



# Urbanization reduces soil microbial network complexity and stability in the megacity of Shanghai

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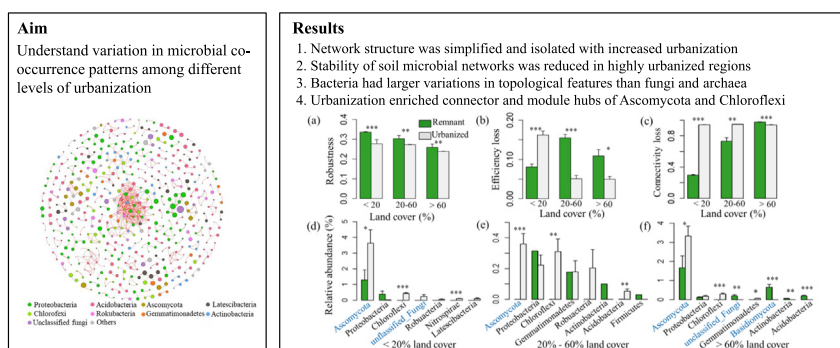
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## HIGHLIGHTS

- We studied microbial networks from 258 sites across an urbanization gradient.
- Network structure was simplified with increased urbanization.
- Stability of soil microbial networks was reduced in highly urbanized regions.
- Bacteria had larger variations in topological features than fungi and archaea.
- Urbanization enriched connector and module hubs of Ascomycota and Chloroflexi.

## GRAPHICAL ABSTRACT



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## ABSTRACT

Urbanization is altering the co-occurrence networks of ecological communities that are critical to maintaining ecosystem functions and services. Soil microbial communities play key roles in various ecosystem processes, but how soil microbial co-occurrence networks respond to urbanization is unclear. Here we analyzed co-occurrence networks in soil archaeal, bacterial, and fungal communities from 258 soil sampling sites across the megacity of Shanghai along large urbanization gradients. We found that topological features of microbial co-occurrence networks were strongly altered by urbanization. In particular, microbial communities in more urbanized land-use and highly impervious land cover had less connected and more isolated network structures. These structural variations were accompanied by the dominance of connectors and module hubs affiliated with the Ascomycota in fungi and Chloroflexi in bacteria, and there were greater losses in efficiency and connectivity in urbanized than in remnant land-use in simulated disturbances. Furthermore, even though soil properties (especially soil pH and organic carbon) were major factors shaping topological features of the microbial networks, urbanization still uniquely explained a proportion of the variability, particularly those describing network connections. These results demonstrate that urbanization has clear direct and indirect effects on microbial networks and provide novel insights into how urbanization alters soil microbial communities.

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## 1. Introduction

Urbanization is accompanied by increased urban land-use and an extensive modification of biotic communities and abiotic environments (Turner et al., 1993; McKinney, 2006). Ecological studies of urbanization effects on biotic communities have mostly focused on biodiversity crisis (e.g., increased biotic homogenization and generalist species) (Aronson et al., 2014; Sol et al., 2017; Merckx and Van Dyck, 2019), but co-occurrence patterns among species can also be altered by urbanization (Barberán et al., 2012; Valiente-Banuet et al., 2015; Fontúrbel et al., 2021). The co-occurrence patterns can reflect direct and indirect interactions between species and the functional role of coexisting species in the environments (Barberán et al., 2012). Documenting the alterations of co-occurrence patterns in response to urbanization is thus critical for preserving ecosystems (Ramalho and Hobbs, 2012; Vacher et al., 2016; Landi et al., 2018). Since soil microbes play a central role in numerous biogeochemical processes and determine ecosystem functioning (Wall et al., 2015), understanding how urbanization affects co-occurrence patterns in soil microbial communities may be particularly relevant for understanding assembly mechanisms and ecological consequences of microbial communities in the human-modified landscapes.

Network analyses have been widely used to investigate co-occurrence patterns among species in complex environments (Rooney et al., 2006; Maruyama et al., 2019; Schneiberg et al., 2020). The co-occurrence patterns can be represented by a network of nodes linked by edges of varying strength, which corresponds to the frequency of paired species presence at a site (Newman, 2003; Araújo et al., 2011). For instance, studies on macroorganisms (e.g., plant-pollinator and animal communities) show that network features shift with urbanization. A high proportion of impervious land cover (hereafter, we use land cover for simplification) may serve as a physical barrier, impeding interactions or connections between species (Unfried et al., 2013; Wenzel et al., 2020; Pantel et al., 2022). Co-occurrence networks in more urbanized areas can also be dominated by generalist species, leading to a structurally less diverse community and consequently unstable networks (Rooney et al., 2006; Rodewald et al., 2014; Landi et al., 2018; Maruyama et al., 2019; Schneiberg et al., 2020). However, how features of microbial co-occurrence networks change with urbanization is not yet clear.

Soil microbial co-occurrence networks can be modified by land-use changes in previous studies (Lupatini et al., 2014; Karimi et al., 2019; Yang et al., 2022). For instance, land-use transitions from natural forests to agroecosystems often relate to reduced edge density (percentage of observed to possible links among taxa within a network) in soil microbial co-occurrence networks (Creamer et al., 2016). Meanwhile, increased crop intensity appears to increase modularity (how strongly taxa are compartmentalized into groups of interacting taxa or modules) (Kay et al., 2018; Karimi et al., 2019). However, urban ecosystems host mosaics of land-uses, and human impacts within the same land-use can be dramatically different due to disturbances from the surrounding urban matrix (Ramalho and Hobbs, 2012). The few studies that have examined the microbial co-occurrence networks in urban ecosystems suggested that the topological feature variations can be linked to the degree of urbanization represented by population density and park age (Wang et al., 2018a; Yan et al., 2021). However, it is unclear how these findings translate to heterogeneous urban environments with multiple land-use categories and varying land cover intensities.

