Fungal-bacterial associations in urban allotment garden soils

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ABSTRACT

The soil microbiome in urban agriculture has not received much attention to date despite its important role in soil functionality. In this work, we evaluated the composition and diversity of fungal and bacterial communities through DNA extraction and ITS/16S marker gene sequencing in 40 soil samples collected from 10 urban allotment garden areas in the city of Santiago de Compostela (northwestern Spain). Despite anthropogenic activities are expected to affect negatively the microbial diversity, the richness of both fungal and bacterial communities was comparable to that of soils from other urban land-use categories with lower anthropogenic influence, i.e., urban forests, urban grasslands, and urban agricultural fields. However, the deterministic effect of soil properties and geographical distances was almost negligible in the surveyed allotment gardens. Neutral community models confirmed that the fungal and bacterial communities followed a random distribution (pseudo-$R^2_{fungi|bacteria}$ = 0.653 | 0.898) and that they were more random (tNST$_{fungi|bacteria}$ = 0.54 | 0.74) compared to the other abovementioned land-use categories (tNST$_{fungi|bacteria}$ = 0.46 | 0.67, p ≪ 0.001). Network analysis showed that in contrast to natural soils with lower anthropogenic influence, microbial associations formed very small modules; and frequently, microbial units remained unconnected. Taken together, our findings provide evidence that the soil microbial communities in the studied urban allotment gardens comprised a random assortment of microbes and their interactions, thereby supporting potential implications of anthropogenic activity for soil health and ultimately ecosystem functionality.

1. Introduction

Food production within cities has been increasing in importance during the last decades in parallel to the rise in urban population (Hallett et al., 2016). This has served as a way to secure a supply of food in urban areas since it is estimated that, in 2050, 80% of the world's food will be consumed in cities (Lal, 2020). This can be especially relevant in the context of climate change or other shocks to the food system (e.g. pandemics), when cross-regional or even regional transport of foods may be affected or even disrupted. Urban agriculture contributes to a low carbon economy as a result of shorter supply chains, provides food security and food sovereignty, and offers spaces for recreation and community building as well as education opportunities toward sustainability and social inclusion (Dinís Ferreira et al., 2018). In this sense, it is recognized as an important tool to advancing Sustainable Development Goals of the United Nations, especially no poverty, zero hunger, clean water, sustainable cities, climate action and life on land.

Soil is a key element in urban agriculture, but common practices applied in urban allotment gardens can make their soils very different from other soil types. Due to their location within cities, they often comprise of highly disturbed and manipulated materials altered through mixing, filling, transportation, and other perturbations caused by construction-related activities (Levin et al., 2017). These plentiful anthropogenic influences interrupt ecological gradients and connect habitats across small to medium scale that are naturally unrelated, thereby causing urban soils to lack naturally occurring spatial logic (Schmidt et al., 2017). This results in soils that present low fertility and functionality, with high amounts of artefacts and coarse fractions, high levels of compaction and/or sealing, low contents in organic matter and potential presence of organic and inorganic pollutants (Morel et al., 2015). However, knowledge about the properties of these soils has not received enough attention to date. Existing studies on urban allotment gardens have primarily dealt with the transference of inorganic contaminants to crops (López et al., 2019; Weber et al., 2019; Paradelo

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et al., 2020; Paltseva et al., 2022); while the biological component has hardly been the focus (Igalavithana et al., 2017; Grierson et al., 2023; Fan et al., 2023).

Soil biodiversity is a key contributor of ecosystem functioning, providing essential ecosystem services in urban greenspaces (Guilland et al., 2018; Fan et al., 2023). Based on a global survey across 56 cities, Delgado-Baquerizo et al. (2021) found that urban parks and large residential gardens harbor higher proportions of genes associated with human pathogens, greenhouse gas emissions, faster nutrient cycling, and more intense abiotic stress than adjacent natural terrestrial ecosystems. Moreover, these authors reported a global convergence in terms of reduced geographic variation in the soil microbiome of these urban greenspaces. The lack of spatial logic typical for urban soils (Schmidt et al., 2017) and similar pressures associated with urbanization may result in biotic homogenization (McKinney, 2006, 2008).

Microbial interactions are considered another key contributor for soil functionality. Generally, fungal-bacterial interactions have far-reaching relevance, including their influence on biogeochemical processes and plant growth (Deveau et al., 2018). Their interactions may provide selective advantage and they may change depending on environmental conditions, such as nutrient availability and soil pH (Velez et al., 2018). Nonetheless, up to now few studies on soil microbial communities have been conducted in urban allotment gardens, which differ in surface area or gardening practices, compared to other urban green areas. Consequently, there is still scarce information about the microbial community composition and diversity, as well as fungal-bacterial interactions in these urban greenspaces. Following this rationale, we (i) assessed the soil physicochemical properties of urban allotment gardens and determined the composition of fungal and bacterial communities; (ii) identified the major factors that shape the soil microbial communities; (iii) explored microbial occurrence patterns and fungal-bacterial associations in these urban greenspaces. For this, we selected 10 urban allotment garden areas in Santiago de Compostela (Spain), where some of the first projects of urban allotment gardens in Spain have been implemented. For comparison purposes, we considered other urban land-uses with lower anthropogenic influence including urban forests, urban grasslands and urban agricultural fields within the vicinity of the surveyed allotment areas. An overall description of these other urban land-use categories and the details on sample collection are given in Gómez-Brandón et al. (2022). We hypothesized that (i) the variation in the microbial community composition can be explained by allotment garden area and soil conditions, i.e. also due to garden management practices, and geographical distances. Supporting this, (ii) we expected that allotment gardens´soil microbial community composition will differ from the nearby urban land-uses with lower anthropogenic influence. Moreover, (iii) we expected a lower microbial diversity in the urban allotment garden soils, compared to the other land-use categories under less anthropogenic influence. Ultimately, (iv) we expected the typical soil heterogeneity of urban soils to influence the fungal-bacterial associations and their degree of complexity in the studied allotment gardens. Therefore, in a broader perspective, we asked in what way anthropogenic activity influences the soil and its microbiology.

2. Materials and methods

2.1. Study area

The urban allotment garden areas studied here were located in the city of Santiago de Compostela and the neighboring municipality of Ames, in the northwest of the Iberian Peninsula (Fig. S1). Details about the soils from other urban land-use categories and sample collection are given in Gómez-Brandón et al. (2022). Overall, the climate of the area is warm and wet, classified as temperate oceanic climate (Cfb) according to the Köppen−Geiger Climate Classification (Kottek et al., 2006). The city of Santiago includes former natural and agricultural soils that have been preserved with little modification during urban growth (Leptic and

Haplic Umbrisols). Soils constructed with human-altered and transported materials (Skeletic Regosols and Urbic Technosols); and those sealed by pavement or concrete for the construction of urban infrastructures (Ekranic Technosols; Paradelo et al., 2022) are also present within the city.

