's Take3™ Multi-Volume Plate (SinergyTM Multi-Mode Microplate Reader, Bio-Tek Instruments, Inc.). The amount of DNA in each sample was determined by Qubit fluorometric quantitation prior to real-time PCR.

Real-time PCR analysis was performed to determine the 16S rRNA gene copy number of bacteria and the 18S rRNA gene copy number of fungi by using the primer pairs 1055f/1392r (bacteria, Bardelli et al., 2017) and FF390/FR1 (fungi, Prévost-Bouré et al., 2011), respectively. The samples were run against serial 10-fold dilution curves of standards consisting of genomic DNA extracted from cultures of *Nitrosomonas europaea* (DSMZ 21879) for bacteria, and *Fusarium solani* (DSMZ 10696) for fungi. DNA concentrations of the standards were measured using Qubit fluorometric quantitation and freshly prepared for each run. Each reaction had a total volume of 20 μL and contained 1 ng of DNA template, 0.4 μM of each forward and reverse bacterial or fungal primer, 0.6 mg mL^{-1} BSA, distilled water (RNase/DNase free), and 10 μL of 1× Luminaris HiGreen qPCR Master Mix, High ROX (Thermo Scientific). Samples and standards were run in triplicate on a StepOnePlus (Applied Biosystems). The cycling conditions were as follows: after an initial denaturation at 94 °C (bacteria) and 95 °C (fungi) for 10 min, thermal cycling comprised 40 cycles of 20 s at 95 °C, 15 s at 58 °C and 30 s at 72 °C for bacteria; and 15 s at 95 °C, 30 s at 50 °C and 30 s at 72 °C for fungi (Bardelli et al., 2017). To check for product specificity and potential primer dimer formation, runs were completed with a melting analysis starting from 60 °C to 95 °C with temperature increments of 0.25 °C and a transition rate of 5 s.

2.3.4. DNA sequencing and microbiome analyses

For these analyses, 22 samples were selected from the total set (indicated with a "#" symbol in Table S1). The selected samples are representative of all land uses considered: five urban grasslands, six urban forests, eight urban garden soils, and three periurban agricultural soils.

To characterize both bacterial and fungal microbiomes associated with the urban soil samples, the 16S rRNA and ITS marker regions were sequenced. Libraries were prepared following the two-step PCR Illumina® MiSeq instrument (Illumina®, San Diego, CA, USA) using 2 × 300 paired-end reads, followed by amplification and sequencing of the 16S V4 gene region and the ITS1 region (primers under patent WO2017096385; Becares and Fernandez, 2018). The primer-removed raw sequences can be found in NCBI's SRA under PRJNA742168. The bioinformatic processing of reads included the merging of forward and reverse paired reads to create robust amplicons, using Vsearch (Rognes et al., 2016) with minimum overlaps of 100 nucleotides and merge read sizes between 70 and 400 nucleotides. OTU clustering was performed at 97% sequence identity, followed by quality filtering through denovo chimera removal using the UCHIME algorithm (Edgar et al., 2011). Taxonomic annotation was performed using the SINTAX algorithm (Edgar, 2016), which uses k-mer similarity to identify the top taxonomy candidate, after which we retained results where the species level had a score of at least 0.7 bootstrap confidence. We used the SILVA database version 132 (Glöckner et al., 2017) and UNITE database version 7.2 (Nilsson et al., 2019) as taxonomic references. The bacterial and fungal data were transformed into compositional tables giving the proportionate abundance of sequencing reads per sample. From there, all

analyses were performed in R (version 4.0.2, R Core Team, 2020). Across all soil samples 973 bacterial and 1381 fungal units were detected.

The medium read abundance of each taxonomic unit within each sample group was calculated. Compositions were illustrated using R package compositions (version 2.0, van den Boogaart et al., 2020) and RColorBrewer (version 1.1–2, Neuwirth, 2014). Phyla and classes, respectively, composed of <20 taxonomic units were summarized to "others".