Here, we conducted co-occurrence network analyses using high-throughput sequencing datasets of soil archaea, bacteria, and fungi from 258 sites across the megacity of Shanghai (Fig. S1). The extensive sampling sites span the entirety of rural-urban gradients and include four typical land-use categories across the city, thereby providing an ideal system for understanding how microbial co-occurrence patterns respond to different levels of urbanization. We calculated land cover from high-resolution remotely sensed images. Detailed soil and vegetation parameters from each site were also collected. First, we hypothesized that microbial co-occurrence patterns would respond to urbanization (Ramalho and Hobbs, 2012). We predicted that increasing urbanization would lead to a less connected and more modulated network structure (Creamer et al., 2016; Kay

et al., 2018; Karimi et al., 2019), and urbanization would uniquely explain a proportion of the variation in the network properties (Xu et al., 2014; Wang et al., 2018a; Boeraeve et al., 2019). Second, we hypothesized that variation in co-occurrence patterns in response to increased urbanization would favor certain taxa that are well-adapted to urban environments (Rooney et al., 2006; Yuan et al., 2021). We predicted that there would be directional changes in taxonomy in the microbial co-occurrence networks (Banerjee et al., 2019), and these changes would lead to reduced stability of the co-occurrence networks (Rooney et al., 2006; Yuan et al., 2021), particularly in highly urbanized regions.

## 2. Material and methods

### 2.1. Study sites and sample collection

The study area of Shanghai (120° 52'–122° 12' E, 30° 40'–31° 53' N) is located in eastern China on the southern estuary of the Yangtze River. As a center of finance, trade, and shipping in China, Shanghai has experienced a dramatic urban expansion over the past four decades. The urbanization of the city of Shanghai has dramatically modified the landscape and is reshaping the structure of organisms living therein (Zhao et al., 2016; Wang et al., 2020; Liu et al., 2022). For instance, the urbanized land area increased more than threefold from 1984 to 2014, with an average expansion rate of 11 % annually (Zhao et al., 2016).

Across the city, we established a biodiversity monitoring network based on 3 km regular grids over a 6340.5 km<sup>2</sup> area. Using a sampling density of 3:1 to represent the varied landscape heterogeneity within and outside the city core area (<16 km radius from the city center), 258 monitoring sites (20 m × 50 m) were established (Wang et al., 2020). Soil samples for DNA isolation were obtained from each site by equally mixing soil replicates from three subplots (2 m × 2 m) along the diagonal line from southeast to northwest. For each soil replicates, eight soil physicochemical properties, including pH, soil water content (SWC), total organic carbon (TOC), total nitrogen (TN), total phosphorus (TP), total potassium (TK), and inorganic nitrogen (IN: NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup>) were measured. Plant communities were identified to gain the plant diversity matrices (Table S1). Detailed information concerning soil samplings, storage, physicochemical analysis, and plant survey were reported previously (Liu et al., 2022).

### 2.2. Characterization of urbanization

Land-use and land cover differences were used as proxies for urbanization. Remnant sites were unused or urban green spaces with well-developed vegetation. Ruderal sites were chosen based on a recent history of substantial disturbance to the soil profile, typically associated with construction or demolition activity. Vegetation in ruderal sites was spontaneously established, along with some historically planted plants. Cropland sites included several types of agricultural systems. Turf sites included residential and municipal lawns or woodlands maintained by mowing and/or pruning. We considered ruderal, cropland, and turf sites as types of severely human-impacted (i.e., urbanized) land-uses when compared to remnant sites. Land cover was determined as the proportion of impervious surfaces within a 1 km radius of each site (Liu et al., 2022). It is a good indicator of urbanization concerning the effects of urbanization on microbial communities based on our previous study (Liu et al., 2022).

### 2.3. DNA extraction and sequence processing

Total DNA was extracted from 0.5 g homogenized soil from each site following the Mag-Bind Soil DNA Kit protocol (OMEGA). We then amplified the 16S rRNA gene V3-V4 region of archaea and bacteria using primers 338F and 806R and the ITS1 region in fungi using primers ITS5F and ITS2R following the protocol described by Lee et al. (2012) and Tedersoo et al. (2011). Illumina adaptors were attached to the forward and reverse primer. Each sample was amplified in triplicate. Positive PCR products checked on an agarose gel were purified with GeneJET Gel Extraction Kit

(Thermo Scientific) and mixed in equal density ratios. Sequencing was performed using Illumina Miseq ( $2 \times 300$  bp paired-end reads) platform at Personal Biotechnology Co., Ltd. (Shanghai, China).