The soils in the areas where the allotment gardens have been established were originally classed as Haplic Umbrisols (Paradelo et al., 2022), and they were cultivated until the 1980s, when they were abandoned under the pressure of urbanization. In most areas (Belvís de Arriba, Belvís de Abaixo, Campo das Hortas, Brañas de Sar, Santa Marta), the original soils have been recovered for gardens without further modification. However, in other cases (Fontiñas, Caramoniña, Almáciga) the original soils have been heavily modified during urbanization and the current surfaces consist of a layer of imported topsoil over transported materials. Soils in the latter case present shallower A horizons (20–50 cm), while in the case of non-modified soils this horizon exceeds 1-m thick. The simultaneous presence of these two situations in urban allotment garden areas is not unusual and has already been observed in other cities (Charzynski et al., 2018).

All the allotment garden areas are property of the municipality, who lend them to users for periods of 2–4 years, in public programs established progressively at different moments in the last 15 years (Table S1). Individual plots range in surface from 20 to 60 m^2 and are cultivated with at least 8–10 horticultural species. When soils were sampled, most plots were cultivated with onion (11), tomato (8) or pepper (7), with potato and bean on three plots each, chard, lettuce and strawberry in two plots each, and corn and cabbage in one plot. All plots are irrigated and, in most cases, they receive organic fertilization (mostly cow manure).

2.2. Soil sampling

Soils from 40 urban allotment garden plots were sampled in the same day in summer (July 2nd, 2020): four plots were selected randomly within each urban allotment garden area. In each plot, 500-g composite samples were taken with an Edelmann auger from the 0–10 cm layer (the most biologically active), sieved immediately by a 5-mm mesh in the field and separated into two subsamples: one for the analysis of physical and chemical properties and another one for DNA extraction and downstream analyses. The first set of samples were air dried and sieved by a 2-mm mesh in the laboratory and used for subsequent determination of pH, soil texture, C, N and P contents and heavy metal concentrations. The second set of samples were kept at −20 °C and used for molecular analyses. All sampling tools and sieves were washed with 96% ethanol between samplings of each soil.

2.3. Soil physicochemical analyses

Particle size analysis was performed after organic matter destruction with H_2O_2 and washing with 1 N HCl, by dispersion with hexametaphosphate + sodium carbonate solution followed by wet sieving for sand (>0.050 mm), and silt and clay separation by the pipette method. Soil pH was measured in water (soil:solution ratio 1:2.5) and in 0.1 N KCl (soil:solution ratio 1:2.5). Total C and N were analyzed in ground samples using a LECO Truspec CHNS elemental analyzer. Since the soils did not present carbonates, total C equals total organic carbon (OC) in this case. Available P was determined by the Olsen method, after extraction with sodium bicarbonate (Olsen and Sommers, 1982). Total trace elements were determined after microwave-assisted acid digestion of 0.5 g of ground soil with 3 mL of HF and 9 mL of HNO_3 at 180 °C following EPA Method 3052 (USEPA, 1996); the extracts were analyzed for Pb, Cr, Ni, Cu and Zn using flame atomic absorption spectrometry (Varian SpectraAA 220FS).

2.4. DNA extraction, amplification, and sequencing

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 (Sinergy™ Multi-Mode Microplate Reader, Bio-Tek Instruments, Inc.). To characterize both fungal and bacterial communities in the soil samples, the ITS and 16S rRNA marker regions were selected. Libraries were prepared following the two-step PCR Illumina® MiSeq instrument (Illumina®ʸ San Diego, CA, USA) using 2 × 300 paired-end reads, followed by amplification and sequencing of the ITS1 and 16S V4 gene region using custom primers (patent WO2017096385; Becares and Fernandez, 2018).

Raw sequences were deposited in NCBI's SRA under the accession PRJNA759835; and analyzed using mothur v.1.44.1 (Schloss et al., 2009) applying standard protocol. Briefly, reads were paired and screened: for fungi/bacteria, reads longer than 280/500 bp or shorter than 230/200 bp were discarded. Sequences with ambiguous bases or homopolymers longer than 13 bp were removed. Fungal sequences were taxonomically assigned against the UNITE reference database (version 04.02.2020); sequences not assigned to the kingdom of fungi were removed from the dataset. Fungal sequences were clustered by abundance based greedy clustering (method = agc). For bacterial reads, the filtered sequences were aligned to SILVA reference database (v138); sequences not aligning well to the reference were removed from the dataset. Bacterial sequences were pre-clustered, thereby summarizing all sequences < 3 bp differences. After removing chimeric sequences from both the bacterial and fungal datasets using vsearch (v2.13.3), sequences were clustered to operational taxonomic units (OTUs) applying 97% sequence similarity criterion. For fungi, the average sequencing depth was 60,000 reads with a standard deviation of 28,000 reads. The minimum and maximum sequencing depth were 18,000 and 132,000 reads, respectively. For bacteria, the average sequencing depth was 40,000 reads with a standard deviation of 17,000 reads. The minimum and maximum sequencing depth were 11,000 and 81,000 reads, respectively. For each dataset, fungi and bacteria, a frequency OTU table was generated giving the relative read abundances of each OTU in each sample. This abundance-based OTU table was used for all further analysis on the soil microbial communities of the urban allotment gardens.

2.5. Statistical analyses

All statistical data analyses were performed in R 4.0.1 (R Core Team, 2020). We used one-way ANOVA to test differences in general soil properties between garden areas. Before conducting ANOVA, we checked 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 an effect at a level of significance of $p < 0.05$ was found, the Tukey's multiple range test was used to separate groups. The dependency of microbial richness on independent experimental factors (area, land-use, crop) was analyzed using non-parametric Kruskal test. The significances among pairwise sample groups were determined using posthoc Kruskal-Dunn test. In order to determine the relation among soil properties and microbial richness, Spearman correlations were calculated. For posthoc tests and multiple pairwise correlations, the resulting p-values were corrected for multiple comparisons using Benjamini Hochberg correction. Prior to testing if the number of OTUs annotated to a taxonomic class differed between the urban allotment gardens and the other urban land-use categories, we randomly subsampled 14 samples from the allotment garden pool (n = 40) in order to account for the lower sample size in the other urban land-use category group (n = 14). Then, we compared the richness of OTUs within taxonomic classes between the two sample groups using Kruskal test. In order to ensure that the obtained differences did not occur by chance, we reported the average richness across 1000 random subsamples.

The effect of experimental factors and soil properties on the multivariate microbial community composition of the allotment garden soils was analyzed using Adonis2 (Permutational Multivariate Analysis of Variance Using Distance Matrices) from the package vegan (Oksanen et al., 2019). Bray-Curtis dissimilarity was used to create the pairwise distance matrix. Significant differences were considered relevant if $p < 0.05$ and the size effect of the parameter $R^2 > 5\%$. In order to visualize the dissimilarities in microbial composition, Non-metric Multidimensional Scaling (NMDS) was applied.

Venn diagrams were generated to show the number of OTUs shared among sample groups or unique to a sample group. Prior to counting the numbers of OTUs within sets, the OTU tables were filtered, keeping only those OTUs present in at least 3 (out of 5) samples within the same allotment garden area. In the same manner, the fungal and bacterial community composition from the allotment garden soils was compared to those from other urban land-use categories with lower anthropogenic influence including urban forests, urban grasslands and urban agricultural fields within the vicinity of the surveyed allotment garden areas.