For Venn diagrams the taxonomic units representative for a sample group were defined as those taxonomic units that were detected in at least 80% of the samples within a sample group. Please note that the sampling size differs among sample groups and can impact on the absolute numbers. The data were illustrated using deepvenn (Hulsen et al., 2008).

The variations within sample groups and the Bray-Curtis dissimilarities of microbiome compositions were illustrated using non-metric multidimensional scaling (NMDS). Analyses were performed using R package vegan (version 2.5–6; Oksanen et al., 2019). For both the bacterial and fungal datasets the algorithm converged in less than 20 iterations. Stress was 0.081 and 0.185 for the bacterial and fungal analyses, respectively. Using permutational multivariate analysis of variance of distance matrices (adonis, 999 permutations) the effect of land use and physicochemical properties on the microbiome compositions was assessed. For both the bacterial and fungal microbiomes, the land use and physicochemical properties were correlated to the Bray-Curtis dissimilarity between samples.

2.4. Statistics

We used one-way ANOVA to test the impact of land use on microbial activity, enzymatic measurements, and microbial abundances. Before conducting ANOVA, we checked the absence of spatial autocorrelation of the data calculating Moran's I using the *ape* package for R (Paradis and Schliep, 2019), the homogeneity of variance using the Levene test, and the normality of data using the Shapiro-Wilk test. Data that did not pass the normality test were log-transformed for ANOVA. When a significant effect of land use at a level of significance of $P < 0.05$ was found, the Tukey's multiple range test was used to separate groups. Pearson's correlation coefficients between all the soil properties analyzed were also calculated, with significance adjusted for multiple comparisons. Principal component analysis (PCA) was employed for the characterization of the relation between soil physicochemical properties and the measures of basal respiration, enzymatic activities and microbial abundances. This analysis was conducted using the R packages *factoextra* (Kassambara and Mundt, 2017) and *FactoMineR* (Lê et al., 2008). All statistical analyses were performed using the R statistical package for MacOSX version R 3.1.3 (R Core Team, 2020) and the package R Commander version 2.6–1 (Fox and Bouchet-Valat, 2019).

3. Results and discussion

3.1. Microbial abundance and enzymatic activity in urban soils

We found a high variability in microbial respiration, enzymatic activities, and microbial abundances across the different urban soils of the city of Santiago (Tables 1, S2), taking into account their relatively small spatial dispersion, with coefficients of variation that range between 54% and 137%. In particular, respiration ranged between 1.3 and 66.3 mg C-CO_2 kg^{-1} day^{-1} with a mean value of 17.7 mg C-CO_2 kg^{-1} day^{-1}. They are in the lower range of those values observed for natural grasslands and forests in the region of Santiago (36 mg C-CO_2 kg^{-1} day^{-1} for grasslands, and 62 mg C-CO_2 kg^{-1} day^{-1} for oak forests) but in the same range as croplands: 5.5–32.8 mg C-CO_2 kg^{-1} day^{-1} with 16.1 mg C-CO_2 kg^{-1} day^{-1} on average (Trasar-Cepeda et al., 2008). Lower respiration rates in urban soils than in natural forest and grassland soils have also been observed by Sushko et al. (2019). Nevertheless, Demina et al. (2018) observed increases in urban soils with respect to croplands, but decreases with respect to forest and grassland, whereas Ivashchenko et al. (2014) observed higher respiration rates in some urban soils (recreational and residential areas) than in lowlands and industrial zones of Moscow region. These disagreements in the literature likely reflect the high heterogeneity in conditions and soil properties in urban ecosystems.