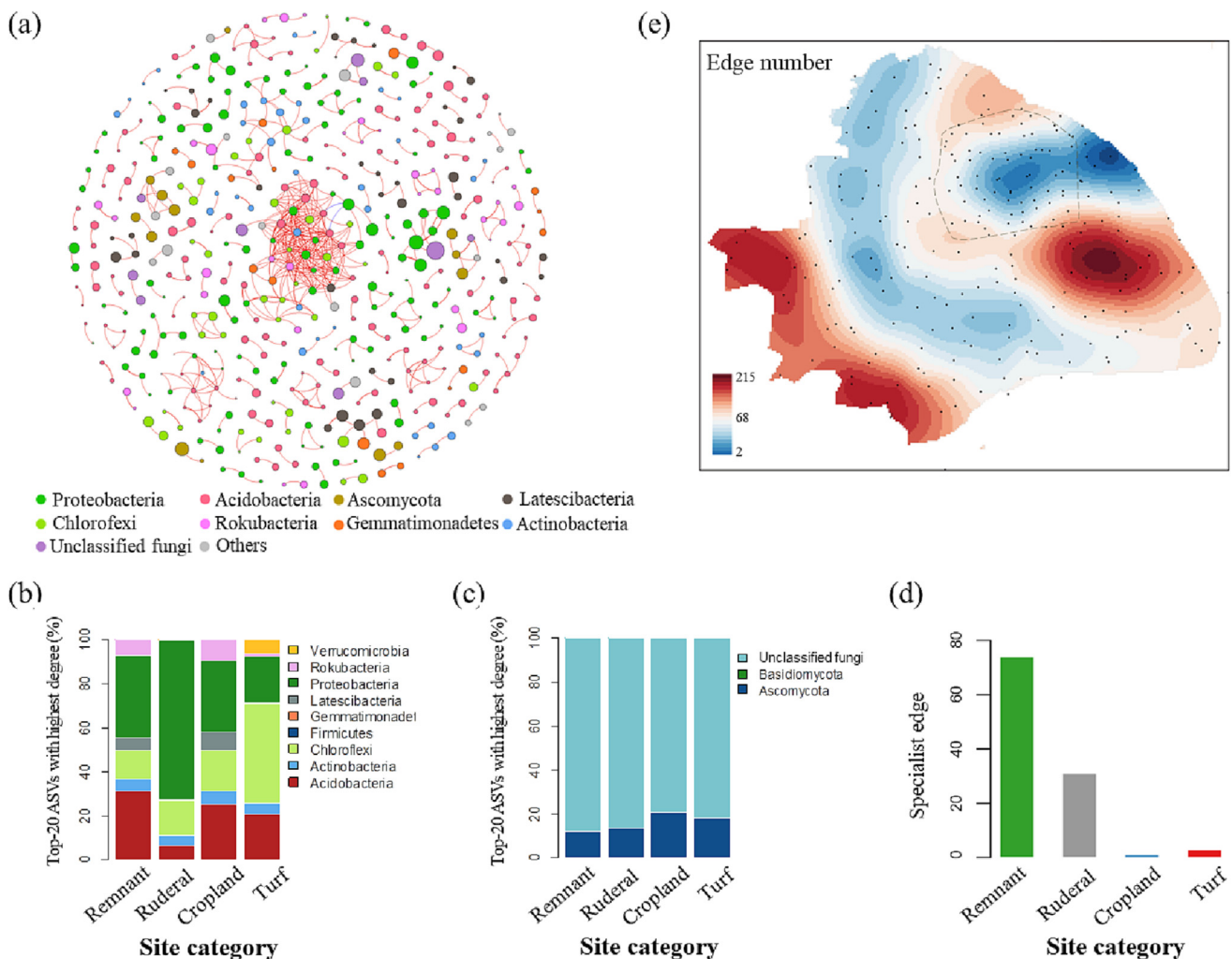
The QIIME2 pipeline (version 2019.10) and the DADA2 plugin with default settings were used to process the raw reads (Bolyen et al., 2019). Taxonomy was assigned to representative ASV (amplicon sequence variants) using SILVA 132 (Quast et al., 2012) for archaea and bacteria and UNITE v8.0 database (Abarenkov et al., 2010) for fungi. Singletons (ASVs with only one sequence read across all samples) were excluded from the data sets.

#### 2.4. Network construction and topological feature analysis

Microbial co-occurrence networks were constructed based on the 16S rRNA and ITS gene amplicon sequencing data. We filtered ASVs that appeared in less than five sites and with less than ten sequences across 258 samples to remove poorly represented ASVs (Barberán et al., 2012; Wang et al., 2018b). After filtering, 2396 bacterial and 867 fungal ASVs were retained for network analysis. They belonged to four land-use categories, including 49 remnant, 18 ruderal, 95 cropland, and 96 turf sites (Fig. S1). The Sparse Correlations for Compositional data (SparCC) approach was used to treat possible compositionality bias in the data (Friedman and Alm, 2012). The bootstrapped estimates of correlation coefficients and  $P$  values were obtained by using the SparCC algorithm with the R package

SpiecEasi (Friedman and Alm, 2012; Kurtz et al., 2015). The cutoff of correlation coefficients was determined as 0.6 through random matrix theory-based methods using R package RMThreshold (Luo et al., 2006). Various network topological features (Table S2) were calculated with the R package igraph (Csardi and Nepusz, 2006). Nodes were further classified as peripheral, connectors, module hubs, and network hubs by comparing the within-module degree and among-module degree (Guimerà and Amaral, 2005). We visualized the network with Gephi (<http://gephi.github.io/>). Nodes that appeared in only one land-use category were defined as land-use specific. Land-use specific edges were assessed by omission score as described by Lima-Mendez et al. (2015).

To statistically evaluate urbanization impacts on the stability of microbial networks, networks were constructed from remnant and urbanized land-uses (including ruderal, cropland, and turf sites) along a land-cover gradient (<20 %, 20–60 %, and >60 %). The cutoff of correlation coefficients was determined as 0.65 through random matrix theory-based methods (Luo et al., 2006). After network construction, network-level topological features, network randomization, network visualization, and nodes' role in the networks were performed (Vázquez et al., 2009). Connectors and hubs strongly support the structure of ecological networks (Faust et al., 2015; Toju et al., 2018). Thus, we calculated the relative abundance of the connectors and hubs in each network at the phylum level. We calculated multiple stability indices. Network robustness measured the proportion



**Fig. 1.** The co-occurrence network of urban soil microbes. a, Visualization of constructed co-occurrence network. The size of each node is proportional to the relative abundance of each ASV. A red link indicates positive correlation and a blue link indicates negative correlation. b–c, Top 20 ASVs with the highest degree included in the network for (b) bacteria and (c) fungi. The relative abundance of the top 20 bacterial ASVs with the highest degree varied across four land-uses ( $P < 0.05$ ). d, Number of edges significantly associated with different land-use categories. e, The spatial distribution of network edge number (connectivity) across the city. Points represent sampling locations. Circle on the map shows the highly urbanized core area of Shanghai city (<16 km radius from the city center).

of species that remained in the network after the random removal of network species or targeted removal of module hubs. Efficiency loss was used to measure the maximum decrease in efficiency (the number of edges in the shortest path over all pairs of nodes) loss when a single node was deleted from the network (Yuan et al., 2021). Connectivity loss measured loss of edges number or the total number of links under degree and betweenness-based removal of 10 % of nodes using R package NetSwan (<https://cran.r-project.org/web/packages/NetSwan/>).