The effect of geographical distances on community dissimilarities was assessed by principal coordinates of neighbor matrices (PCNM) (Borcard and Legendre, 2002). Briefly, the Euclidean distances among sampling sites were reduced to positive eigenvalues applying the pcnm command of the vegan package (Oksanen et al., 2019). Variation partitioning via vegan's varpart function was applied to quantify the effects of environmental variables and geographical distances on the microbial community. Prior to variation partitioning, both the environmental and geographical dataset were reduced: from correlated environmental variables (|correlation coefficient| > 0.8) we selected one representative variable, i.e. the variable with the lowest correlation coefficient compared to all other environmental variables. The dataset subjected to variation partitioning contained the following variables: pH (in water), total OC, CN ratio, available P, Pb, Cr, soil texture and crop. For the geographical data, we calculated the influence of PCNM vectors on the microbial composition using vegan's RDA function and selected the relevant vectors by forward model selection. Variations in these two matrixes, namely relevant geographical PCNM vectors and environmental soil variables, were used to explain variances in pairwise Bray-Curtis dissimilarities among soil fungal and bacterial communities, respectively. Significances of the main predictors in variation partitioning (soil physicochemical variables, PCNM vectors representing geographical distances) were tested using vegan's RDA.

The stochastic and deterministic effects on the microbial community structure detected in the soil samples were quantified and compared by normal stochasticity ratio (NST) implemented in the corresponding R package NST (Ning et al., 2019). In addition, we calculated neutral community models following the framework of Sloan et al. (2006) as applied by Burns et al. (2016). Here, the model parameter "m" can be interpreted as migration rate, i.e. a measure of dispersal limitation.

Prior to calculating fungal-bacterial association networks, data were filtered to only those OTUs detected across allotment garden areas and crops. In other words, an OTU had to be detected in at least two different areas and two different crops growing in the allotment garden. This filtering approach was used because we wanted to calculate a network that is specific for allotment garden soils and independent of areas and crops. Using this approach, also those OTUs unique to a single sample were omitted from the network analysis. In total, a number of 100 and 447 fungal and bacterial OTUs, respectively, were subjected to network analysis. The associations were inferred using Julia programmed software FlashWeave (Tackmann et al., 2019) as it offers the possibility to integrate experimental factors and environmental variables in addition to OTUs. In addition, we used SpiecEasi (Kurtz et al., 2015) and SparCC (Friedman and Alm, 2012) for network inference. The network was visualized in R using package igraph (Csardi and Nepusz, 2006).
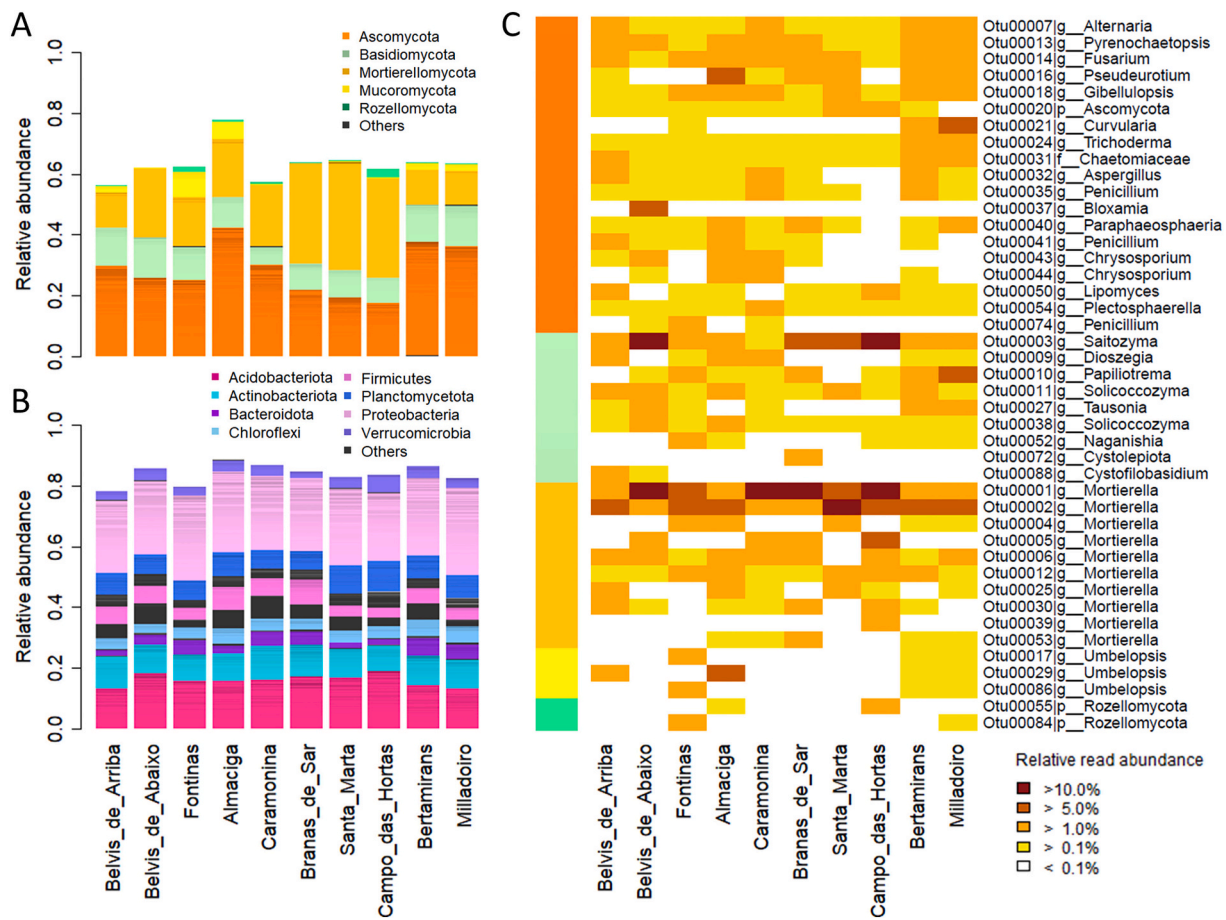
Fig. 1. Bacterial and fungal composition of the urban allotment gardens of the city of Santiago. Taxonomic composition of fungal (A) and bacterial (B) soil communities based on relative read abundances. The median relative read abundance of OTUs across four samples from the same area independently of the crop was illustrated. The difference to 1 can therefore be seen as a measure for within-group heterogeneity. (C) Heatmap illustrating the top ten most abundant fungal OTUs: From each allotment garden area and across all allotment garden areas, the top ten most abundant OTUs were identified and their respective relative read abundances were visualized.