In general, the enzymatic activities were inter-correlated and correlated positively with basal respiration (except for alkaline phosphomonoesterase, Fig. 2A, Table S3) in the surveyed urban soils. We also found a positive correlation between all measures of biological activity and total organic carbon (OC) and N concentrations (Fig. 2A, Table S3), thus showing a clear relationship of microbial activity and soil organic

Table 1
Summary of soil biological properties. Basal respiration, enzymatic activities and microbial abundances in the urban soils from the city of Santiago de Compostela (NW Spain). Significance of the effect of land use is indicated as follows: * significant at a *P*-value of 0.05; ** significant at a P-value of 0.01; *** significant at a P-value of 0.001. Different letters within each column indicate statistically significant differences between land uses in the Tukey test at $p < 0.05$. Abbreviations: αGlu: α-glucosidase; βglu: β-glucosidase; cell: cellobiohydrolase; xyl: xylosidase; ßgal: β-galactosidase; uroni: α-glucuronidase; prot: serin-like protease; chit: chitinase; leu: leucine-aminopeptidase; acP: acid phosphomonoesterase; alkP: alkaline phosphomonoesterase; phyt: phytase; aryS: arylsulphatase.

	Basal respiration (mg CO_2-C kg^{-1} d^{-1})	Enzymatic activities (nmol MUF g^{-1} soil dw h^{-1})													Bacterial 16S rRNA gene copy number	Fungal 18S rRNA gene copy number
		C cycle						N cycle			P cycle			S cycle		
		αGlu	ßGlu	cell	xyl	ßgal	uroni	prot	chit	leu	acP	alkP	phyt	aryS		
Urban grassland	16 ± 8	14 ± 6	84 ± 49	8 ± 5	24 ± 32	36 ± 18	10 ± 3	15 ± 8	53 ± 27	283 ± 147	537 ± 261	377 ± 238a	7 ± 4	403 ± 279	(1.8 ± 1.3)·10^{10} b	(1.5 ± 1.3)·10^{8}
Urban forest	23 ± 18	21 ± 25	83 ± 90	8 ± 9	29 ± 11	41 ± 48	13 ± 13	14 ± 9	112 ± 165	361 ± 389	747 ± 833	332 ± 238a	7 ± 3	440 ± 412	(8.8 ± 5.0)·10^{9} a	(1.1 ± 0.7)·10^{8}
Urban garden	16 ± 14	7 ± 2	41 ± 21	4 ± 1	12 ± 3	15 ± 5	8 ± 1	15 ± 5	33 ± 13	238 ± 106	293 ± 117	673 ± 236b	6 ± 2	223 ± 181	(5.7 ± 7.0)·10^{9} a	(6.1 ± 5.6)·10^{7}
Periurban cropland	14 ± 9	6 ± 1	28 ± 29	3 ± 1	7 ± 4	16 ± 6	6 ± 2	11 ± 4	17 ± 9	179 ± 49	317 ± 62	356 ± 141a	6 ± 2	303 ± 141	(3.9 ± 1.5)·10^{9} a	(6.5 ± 6.4)·10^{7}
ANOVA																
Land use																
F	1.2	2.3	2.1	2.2	2.5	2.4	1.7	0.35	2.5	0.91	2.1	4.6	0.33	1.1	5.7	2.1
p	0.3	0.07	0.11	0.12	0.07	0.08	0.19	0.79	0.07	0.44	0.10	0.01**	0.80	0.34	0.002**	0.11