## 2.5. Statistical analysis

We generated sub-networks for each soil sample by extracting ASVs associated with specific samples and all edges among them in the co-occurrence network from the 258 sites. To extend our results beyond the 258 sites directly assayed, we also predicted spatial distribution of the network-level topological features across the city using Ordinary Kriging interpolation implemented in R package gstat (Pebesma, 2004). We then grouped each sub-network by land-use category and used Kruskal-Wallis rank-sum test to determine the different topological features. The relationship between topological features and land cover was estimated by linear regression. Differences in network topological features, mean relative abundance of taxa, and stability between remnant sites and urbanized land-uses were examined by two-sided *t*-test. To determine the importance of environmental factors (Table S1), including land-use differences (land-use category and land cover), soil properties (pH, SWC, TOC, TP, TK, IN, C/N), plant diversity (richness and abundance of native and exotic woody and herbaceous plants) for network-level topological features, canonical variance partitioning was used (Karimi et al., 2018). The principal coordinates of neighbor matrix (PCNM) approach was used to describe the spatial relationship between samples (Dray et al., 2006), and only PCNMs with a significant Moran index were selected for canonical variance partitioning ( $P < 0.05$ ). All variables were standardized to guarantee approximated Gaussian and homoscedastic residual distribution of the models. Forward selection using the function ordistep in R package vegan (Oksanen et al., 2010), starting from a full redundancy analysis (RDA) model, was first applied to select significant variables. The explained variance (independent and interactions between the significant variables) was determined by

canonical variation partitioning and the adjusted  $R^2$  with redundancy analysis (Ramette, 2007). Statistical significance of the independent effects was assessed from 1000 permutations of the final model. The variance explained by each group of variables was computed as the sum of the variance explained by all independent effects.

## 3. Results

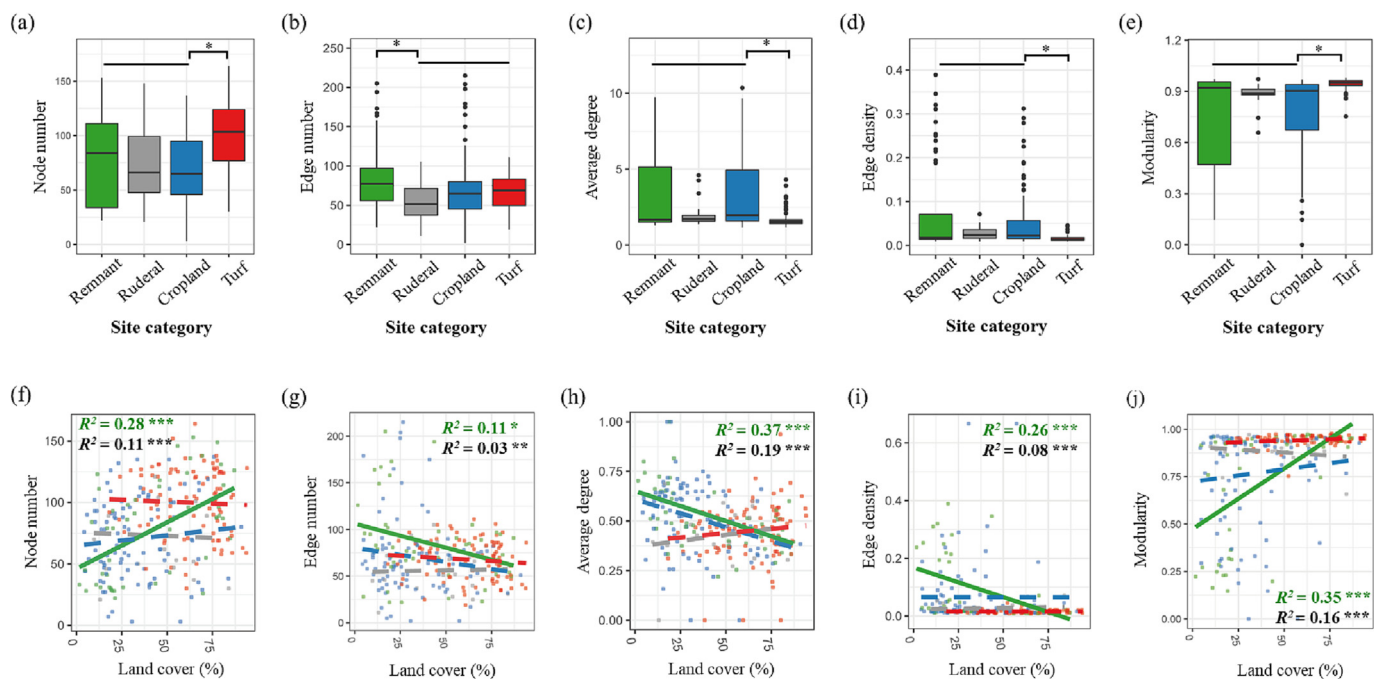
### 3.1. Topological features of co-occurrence network

The co-occurrence network across 258 sites captured 582 associations (580 positive and two negative edges) among 455 (94.2 %) bacterial and 28 (5.8 %) fungal ASVs (Fig. 1a). No archaea were retained in the network. The network followed a scale-free distribution where most nodes had only a few connections and only a few nodes possessed a relatively high number of links, as indicated by the curve of node degree fitting the power-law distribution well (Fig. S2). Meanwhile, the network had a short average path length (2.24 vs.  $6.67 \pm 0.14$ ) but high average transitivity (0.59 vs.  $0.003 \pm 0.002$ ) and modularity (0.83 vs.  $0.71 \pm 0.007$ ) compared to the random network (Table S3). These results suggested a non-random co-occurrence pattern of the soil microbial communities across the city.

The nodes' roles and topological features did not show land-use specific variation. Of the 483 nodes, none of them could be classified as network hubs or connectors, and only two were module hubs (Fig. S3), from unclassified Chloroflexi (relative abundance = 0.238 %) and Acidobacteria (relative abundance = 0.034 %), respectively. Node-level topological features did not show land-use specific variation, except for the top 20 bacterial ASVs with the highest degree, betweenness centrality, closeness centrality, and transitivity (Fig. 1b–c and Figs. S4–S5). Of the 582 edges, 19.1 % were land-use specific, including 74 edges in remnant, 32 edges in ruderal, two in cropland, and three in turf sites (Fig. 1d).