3. Results and discussion

3.1. Community composition in urban allotment gardens of Santiago de Compostela

Ascomycota and Mortierellomycota accounted for the highest proportion of fungi in our overall dataset (Fig. 1A). Ascomycota are commonly found in soil and often have the ability of wind dispersal, thereby making them more likely to be observed across locations (Eigidi et al., 2019). Mortierellomycota represents one of the most common soil-dwelling fungi and can be present in rhizosphere and plant tissues (Ozimek and Hanaka, 2021), which could explain why this phylum is abundant in the soils from the surveyed allotment gardens. Indeed, ten OTUs annotated to the genus *Mortierella* were within the top ten most abundant fungal OTUs within each allotment garden area (Fig. 1C). Any member of Mortierellomycota, especially *Mortierella*, are known as valuable plant-growth promoting fungi; due to their ability to degrade pollutants, they are typically associated with healthy soils (Büttner et al., 2021). Additionally, our dataset contained Basidiomycota (Fig. 1A), with their vast majority belonging to Agaricomycetes (362/927 Basidiomycota OTUs) and Tremellomycetes (67/927 Basidiomycota OTUs). Within the top ten most abundant fungal OTUs, one out of ten Basidiomycota OTUs was affiliated to the order Agaricomycetes; and the remaining nine Basidiomycota OTUs all belonged to the order Tremellomycetes (Fig. 1C). It must be noted here that these basidiomycete yeasts do not rely on mycorrhizal structures and might therefore cope

better with smaller scales coming with heterogeneous environments that lack spatial logic. Therefore, this lifestyle might give them a competitive advantage over other fungi in the surveyed allotment garden soils. On average, we observed a value of 4.1 for the richness ratio of Ascomycota to Basidiomycota, exceeding those values reported for natural ecosystems by Tedersoo et al. (2014). These authors recorded the highest ratio of 1.86 in grassland and shrubland ecosystems, while the lowest value of 0.88 was detected in tropical deciduous forests. The increased dominance of Ascomycota with regard to Basidiomycota in the allotment gardens suggests that the soils from these urban greenspaces support a greater proportion of fast-growing organisms – when compared to those with lignin-degrading ability - that could take advantage of the often fertilized and irrigated conditions (Delgado-Baquerizo et al., 2021).

Other phyla like Mucoromycota and, to a lesser extent, Rozellomycota were also present in our allotment garden areas (Fig. 1A). The Mucoromycota are major decomposers of soil organic matter and widely found on organic substrates including vegetable matter, crop debris or composted materials (Kuramae et al., 2012; Hernández-Lara et al., 2022), which can be used for the management practices in the allotment gardens.

With regards to bacteria, the most abundant phyla found in the urban allotment gardens of the city of Santiago de Compostela belonged to Proteobacteria, Acidobacteriota and Actinobacteriota followed by Bacteroidota, Verrucomicrobia, Firmicutes, Chloroflexi and Planctomycetota (Fig. 1B). These findings are in line with previous studies performed in urban and peri-urban environments (Huot et al., 2017; Wang et al.,

Table 1

Summary of physicochemical properties of the urban garden soils (mean ± standard deviation, n = 4). Significance of differences between garden areas 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 areas in the Tukey test at p < 0.05.

	pH$_w$	Total OC	Total N	C/N	Available P	Sand	Silt	Clay	Texture	Pb	Cu	Zn	Ni	Cr
		g kg⁻¹	g kg⁻¹		mg kg⁻¹	%				mg kg⁻¹				
Belvís de Arriba	5.8 ± 0.5[ab]	28 ± 4[a]	2.3 ± 0.3[a]	12 ± 1[a]	77 ± 19[b]	56 ± 5[ab]	29 ± 4[cd]	15 ± 1[bde]	Sandy loam	185 ± 55[b]	42 ± 5[ab]	184 ± 5[cd]	30 ± 1[bd]	19 ± 1[cd]
Belvís de Abaixo	6.3 ± 0.3[ab]	37 ± 9[a]	2.7 ± 0.6[a]	14 ± 1[a]	71 ± 10[b]	51 ± 1[a]	33 ± 1[d]	16 ± 1[cde]	Loam	343 ± 77[c]	83 ± 16[bc]	238 ± 27[de]	39 ± 3[e]	22 ± 2[de]
Fontiñas	6.1 ± 0.4[ab]	63 ± 9[c]	4.1 ± 0.4[bc]	15 ± 1[b]	26 ± 14[a]	65 ± 1[cd]	18 ± 2[ab]	18 ± 2[e]	Sandy loam	64 ± 3[a]	11 ± 5[a]	65 ± 13[a]	30 ± 1[bd]	6 ± 1[a]
Almáciga	5.9 ± 0.5[ab]	40 ± 13[ab]	3.0 ± 0.8[ab]	13 ± 1[a]	62 ± 15[b]	68 ± 6[d]	21 ± 5[ab]	12 ± 1[a]	Sandy loam	87 ± 20[a]	19 ± 4[a]	89 ± 14[ab]	25 ± 1[ab]	14 ± 4[bc]
Caramoniña	6.6 ± 0.1[b]	38 ± 8[ab]	2.8 ± 0.4[a]	14 ± 1[ab]	62 ± 3[b]	63 ± 5[bcd]	25 ± 4[d]	13 ± 1[abc]	Sandy loam	136 ± 55[ab]	109 ± 58[c]	153 ± 8[bc]	34 ± 1[cde]	15 ± 0.4[bcd]
Brañas de Sar	6.7 ± 0.5[b]	56 ± 3[bc]	4.2 ± 0.2[c]	13 ± 1[a]	54 ± 13[ab]	52 ± 2[a]	33 ± 2[bc]	15 ± 1[bde]	Sandy loam	76 ± 21[a]	36 ± 5[ab]	119 ± 20[ac]	36 ± 2[de]	30 ± 3[f]
Santa Marta	5.7 ± 0.1[ab]	43 ± 9[ab]	3.4 ± 0.7[ac]	13 ± 1[a]	27 ± 12[a]	57 ± 3[ac]	29 ± 2[cd]	14 ± 2[ad]	Sandy loam	89 ± 9[a]	32 ± 3[ab]	106 ± 22[ab]	34 ± 3[cde]	26 ± 3[ef]
Campo das Hortas	5.9 ± 0.3[ab]	38 ± 3[ab]	2.8 ± 0.2[a]	13 ± 1[a]	83 ± 20[b]	50 ± 5[a]	33 ± 4[d]	17 ± 1[de]	Loam	343 ± 37[c]	129 ± 28[c]	298 ± 87[e]	39 ± 6[e]	27 ± 5[ef]
Bertamiráns	6.5 ± 0.8[ab]	45 ± 10[ac]	3.4 ± 0.7[ac]	13 ± 0.2[a]	28 ± 7[a]	64 ± 1[bcd]	21 ± 2[ab]	15 ± 1[bde]	Sandy loam	58 ± 1[a]	11 ± 3[a]	80 ± 16[ab]	29 ± 5[bc]	9 ± 1[ab]
Milladoiro	5.5 ± 0.5[a]	39 ± 4[ab]	2.9 ± 0.3[ab]	13 ± 1[a]	27 ± 12[a]	71 ± 1[d]	16 ± 1[a]	13 ± 1[ab]	Sandy loam	53 ± 2[a]	11 ± 4[a]	47 ± 4[a]	19 ± 1[a]	10 ± 4[ab]
ANOVA														
F	3.4	6.7	5.6	4.4	11.7	16.7	18.7	8.7		35.1	16.8	26.2	18.6	33.9
p	<0.006**	<0.001***	<0.001***	<0.001***	<0.001***	<0.001***	<0.001***	<0.001***		<0.001***	<0.001***	<0.001***	<0.001***	<0.001***

2017; Delgado-Baquerizo et al., 2021; Stephanou et al., 2021; Yan et al., 2021), supporting that the dominant phyla comprising the bacterial communities are frequent in different soil types. While the top ten most abundant fungal OTUs exceeded 10% of the total read abundances (Fig. 1C), none of the top ten most abundant bacterial OTUs surpassed 5% of the total read abundances (Fig. S2). They mainly belonged to different genera of Proteobacteria and interestingly to the family Nitrosospheraceae, which comprises ammonia-oxidizing archaea within the phylum of Crenarchaeota.