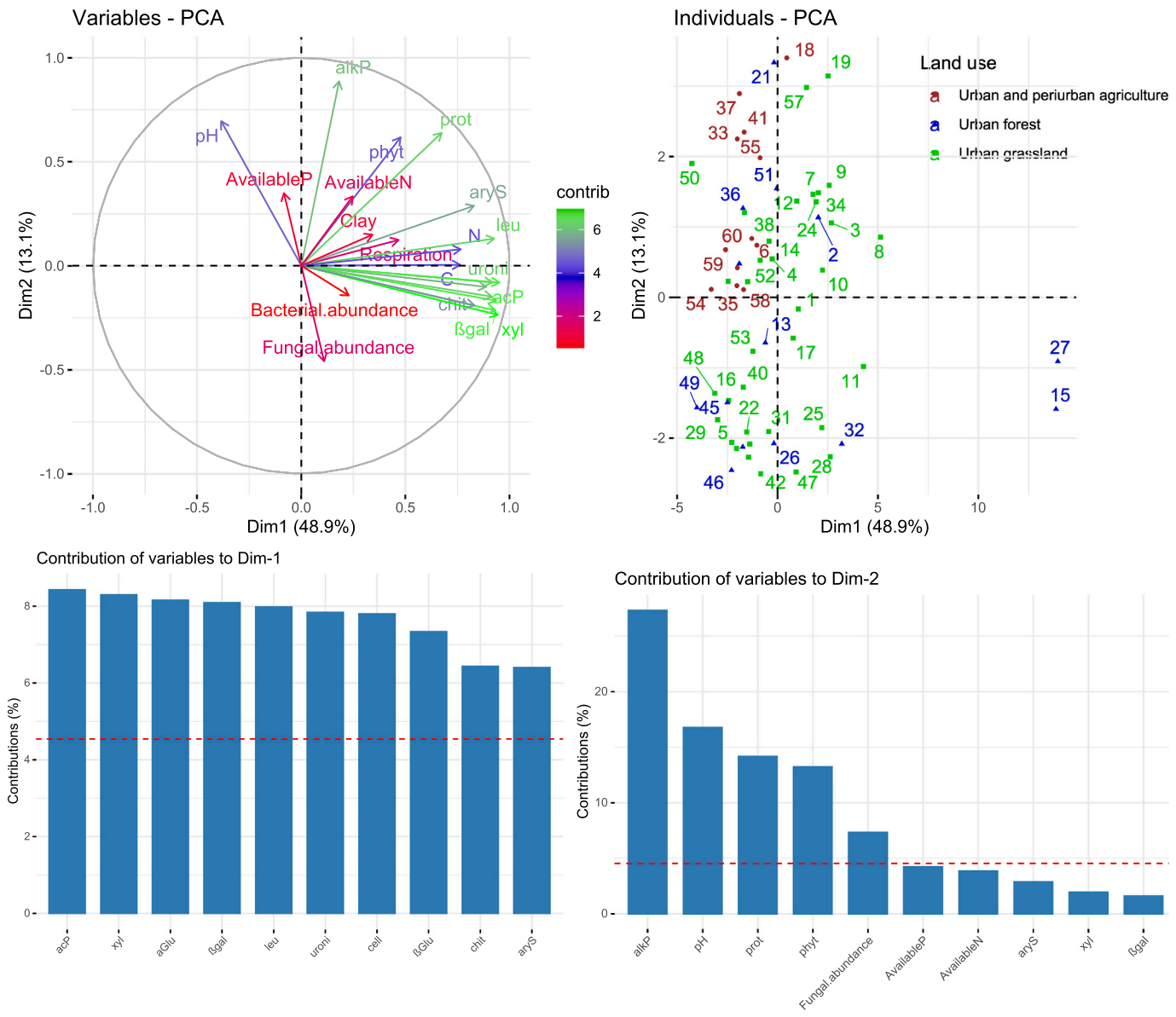

Fig. 2. Results of principal component analysis (PCA) of measures of basal respiration, enzymatic activities and microbial abundances across the urban soils from the city of Santiago de Compostela. A) Graph of variables contribution to the principal axes. B) Graph of individuals. C) Contribution of variables to the first two extracted components. Abbreviations: αGlu: α-glucosidase; βglu: β-glucosidase; cell: cellobiohydrolase; xyl: xylosidase; ßgal: β-galactosidase; uroni: α-glucuronidase; prot: serin-like protease; chit: chitinase; leu: leucine-aminopeptidase; acP: acid phosphomonoesterase; alkP: alkaline phosphomonoesterase; phyt: phytase; aryS: arylsulphatase.

matter. In the Principal Component Analysis (Fig. 2), these variables composed the first axis, which explained almost 50% of the overall variance in the dataset. Taken together, this reinforces the use of enzymatic activities as indicators of soil quality changes in urban areas, as pointed by other authors (Lorenz and Kandeler, 2006; Wang et al., 2011; Lemanowicz et al., 2020; Demina et al., 2018).