### 3.2. Land-use and land cover differentiated topological features

Most of the examined topological features were distinctly impacted by land-use categories. Predicted spatial distribution maps of the network topological features showed variations correlated with the distances to the



**Fig. 2.** Land-use and land cover changed network topological features, including node number (a, f), edge number (b, g), average degree (c, h), edge density (d, i), and modularity (e, j). Different colors represent different land-use categories: green, remnant; gray, ruderal; blue, cropland; red, turf. Solid lines in (f–j) indicate significant linear associations between topological features and land cover of remnant sites and all land-uses (dark): \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ .

**Table 1**

Topological features of microbial networks and their random networks from remnant and urbanized sites along three levels of land cover intensity (&lt;20 %, 20–60 %, and &gt;60 %).

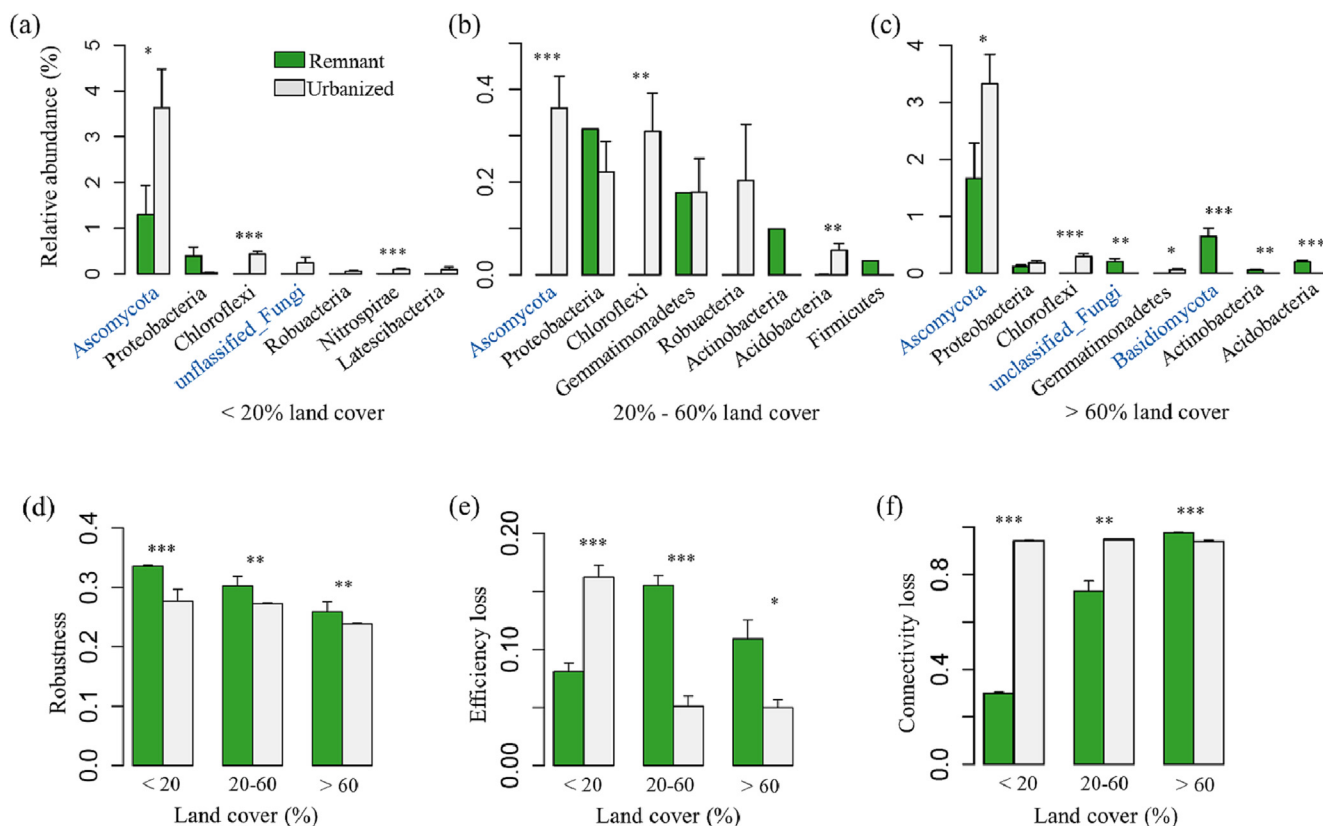
	Empirical networks							Random networks		
	Node number	Edge number	Average degree	Edge density	Average path distance	Transitivity	Modularity	Average path distance	Transitivity	Modularity
Remnant <20 %	348	<b>1112</b>	<b>6.391</b>	<b>0.018</b>	<b>4.610</b>	<b>0.357</b>	<b>0.619</b>	3.288 ± 0.034	0.028 ± 0.006	0.342 ± 0.005
Urbanized <20 %	394	<b>482</b>	<b>2.447</b>	<b>0.006</b>	<b>9.611</b>	<b>0.192</b>	<b>0.877</b>	5.745 ± 0.111	0.001 ± 0.002	0.723 ± 0.007
Remnant 20–60 %	<b>438</b>	<b>862</b>	<b>3.936</b>	<b>0.009</b>	<b>5.694</b>	<b>0.236</b>	<b>0.715</b>	3.941 ± 0.052	0.012 ± 0.004	0.493 ± 0.006
Urbanized 20–60 %	<b>535</b>	<b>776</b>	<b>2.901</b>	<b>0.005</b>	<b>7.078</b>	<b>0.210</b>	<b>0.813</b>	5.087 ± 0.069	0.002 ± 0.002	0.644 ± 0.006
Remnant >60 %	597	<b>708</b>	<b>2.372</b>	0.004	<b>10.629</b>	<b>0.141</b>	<b>0.894</b>	6.456 ± 0.118	0.001 ± 0.002	0.754 ± 0.005
Urbanized >60 %	625	<b>882</b>	<b>2.822</b>	0.005	<b>7.059</b>	<b>0.178</b>	<b>0.781</b>	5.036 ± 0.078	0.003 ± 0.002	0.657 ± 0.005