3.2. Microbial communities of allotment garden soils were unrelated to several well-described environmental drivers

We hypothesized that the variation in the soil conditions due to garden management practices and geographical distances (Table 1) will be accompanied by compositional community changes across the studied allotment gardens. In line with our hypothesis, the microbial community composition differed among the soils from the different allotment garden areas, and between samples collected within the same area (Table 2, Fig. S3). By using permutational analysis of variance, 23% and 20% of variance was assigned to the allotment garden identity (p$_{Adonis}$ = 0.001) with respect to fungi and bacteria, respectively. Despite these locational differences, this variation was mostly unrelated to the soil properties (Adonis R^2 < 0.05; Table 2). Only soil pH was found to explain a significant portion of variance within the bacterial community (13.3% variance, p$_{Adonis}$ = 0.001), which is consistent with previous studies (Joyner et al., 2019; Eldridge et al., 2021; Delgado-Baquerizo et al., 2021; Gómez-Brandón et al., 2022). This, however, did not hold true for fungi (R$^2_{Adonis}$ < 0.05, Table 2), despite soil pH covered a wide range from 4.1 to 7.1 in the studied allotment garden areas (Table 1). As soil pH is usually considered the major driver of the fungal community composition, this further underlines that ubiquitously distributed generalist fungal species might be favored in the allotment garden soils.

Vegetation might be another major driver of community compositional changes. Although the type of crop planted by the gardener explained a high portion of variance (fungi: 19%, bacteria: 17%), its effect was insignificant (p$_{Adonis}$ > 0.14, Table 2). This might be due to the high variety of crops planted, which could not be sufficiently captured by the sampling size of our study. In addition, gardeners might vary the crops grown frequently, thereby potentially introducing various historical effects which could not be captured here. Therefore, the influence of different crops on allotment garden soils' microbial community composition needs further attention.

Likewise, there was no correlation between the soil microbial communities and the trace metal contents in the current study (Table 2). This is in disagreement with earlier studies in which heavy metal/metalloid pollution led to a change of microbial species composition, and the replacement of tolerant microorganisms by more susceptible microorganisms (Guo et al., 2017; Li et al., 2020a; Hu et al., 2021; Stephanou et al., 2021).

Taken together, the fact that the majority of environmental variables – except for the allotment garden area, and the soil pH in the case of bacteria – played a negligible role in microbial community assembly does not fully support our hypothesis. It also contrasts with other studies in which soil physicochemical properties are reported as important drivers underlying variation in the composition of microbial communities, both in general and in urban areas in particular (Fierer and Jackson, 2006; Delgado-Baquerizo et al., 2021). The overall high organic matter and nutrient content of the urban garden soils from Santiago (Table 1) might have attenuated the ecological pressure on microbial communities imposed by soil nutrient limitation.

Using variation partitioning, we tested if in multivariate scale, the soil physicochemical properties and the geographical distances could explain the compositional variation in the microbial communities of the studied allotment garden areas. For bacteria, the variance within the soil

Table 2

Dependency of the fungal and bacterial community composition on experimental factors and soil physicochemical properties measured in the allotment gardens of Santiago de Compostela. Adonis analysis was performed on 999 permutations. Significance codes: *** = 0.001; ** ≤ 0.01; * ≤ 0.05.