Moreover, we observed that the urban soils from the city of Santiago had much higher activities of acid and alkaline phosphomonoesterases, arylsulphatase, leucine-aminopeptidase and β-glucosidase than for the other enzymes measured (Table 1). These particular enzyme activities have already been shown as good reflectors of soil environmental conditions and changes in agricultural soils owing to their rapid response to alterations in management, land use and/or conservation practices (Adetunji et al., 2020). However, in our case we only detected a significant influence of land use for alkaline phosphomonoesterase (p = 0.006**; Table 1). The urban garden soils had the highest values for this enzyme activity, probably due to a combination of a higher soil pH (mean of 6.2 in urban gardens against 5.4 in urban grasslands and 5.6 in urban forests) and higher available P contents (72 mg P kg^{-1} in urban gardens against 46 mg P kg^{-1} in urban grassland and forests) compared to the rest of land uses (Table S1). These differences in soil properties are a consequence of agricultural practices including organic fertilization and liming that are practiced in the region to counteract the inherent acidity and low P fertility of Galician soils (Calvo de Anta et al., 1992). In fact, we also found a significant positive correlation of phosphomonoesterase activity with pH and available P (Table S3) and these three variables mainly composed the second PC (Fig. 2A). Together with the fungal abundance and to a lesser extent to bacterial abundance, this axis tended to set apart the cultivated urban and periurban soils from the rest, even though there was not a clear differentiation among land uses (Fig. 2B).

3.2. Microbiome composition in urban soils

We selected a representative subset of urban soil samples from the different land-uses for the microbiome analysis. Consistent with the findings of microbial abundance and enzymatic activities, only 34% and 18% of the compositional variation of bacterial and fungal communities among samples could be attributed to differences in land use (Fig. 3B, D). Despite the high heterogeneity among samples, there was a relatively large core microbiome across all of the land-use categories and a few OTUs were robustly represented in only one sample group (Fig. 3A, C). The smaller percentage of fungi detected as fungal core compared to bacteria (17.6% versus 32.9% of all OTUs included in the Venn analysis for fungi and bacteria, respectively), however, might be directly linked to the land-use categories. High patchiness, heterogeneity, and lack of spatial organization typical of urban soils are expected to interfere with fungal mycelium, which can largely contribute to the fungal biomass in the soil, and consequently, to fungal DNA. These results are in agreement with studies reporting significant effects of tillage practices on microbial community composition in agricultural soils (e.g., Anderson et al., 2017) and strong spatial dependencies of fungal communities across regions despite similar agricultural practices (Hannula et al., 2021).

Proteobacteria, Acidobacteria, Actinobacteria and Verrucomicrobia accounted for the highest read percentages across all urban soil samples (Table S4). Previous studies across a range of locations and soil types found these phyla as dominant of the urban soil microbiome (McGuire et al., 2013; Huot et al., 2017; Lee Joyner et al., 2019). With regard to the fungal community composition, the phyla Ascomycota, Basidiomycota and Glomeromycota accounted for the highest abundances in the studied urban soils (Table S5). At higher taxonomic ranks, the fungal compositions were more homogeneous compared to the bacterial ones. However, at lower taxonomic ranks, individual samples were heterogeneous and patchy (Fig. 3D).

Unlike fungi, we observed a slight rise in both bacterial richness and Shannon diversity from urban forests to urban grasslands, periurban croplands and urban gardens (Table 2), which could be tentatively related to an increasing sequence of human management intensity in the same direction as previously reported by Stephanou et al. (2021). Interestingly, the fungal richness was comparable to the bacterial one (Table 2). In any case, further studies are necessary to investigate the precise effect of anthropogenic activity on soil microbial diversity in urban environments.