Significant differences (two-sided *t*-test,  $P < 0.001$ ) between remnant and urbanized sites in network topological features are bolded.

city center (Fig. 1e and Fig. S6). The post-hoc Dunn tests showed that the edge number was significantly higher in remnant sites than in other land-uses (Fig. 2b,  $P < 0.05$ ). Meanwhile, turf sites tended to have a higher node number, modularity, and cluster number but lower average degree, edge density, average path length, and closeness centrality than other land-uses (Fig. 2a–e and Fig. S7, Dunn's test,  $P < 0.05$ ). Further, the node number, cluster number, and modularity significantly increased, but the average degree, edge density, average path length, and closeness centrality reduced with increasing land cover (Fig. 2f–j and Fig. S8, Dunn's test,  $P < 0.05$ ).

Consistent with the results from 258 sites, microbial co-occurrence networks were generally less connected and more modular in urbanized than in remnant land-use, except for modularity in sites with >60 % land cover (Table 1 and Fig. S9). We found that over 57 % phyla were enriched in networks constructed from urbanized land-use compared to networks from

remnant sites. Connectors and module hubs belonging to Ascomycota in fungi and Chloroflexi in bacteria showed a consistent trend of increased proportion in urbanized than in remnant land-use (Fig. 3a–c and Table S4, Welch's *t*-test,  $P < 0.05$ ). Under random species removal, the networks in remnant sites had significantly higher robustness than in urbanized land-uses (Fig. 3d,  $P < 0.01$ ). Network robustness under the target removal of module hubs could not be calculated due to limited module hubs (Table S4). Meanwhile, the maximum efficiency loss in microbial networks was on average higher in remnant sites ( $0.12 \pm 0.011$ ) than in urbanized land-uses ( $0.08 \pm 0.007$ ) but was highest in urbanized land-uses with <20 % land cover and decreased significantly with increased land cover ( $r^2 = 0.33$ ,  $P = 0.001$ ; Fig. 3e). Further, the connectivity loss induced by simulated species elimination (degree and betweenness based) was on average lower in remnant sites ( $0.67 \pm 0.007$  and  $0.68 \pm 0.016$ , respectively) than in urbanized land-uses ( $0.94 \pm 0.001$  and  $0.96 \pm 0.001$ ) and



**Fig. 3.** Land-use and land cover changed composition and stability of networks. a–c, The relative abundance of connectors and module hubs at phylum-level between remnant and urbanized sites among three levels of land cover. Names of bacterial and fungal phyla were colored in black and blue, respectively. d–f, Network stability assessed by robustness, efficiency loss and connectivity loss for microbial communities between remnant and urbanized sites among three levels of land cover. Robustness measured as the proportion of taxa that remained in the networks after 50 % of the taxa was randomly removed. Efficiency loss measured the maximum decrease in efficiency loss when a single node was deleted from the network. Connectivity loss measured loss of edge number under degree based removal of 10 % nodes. The bars represent standard errors. Each error bar corresponds to the standard deviation of 100 repetitions of the simulation in d–f. Significant comparisons (two-sided *t*-test) between remnant and urban land-uses are indicated by \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ .

increased significantly with increased land cover in remnant sites ( $r^2 = 0.95, P < 0.001$  and  $r^2 = 0.98, P < 0.001$ ) (Fig. 3f and Fig. S10).

### 3.3. Environmental effects on network topological features

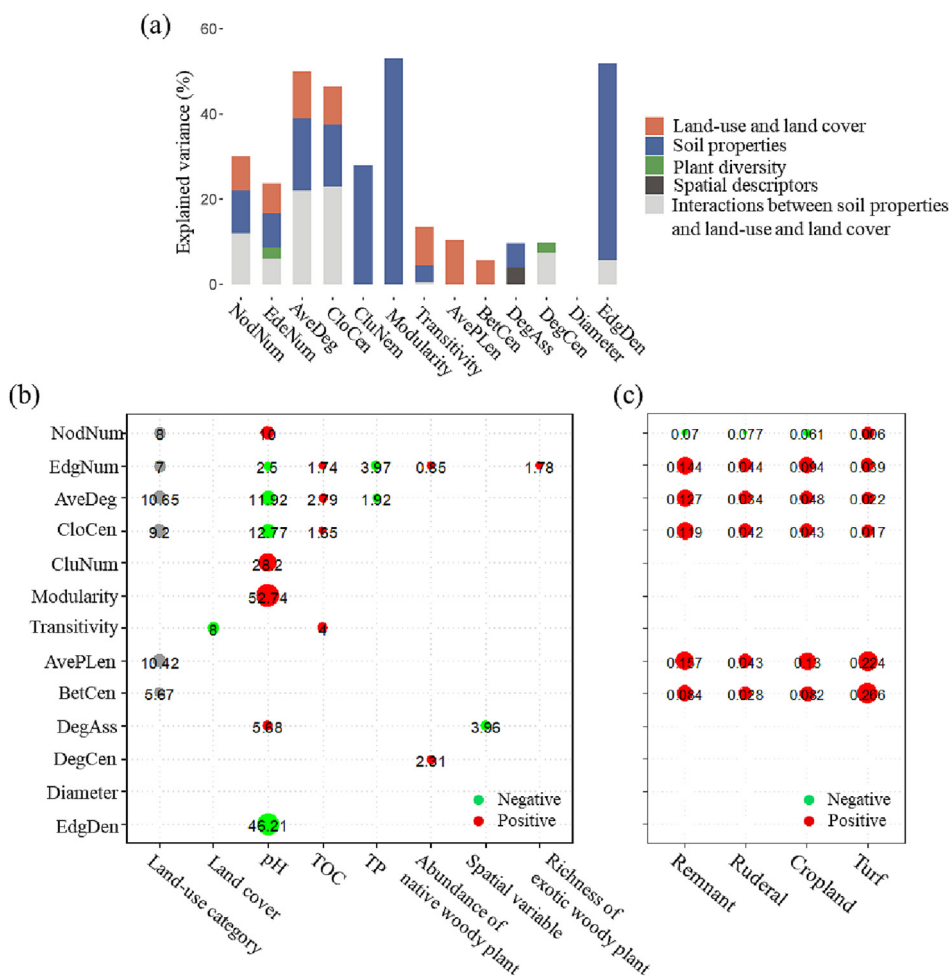
The results showed that soil properties were major factors shaping topological features of the microbial networks, but urbanization still uniquely explained a proportion of the variability, particularly those describing network connections. The total explained variance in network topological features ranged from 5.7 % (betweenness centrality) to 52.7 % (modularity) (Fig. 4a). Land-use and land cover uniquely explained 5.7 %–10.02 % of the variation in network topological features, and also contributed to 0 %–23.05 % of variance through interactions with soil properties. Soil properties independently explained 4.0 %–52.7 % of the variation in the topological features and were the major factor for modularity, edge density, and cluster number (explaining 52.7 %, 46.2 %, and 28.2 % of the variance). Plant diversity significantly contributed to the variation in edge number (1.8 % of the variance) and degree centralization (2.3 %) across samples. Spatial variables contribute to 4.0 % of the variance in degree assortativity.