|	Variable	Df	SumsOfSqs	R^2	F.Model	Pr(>F)	
Fungi	pHw	1	0.5416	0.04898	2.6224	0.001	***
	C	1	0.4566	0.04129	2.2106	0.002	**
	N	1	0.3128	0.02828	1.5144	0.049	*
	CN	1	0.296	0.02677	1.4333	0.073	
	P	1	0.4996	0.04517	2.4188	0.001	***
	Sand	1	0.5447	0.04925	2.6372	0.001	***
	Silt	1	0.3498	0.03163	1.6937	0.027	*
	Clay	1	0.2656	0.02402	1.286	0.144	
	Pb	1	0.286	0.02587	1.3849	0.088	
	Cu	1	0.4151	0.03754	2.0099	0.002	**
	Zn	1	0.3048	0.02757	1.4759	0.059	
	Ni	1	0.2476	0.02239	1.199	0.224	
	Cr	1	0.2444	0.0221	1.1834	0.213	
	Area	9	2.5647	0.23192	1.3798	0.007	**
	Crop	9	2.0769	0.18781	1.1173	0.196	
	Residual	8	1.6523	0.14941			
Bacteria	pHw	1	0.8572	0.13334	8.5635	0.001	***
	C	1	0.2714	0.04221	2.711	0.004	**
	N	1	0.134	0.02084	1.3384	0.179	
	CN	1	0.2178	0.03387	2.1755	0.015	*
	P	1	0.2242	0.03487	2.2394	0.013	*
	Sand	1	0.3311	0.0515	3.3073	0.001	***
	Silt	1	0.1296	0.02016	1.2948	0.21	
	Clay	1	0.1488	0.02315	1.4865	0.118	
	Pb	1	0.2507	0.039	2.5047	0.006	**
	Cu	1	0.179	0.02784	1.788	0.056	
	Zn	1	0.1019	0.01586	1.0183	0.409	
	Ni	1	0.1122	0.01745	1.1206	0.311	
	Cr	1	0.1024	0.01592	1.0224	0.416	
	Area	9	1.4784	0.22997	1.641	0.006	**
	Crop	9	1.0895	0.16946	1.2093	0.146	
	Residual	8	0.8008	0.12457			
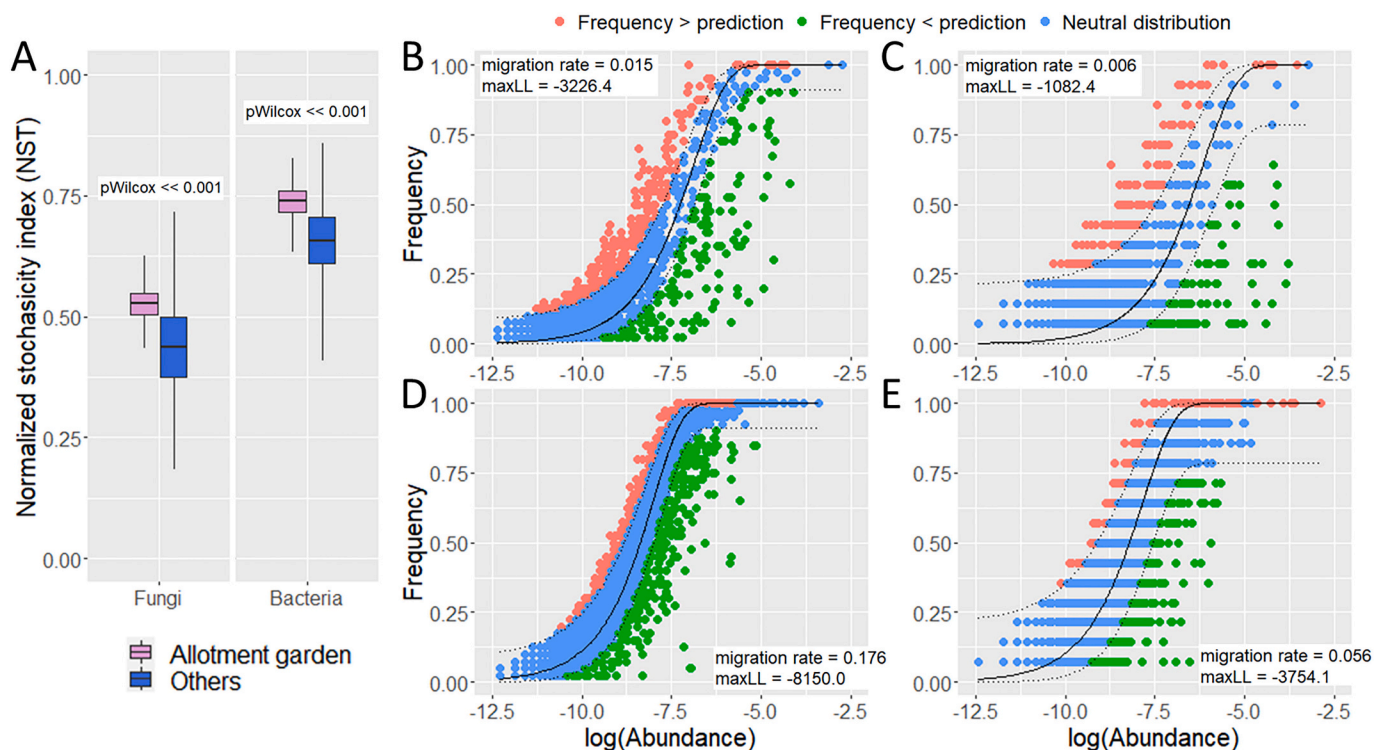
Fig. 2. Level of stochasticity based on calculation of the taxonomic neutral stochasticity index (tNST) (A); and neutral community models (B–E) for the urban allotment garden soils (B = fungi, D = bacteria) and those from other land-use categories (urban forest, urban grassland and urban agricultural fields) within the city of Santiago de Compostela (C = fungi, E = bacteria). OTUs represented by blue points follow the neutral community model. Red points indicate OTUs that are observed more frequently than predicted by the model, and likely selected for in the ecosystem. Green points refer to OTUs that are observed less frequently than expected by the model, and likely replaced by members of other OTUs.
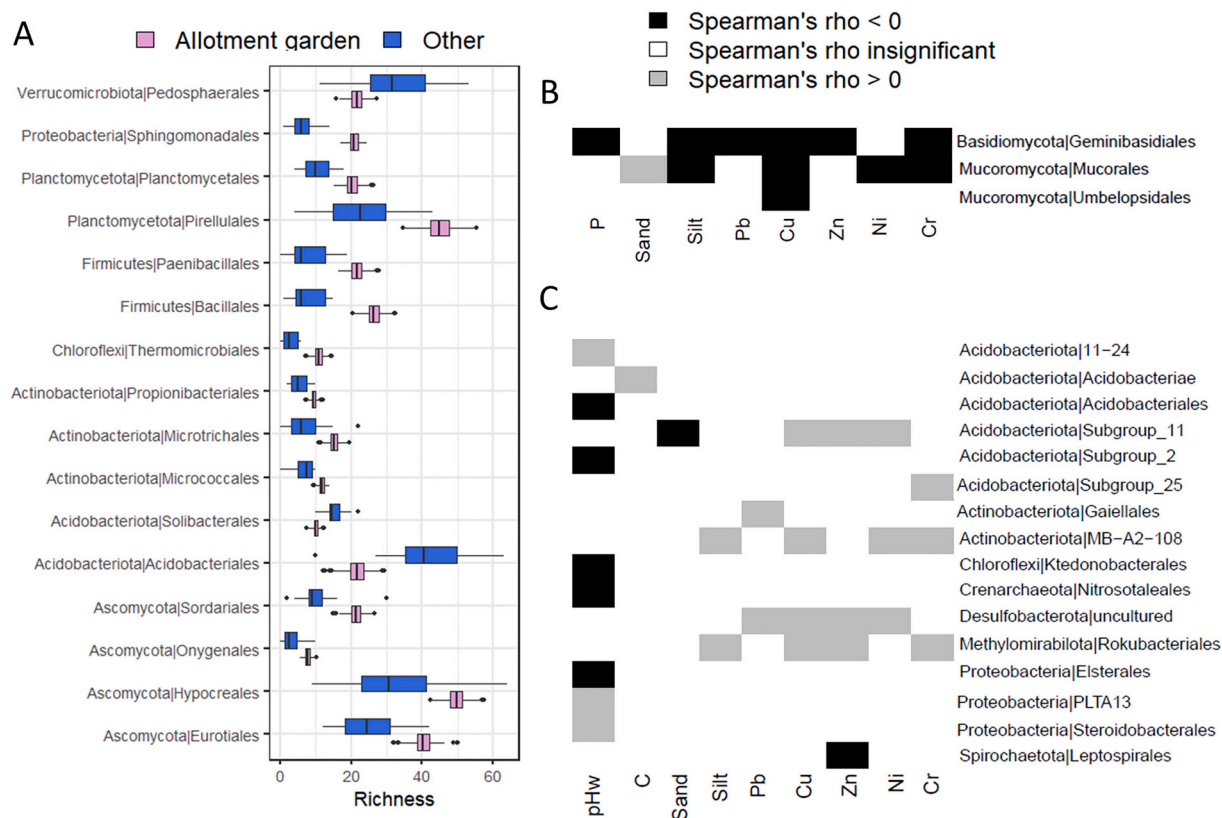
Fig. 3. Microbial diversity. (A) The microbial OTU richness in the allotment garden soils was compared to the one from other urban land-use categories within the city of Santiago de Compostela (urban forests, urban grasslands and urban agriculture). The bars visualize all fungal and bacterial classes with a richness >10 OTUs in at least one of the sample groups and significant differences in richness between both sample groups in at least 90% of comparisons (Kruskal test on 1000 permutations). (B, C) Spearman correlation coefficients between the OTU richness within classes and environmental variables were calculated. All significant coefficients across all fungal (B) and bacterial (C) orders were displayed.

properties and geographical distances accounted for 21% ($p = 0.01$) and 7% ($p = 0.018$). Only 1% of this variance was jointly explained by both factors. For the fungal community, neither the soil properties nor the geographical distances among the allotment areas accounted for a significant portion of variance. This suggests that the soil microbial communities in the surveyed allotment gardens were largely independent of these ecological forces. In this regard, anthropogenic conversion of natural to agricultural land has been found to weaken the correlation between environmental conditions and soil biodiversity (Manning et al., 2015; Li et al., 2020b). These latter authors observed that, in contrast to natural habitats, both alpha- and beta-diversity levels were not driven by climatic differences in agricultural systems. This partly indicates that agriculture-related activities could erase or reduce climatic constraints on soil biodiversity.