3.3. Soil properties explaining activity and composition of microbial communities in urban soils

Soil physical and chemical properties distinctly influenced microbial

Table 2
Indicators of bacterial and fungal diversity in urban soils grouped by land use. The number of different bacterial and fungal units (richness; OTUs) and the Shannon index were calculated for each of the samples. Values are given as means and standard deviation of the sample groups. Normal and bold letters indicate significant differences on a 90% and 95% confidence interval, respectively.

	Richness		Shannon		n
	Bacteria	Fungi	Bacteria	Fungi	
Urban grassland	321 ± 71^{ac}	323 ± 59	4.0 ± 0.13^{ac}	3.3 ± 0.9	5
Urban forest	302 ± 36^{ab}	317 ± 87	4.0 ± 0.33^{ab}	3.2 ± 0.7	6
Urban garden	414 ± 45^{bc}	303 ± 62	4.5 ± 0.23^{bc}	3.6 ± 0.7	8
Periurban cropland	339 ± 25^{abc}	235 ± 28	4.1 ± 0.21^{abc}	3.7 ± 0.1	3

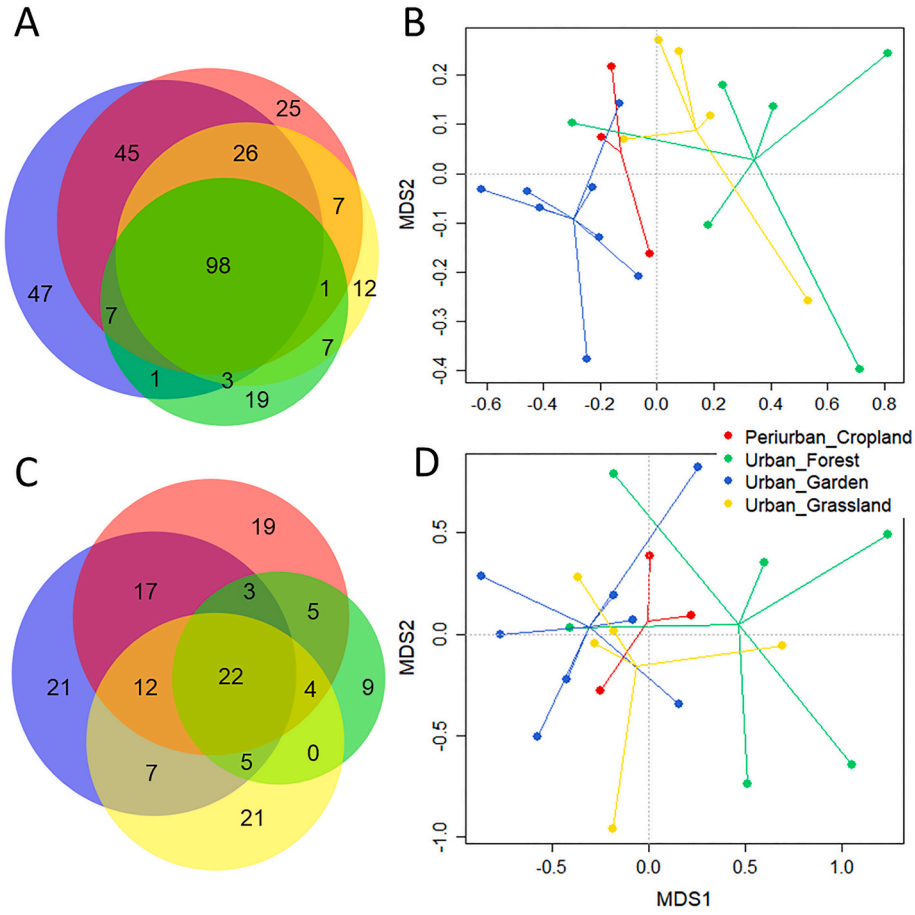

Fig. 3. Microbiome overview of the studied urban soils. The top and bottom rows refer to the composition of bacterial and fungal communities, respectively. In the Venn diagrams (A, C) the microbiome compositions according to operational taxonomic units (OTUs) were compared. The figures were generated using the online tool deepvenn Tim Hulsen. In the NMDS plots (B, D) the Bray-Curtis similarities of the individual samples were illustrated. Samples belonging to the same sample group were connected to illustrate the groups' variation in a comparative manner.