The contribution and effect of examined environmental variables can be ranked as soil pH > land-use category > TOC > land cover > TP > spatial variable > abundance of native woody plant > richness of exotic woody plant (Fig. 4b and Table S3). Soil pH was positively related to modularity,

node number, cluster number, and degree assortativity and negatively related to edge number, average degree, and closeness centrality. Land-use differences significantly influenced network topological features, including node number, edge number, average degree, closeness centrality, average path length, and betweenness centrality. Meanwhile, the effects were stronger in remnant and turf sites than in ruderal and cropland land-use categories. TOC showed positive influences on edge number, average nearest neighbor degree, closeness centrality, and transitivity. TP negatively influenced the edge number and average nearest neighbor degree. An increase in the richness of exotic woody plants and abundance of native woody plants contributed to high edge number and degree centralization.

### 4. Discussion

The co-occurrence network analyses of soil archaeal, bacterial, and fungal communities along the rural-urban gradient allow for predicting changes in microbial co-occurrence patterns in relation to the degree of urbanization. Here, using 258 sites across entire rural-urban gradients, our results show that urbanization impacted the topological features of bacterial and fungal co-occurrence networks (Fig. 2 and Figs. S7–8). Topological features, including node number, edge number, average degree, edge density, average path length, closeness centrality, cluster number, and modularity, were varied among land-use types and showed a linear



**Fig. 4.** Relative contribution of environmental and spatial factors to network topological features. The explained variance corresponds to the sum of adjusted  $R^2$  values of the significant variables ( $P < 0.05$ ) within each group in (a). The explained variance represents the significant contribution of each variable in (b–c). Missing values indicate that no variable of the related group was retained in the model. The colors depict the direction of the standardized partial regression coefficients: red, positive effect; green, negative effect. The size of the circle and values indicate the percentage of variance explained by each variable in (b) and the coefficient of standardized partial regression of each land-use in (c). The coefficients are relative to a reference level grouping of randomly selected 20 samples from the dataset in (c). Abbreviations of variables are as follows: NodNum, node number; EdgNum, edge number; AveDeg, average degree; CloCen, closeness centrality; CluNum, cluster number; AvePLen, average path length; BetCen, betweenness centrality; DegAss, degree assortativity; DegCen, degree centralization; EdgDen, edge density.

relationship with increased land cover, particularly in remnant sites (Fig. 2 and Figs. S7–8). Urbanization effects were also evidenced by spatial patterns of network topology across the city (Fig. 1e and Fig. S6). In our analysis, urbanization combined with edaphic properties was strongly associated with the variation in topological features of soil bacterial and fungal co-occurrence networks (Fig. 4).

Consistent with our expectations, we found that topological features of microbial co-occurrence networks were less complex in more urbanized sites and areas. The topology of co-occurrence networks provides information about direct and indirect relationships between microbes and their interactions with environments (Barberán et al., 2012; Guseva et al., 2022). The results of low edge number, edge density, and average degree but high modularity and cluster number in more urbanized sites and areas (Fig. 2 and Figs. S6–8) thus suggest that microbial communities become less connected and more isolated with increasing urbanization. Similar patterns were also evident after grouping sites into urbanized and remnant land-use. Yet, already isolated microbial communities may not be impacted by increased urbanization (e.g., urbanized land-use with >60 % land cover in Table 1). One explanation for the less connected and more isolated microbial communities is the weak spatial connectivity between soil ecological niches in disturbed soil (Bissett et al., 2013; Zappellini et al., 2015; de Vries et al., 2018; Xue et al., 2020). In urbanized areas, human activities and management practices can disturb soil physical and chemical conditions and thus create fragmented niches for microbes to fill (Pouyat et al., 2007; Delbecque et al., 2022). Another potential explanation is related to the enriched taxa in urban soil. For instance, we revealed the consistent dominance of connectors and module hubs belonging to Chloroflexi and Ascomycota under urbanized land-use (Fig. 3a–c). Chloroflexi in bacteria and Ascomycota in fungi are copiotrophic microorganisms that are adapted to high resource availability (Thompson and Kao-Kniffin, 2019). Members of the Ascomycota can also form symbiotic or parasitic relationships with plants and animals (Egidi et al., 2019). Those taxa are more self-sufficient in terms of cooperated degradation and thus may reduce the direct and indirect interactions or associations within and between microbial communities (Morris et al., 2013).