3.3. Different degrees of anthropogenic activity might affect the soil microbial community

In order to test our second hypothesis, whether increased anthropogenic activity might have affected the soil microbial community composition, we compared the soil microbiomes of the allotment garden areas to those collected from other nearby urban land-use categories within the city of Santiago. These other locations comprise urban forests, urban grasslands, and urban agricultural fields; they are all influenced by human activity (Gómez-Brandón et al., 2022), but to a lesser extent compared to urban allotment gardens. Supporting our hypothesis, we found that the effect of land-use on the soil microbial community composition was significant for both fungi and bacteria, even though the variance explained was low ($R^2_{fungi} = 4.6\%$, $R^2_{bacteria} = 11.8\%$, Fig. S4A, B). Furthermore, we observed that 36.7% of the fungal and 47.1% of the

bacterial OTUs detected in the allotment garden soils coincided with those found in other urban land-uses within the city of Santiago (Fig. S4C, D). Together, these core OTUs accounted for >80% of all reads in both data sets (Fig. S4E, F), thereby indicating that the existing differences detected by Adonis analysis were mainly due to differences in relative abundances rather than qualitative aspects.

3.4. Allotment gardens' microbial communities follow a random distribution

Following the rationale developed above that anthropogenic activities might decrease the environment's deterministic effect on the soil microbial communities, we quantified the degree of stochasticity in the urban allotment garden soils by using the taxonomic normalized stochasticity ratio (tNST) (Ning et al., 2019); together with neutral community models (Sloan et al., 2006). For comparison, we considered the other urban land-use categories (urban forests, urban grasslands, urban agricultural fields) within the city of Santiago de Compostela, which were under less anthropogenic influence than the allotment garden areas.

Supporting our hypothesis, the contribution of stochasticity to the community structure was higher in the allotment gardens compared to the other urban land-use categories for both fungal and bacterial communities ($p_{Wilcox} \ll 0.0001$) (Fig. 2A). Moreover, the comparison to natural soils supports the hypothesized tendency: In a natural soil from France with comparable pH, Romdhane et al. (2021) calculated below 20% stochasticity for the bacterial community using the same tNST measure as we did in our study. However, Shi et al. (2018) observed around 60% stochasticity in agricultural soils in the Tibetan Plateau. Here, the bacterial tNST (allotment garden soils = 0.74, other urban
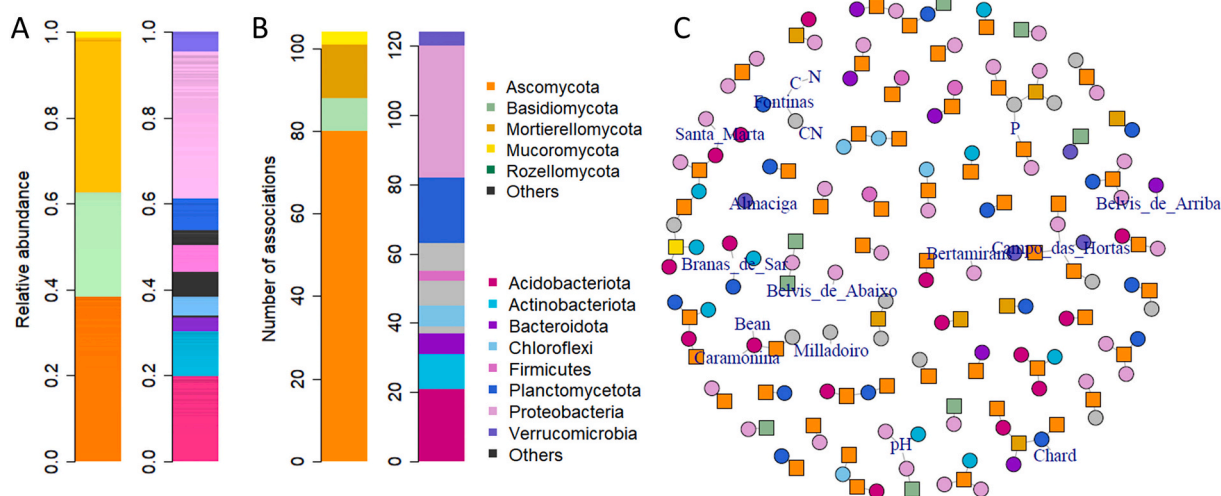
Fig. 4. Fungal and bacterial core communities of urban allotment gardens and their associations. (A) Relative abundances of bacterial and fungal OTUs representative for all sampling areas and crop cultivations across the overall dataset. Those OTUs were used for network inference. (B) Taxonomic distribution of OTU associations. (C) Associations between representative fungal and bacterial OTUs inferred via FlashWeave network calculation.

soils = 0.67, $p_{Wilcox} \gg 0.001$, Fig. 2A) was even higher than that reported for agricultural soils. Guo et al. (2022) also found a level of stochasticity >50% with regards to the fungal community of grasslands, which is comparable to the tNST detected here (allotment garden soils = 0.54, other urban soils = 0.46, $p_{Wilcox} \gg 0.001$, Fig. 2A).

Supporting the high stochasticity in the urban soil microbial communities, the community assembly in both the surveyed urban allotment gardens and the other urban land-use categories satisfied the prediction of the neutral theory model based on Sloan et al. (2006) (Fig. 2B-E, Table S2). The percentage of both fungal and bacterial OTUs that followed the random model (blue points) accounted for a high percentage (>88%); while the percentages of OTUs likely lost from the ecosystem (green points) was low (around 4%) for all of the urban land-use categories in the case of bacterial and fungal communities. Nonetheless, the percentages of OTUs likely selected for by the ecosystem were higher for the allotment garden soils (fungal OTUs = 8.1%, bacterial OTUs = 4.2%) in comparison to other urban soils (fungal OTUs = 6.7%, bacterial OTUs = 2.6%). Whether these differences were meaningful from a functional perspective, it needs to be further tested across urban areas from other cities.

Altogether, both the tNST and good fit of neutral models ($R^2 > 0.65$, Table S2) implies that both bacterial and fungal microbial communities were primarily governed by stochastic processes in the studied allotment garden areas within the city of Santiago. As stated by Guo et al. (2022), a higher stochasticity might be related to a more homogenous soil environment, which could be in line with the fact that urbanization may result in biotic homogenization. Another plausible driving force that might shape the urban microbiome relies on microbial interactions, which will be addressed in the last subchapter.

3.5. Microbial diversity in urban allotment gardens

We hypothesized that the soil microbial richness might be lower in allotment gardens than in the other land-use categories. Against our hypothesis, the allotment gardens' microbial richness was comparable to that in the other urban soils from the city of Santiago de Compostela (bacteria: $p_{Kruskal} = 0.17$, fungi: $p_{Kruskal} = 0.072$). On average, 1300 ± 380 bacterial OTUs were detected across all the urban land-use categories studied here. By trend, the average fungal richness was even higher in the allotment gardens (allotment gardens: 440 ± 82; other urban soils: 380 ± 94 fungal OTUs). Overall, the majority of fungal (74 ± 2.6%) and bacterial classes (63 ± 4.2%) did not differ in OTU richness

between the allotment garden soils and those from the other urban land-use categories. Out of 158 fungal classes, in the allotment gardens 8 ± 2 classes were lower in OTU richness while 21 ± 3 classes were higher compared to the other urban land uses ($p_{Wilcox} = 0.003$). Out of 318 bacterial classes, in the allotment gardens 22 ± 7 classes were lower and 67 ± 10 classes were higher in OTU richness than in the other urban land-use categories ($p_{Wilcox} = 0.003$). Likewise, the OTU richness of abundant classes (>10 OTUs within a class) was usually higher (13/16 orders) in the allotment garden soils than in those from the other urban land-use categories (Fig. 3A).