abundance, activity and composition in the surveyed urban soils from the city of Santiago (Tables S7, S8). Soil pH is known to impose a direct selection pressure on microbial community composition (Lauber et al., 2009). Almost all enzymatic activities presented negative correlations with pH (Table S3). Lorenz and Kandeler (2006) and Lemanowicz et al. (2020) also found negative correlations of soil pH with most enzyme activities. For bacteria, pH in the surveyed urban soils accounted for almost >28% of the variance, while for fungi pH explained less than 9% ($p_{Adonis} = 0.002$). This is in agreement with previous studies in which soil pH appeared as one of the most influential factors shaping bacterial communities in urban environments (Ramírez et al., 2014; Yan et al., 2016; Huot et al., 2017; Hui et al., 2017; Wang et al., 2017; Eldridge et al., 2021; Delgado-Baquerizo et al., 2021). Besides pH, the only other soil properties influencing microbial activity and community composition were related to soil organic matter content: as stated above, total organic C and total N were positively correlated to basal respiration and most enzymatic activities (Table S3). The total organic C content explained some part of the variance in the fungal community composition (7% variance, $p_{Adonis} = 0.010$; Table S8), whereas for bacteria, the total N content was more influential (8% of variance, $p_{Adonis} = 0.025$; Table S7).

It is likely that the fungal compositional shifts will be influenced to a larger extent by spatial restrictions such as lack of spatial organization and size of individual patches (Wardle et al., 2003; Abrego and Salcedo, 2013; Reese et al., 2016), rather than by other environmental factors. The lack of spatial logic and the high heterogeneity of urban soils might disable the establishment of one dominant fungus that usually occurs in soil. Supporting this, yeasts from the classes Tremellomycetes and Saccharomycetes, which do not build mycelium and are relatively small in cell size compared to other fungi, appeared to be among those fungal groups with largest differences among land uses (Table S6). The same occurred for the class Sordariomycetes (Table S6) that usually includes fungi with a host-associated life style such as endosymbionts, pathogens or saprotrophs.

Finally, we need to be mindful that further studies in other urban areas are needed to confirm these trends, as highlighted by other researches (Schmidt et al., 2017). The assumptions in our work would be valid for example for not very polluted cities with a patchy distribution of green areas, but the results would not be necessarily similar in cities with different characteristics to Santiago. For example, cities where the green surface is concentrated in only one or two large green areas instead of many small green areas, or in cities of a much larger size and/or more densely populated, or heavily polluted, or simply under a different climate.

4. Conclusions

This study shows a high variability in all of the microbiological variables among the soils from the city of Santiago de Compostela, reflecting the typical spatial heterogeneity of urban soils, where soil organic matter was more influential than land use to explain microbial activity and composition. Basal respiration and enzymatic activities were only correlated to organic matter contents and not affected by land use, with the exception of alkaline phosphomonoesterase (which was higher in urban garden soils, where distinct soil chemical conditions have been created by liming and fertilization). The high spatial heterogeneity of urban soils was particularly influential for the community composition of their microbial communities. While bacterial communities were structured in a more homogeneous, land use-dependent manner, fungi appear to be more heterogeneous and less influenced by group-dependent characteristics. Overall, our study provides evidence that soil organic matter is the driving force of biological activity in these urban soils, so measures for protecting, increasing and/or maintaining its contents should be adopted in order to guarantee urban soil functioning and health.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.apsoil.2022.104452.

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