Assessing the efficiency and connectivity loss of microbial co-occurrence networks under simulated disturbance provides valuable insights into the stability of the systems (Thébault and Fontaine, 2010; Wei et al., 2015). Although most previous studies have not directly examined stability, a less connected network structure is suggested to reduce stability of the microbial networks (Mougi and Kondoh, 2012; Karimi et al., 2017). More importantly, an unstable network structure is believed to decrease the number of functions provided by the ecological communities (Thébault and Fontaine, 2010; Morriën et al., 2017). The predominance of positive edges in the microbial network created from the 258 sites indicated cooperation and/or affiliation-dominated relationships between microbial taxa and unstable microbial networks in the urban ecosystem (Coyte et al., 2015). Indeed, we found that the efficiency and connectivity loss under simulated species elimination were higher in urbanized than in remnant land-use (Fig. 3d–f). These results are consistent with our expectations and confirm the negative effects of urbanization on the stability of the microbial co-occurrence networks. Moreover, our results indicate that microbial co-occurrence networks in highly urbanized sites or areas may be stable to disturbances, potentially due to the highly isolated network structure (e.g., networks in the urbanized land-use in Fig. 3d–f), but microbial co-occurrence networks with more complicated interaction or association relationships such as those in remnant sites may be sensitive to disturbances (Karimi et al., 2017).

We observed clear differences in microbial response to urbanization at both the kingdom and phylum levels. Previous studies have shown that archaea appear to be more abundant in relatively acid soils (Leininger et al., 2006; Erguder et al., 2010). Soils throughout the city of Shanghai are generally alkaline (pH = 7.74 ± 0.63 in Table S1). Thus, the survival and fitness of archaea may be limited in these soils, as evidenced by the fact that most archaeal ASVs were not represented by more than ten sequences or appeared in more than five sites. Bacterial network features showed a

more sensitive response to urbanization compared to fungi (Fig. 1b–c and Fig. S5). Although the less accurate assignment of fungal ASVs may have influenced the results, the more sensitive response of bacteria to disturbances than fungi was consistent with other studies (de Vries et al., 2018; Nakayama et al., 2019; Osburn et al., 2021). For example, co-occurrence networks in conditions such as drought (de Vries et al., 2018; Osburn et al., 2021) and management practices like artificial plantation (Nakayama et al., 2019) and agricultural intensification (Banerjee et al., 2019) show greater variation in edge number and modularity in bacterial networks than fungal networks. Higher dispersal capability may facilitate bacterial relocation and their coexistence in fragmented soil environments in the urban ecosystem (Pouyat et al., 2007; Schmidt et al., 2014). Despite the lower dispersal capability of fungi versus bacteria, hyphal networks that facilitate resource acquisition may allow fungi to be less sensitive to environmental change than bacteria (Guhr et al., 2015).

We identified that soil properties, particularly soil pH, total organic carbon, and total phosphorus, were the main factors that explained the topological variations in microbial co-occurrence networks. The high variability in soil physicochemical properties could be explained by urban management practices like the application of fertilizer, irrigation for salt washing, and the use of amendments (Pouyat et al., 2007), resulting in significant dissimilarities between the clusters and, thus, the isolated structure of the networks (Blondel et al., 2008; Wang et al., 2018b). Soil pH was a key driver in shaping microbial communities in natural ecosystems (Fierer and Jackson, 2006). Total organic carbon and total phosphorus were the important soil nutrients determining soil microbial community structure (Cui et al., 2019; Ramírez et al., 2020). We have also observed that the microbial diversity in Shanghai was mainly controlled by soil pH, total organic carbon, and total phosphorus (Liu et al., 2022). These previous studies demonstrated that the soil pH, total organic carbon, and total phosphorus are important for shaping the compositions of microbial communities, whereas our results here supported it at the level of microbial co-occurrences networks. We also found that higher plant community diversity, either by introduction of exotic woody species or by increasing the abundance of native woody plants, appears to promote microbial co-occurrence (Liu et al., 2022). Such a relationship could be explained by the diverse ways that plants affect belowground microbes, either through direct interactions (e.g., plant-mycorrhizal fungi, plant-pathogenic microbes) or by indirect mediation of soil physicochemical properties via litterfall and root exudates (Hooper et al., 2000; Liu et al., 2020).

## 5. Conclusion

Although the co-occurrence relationships in this study were optimized to remove indirect associations, the constructed co-occurrence networks do not prove direct microbial interactions (Blanchet et al., 2020). Similar to macroorganisms, the detected co-occurrence relationships are the results of community assembly, which is influenced by processes such as environmental filtering, species interactions, or stochastic processes (Barberán et al., 2015; Faust, 2021). Instead of representing direct interactions, co-occurrence relationships provide information about the overall organization of the system and are suggested to be sensitive to a wide range of disturbances (Karimi et al., 2017). The comparison of co-occurrence networks along urbanization gradients, thus, provides complementary information about the state and stability of ecological communities and the ecosystem under anthropogenic disturbances in urban environments (Deng et al., 2012; Karimi et al., 2017; Gao et al., 2022; Guseva et al., 2022).

With this work we have demonstrated that urbanization played an important role in structuring soil bacterial and fungal co-occurrence networks. Urbanization can lead to reduced complexity and stability in microbial co-occurrence networks. Moreover, the structural alteration was associated with deterministic variation in the taxonomic identities of networks. Given the central role of microbes in soil functioning, the globally accelerating urbanization is expected to have profound impacts on ecosystems. Further studies linking these variations to changes in functional potential are essential to deeply understand microbial interactions and how such

variations contribute to ecosystem functioning and services in human-dominated ecosystems.

### CRedit authorship contribution statement

Lan Liu: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing - original draft, Writing - review & editing, Funding acquisition. Zhaochen Zhang: Investigation. Xin Wang: Investigation. Ran Zhang: Investigation. Meng Wang: Investigation. Nina Wurzbürger: Methodology, Writing. Junxiang Li: Methodology, Writing - review & editing. Jian Zhang: Methodology, Writing - review & editing, Funding acquisition, Project administration, Supervision.

### Data availability

The data are available from the National Center for Biotechnology Information (NCBI) Sequence Read Archive (<http://trace.ncbi.nlm.nih.gov/Traces/sra/>) under the accession numbers of PRJNA635542.

### 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.scitotenv.2023.164915>.

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