Similar to the community composition, the microbial richness was not correlated with any of the soil physicochemical properties ($p > 0.05$). Also, on the taxonomically finer class level, the richness of only 3/146 fungal and 16/314 bacterial classes was at least correlated to one environmental variable (Fig. 3B, C). As shown for bacterial community composition and soil pH (13% variance, $p = 0.001$, Table 2), this edaphic variable was also correlated to the richness of different bacterial orders (Fig. 3C). Together, these findings further emphasize the disconnectedness between allotment gardens' microbial communities and the soil environment, except for the soil pH. Likewise, Grierson et al. (2023) reported no correlation between alpha diversity and the soil properties for bacterial, fungal and protozoan communities across private backyards and public parks in Tasmania, Australia. Nonetheless, previous existing literature on urban greenspaces have found that edaphic variables appear to be closely related to alpha diversity measurements (Wang et al., 2018; Zhang et al., 2019; Delgado-Baquerizo et al., 2021). For instance, Wang et al. (2018) reported that the alpha diversity of soil bacteria was positively associated with the contents of moisture and nitrate when compared to urban parks and residential sites. These contrasting findings further underscore the clear need for more comprehensive studies to shed light onto how (or if) soil microbial communities are influenced by urban growth, in both regional and large cities.

3.6. Microbial associations in urban allotment gardens

We hypothesized that the high heterogeneity of urban soils, including their lack of spatial logic, might influence microbial associations as well as the complexity of the microbial association network. In this regard, the microbial association network calculated from our allotment garden samples had a high number of individual pairs (i.e., one fungal and one bacterial OTU) and quite few, relatively small

Table 3
Properties of association networks calculated using different tools. f = fungal; b = bacterial. For fb networks, ff and bb associations were excluded. mb = SpiecEasi (meinshausen-buhlmann's neighborhood selection); sparcc = SparCC, flash = FlashWeave. For sparcc networks, the value behind the name indicates the threshold used for filtering the network based on correlation coefficients. degree = average number of connections per OTU; gsize = the total number of associations; diameter = the longest path; transitivity = cluster coefficient; degree~abundance = pearson correlation coefficient of degree and OTU relative abundance; p.cor = significance (p-value) of the correlation coefficient.

	degree	gsize	diameter	Density	transitivity	degree~abundance	p.cor
fb.mb	2.16	591	12	0.0040	0	0.318	2.5E-14
fb.sparcc.0.5	2.22	606	10	0.0041	0	0.460	5.1E-30
fb.sparcc.0.8	0	0	0	0	NA	NA	NA
f.mb	0.30	15	4	0.0030	0	0.290	0.003
f.sparcc.0.5	1.64	82	11	0.0166	0.3490	0.462	1.3E-06
f.sparcc.0.8	0.02	1	1	0.0002	NA	0.018	0.862
b.mb	10.24	2288	5	0.0230	0.0826	0.199	2.3E-05
b.sparcc.0.5	25.86	5779	7	0.0580	0.5253	0.460	5.1E-30
b.sparcc.0.8	0.12	27	7	0.0003	0.4861	NA	NA
fb.flash	1.39	129	8	0.0075	0	−0.025	0.748
b.flash	2.88	650	15	0.0064	0.0017	0.130	0.006
f.flash	1.73	64	9	0.02369493	0	0.255	0.033

modules (Fig. 4C). Supporting our hypothesis, the big modular structures usually reported for natural ecosystems such as forests (Sun et al., 2017), deadwood (Gómez-Brandón et al., 2020), grasslands (Wagg et al., 2019), tundra (Geml et al., 2021), and agricultural fields (Banerjee et al., 2016) were absent from our networks (Fig. 4C). Modularity measures the degree in which a network has subordinate structures and it can be interpreted by the higher the modularity the more resistant the microbiome to disturbance (Siles et al., 2021). Bearing this in mind, the absence of big modules in the allotment gardens´ networks could have important implications not only for the microbes themselves, but also for the plants, soil and ecosystem functioning and services in these urban greenspaces. This calls for comparative studies evaluating the complexity of microbial networks in a broader context considering soils from multiple cities with contrasting populations, vegetation, and climatic conditions.

The taxonomic distribution of bacterial communities in the network matched the overall distribution depicted across the soils from the studied allotment garden areas within the city of Santiago (Fig. 4A, B). In the case of fungi, we found a high number of associations involving Ascomycota (Fig. 4B). It seems that members of this phylum are widespread and might be randomly distributed across the surveyed allotment gardens. Moreover, we found that the sampling areas and not the soil properties were frequently associated with OTUs (Fig. 4C), which suggests that the microbial associations we observed might have been area specific, which is in line with the allotment garden area being a significant predictor for the microbial community (Table 2). This indicates that these associations unlikely follow a more general selective force, which aligns with the geographical distances hardly playing a role in shaping the community composition in these urban greenspaces.

In order to rule out that the lack of connections observed in the network could be a result of the methodological approach, we calculated different sets of networks (Table 3). Further supporting our hypothesis, irrespective of the approach, the density of the network was low (max. 5.8%), which means that the average number of associations between fungi and bacteria was also low. Furthermore, we evaluated if the microbial associations followed a random pattern by analyzing the frequency distribution of OTU connections and their correlation to the relative OTU abundances. With the exception of the fungal-bacterial association network calculated by FlashWeave (Fig. 4C), all other bacterial-bacterial, fungal-fungal and fungal-bacterial networks OTUs were either unconnected or their degree of association was significantly correlated to their relative abundance, indicating a random distribution of associations (Table 3, Fig. S5). Accordingly, the frequency distribution of the OTUs degree of association (Fig. S6) appeared to follow a binomial distribution rather than a power law distribution, which would be expected if associations were not random (Newman, 2003). This further indicates that not only the microbial communities in the

allotment gardens were structured mainly by stochastic processes, but also emphasizes that the associations of microbes were likely random, mainly depending on the community composition at that particular time in that particular area.

4. Conclusions

Overall, we found that the microbial community composition and the microbial richness of soils from the urban allotment gardens were comparable to that in other urban land-use categories within the city of Santiago. Nevertheless, the high abundance of Ascomycota, the occurrence pattern of other fungi not building strong spatial structures and the lack of correlation to soil physicochemical properties, except for soil pH to some extent, indicate substantial differences with regard to natural ecosystems and other urban greenspaces. To this end, the microbial associations were either absent or occurred between only two microbial units in the studied allotment garden areas, while appearing to be rather scarce and likely random. The consequences, however, of this randomness on the ecosystem functioning might be far-reaching and cannot be fully estimated within the scope of this study. Further research merits toward directly monitoring microbial function over-time by using proteomics or transcriptomics approaches in the face of urban growth and in the context of climate change.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.apsoil.2023.104896.

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