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Impact of urbanization on soil microbial diversity and composition in the megacity of Shanghai

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Abstract

Urbanization alters the physicochemical environment on an unprecedented scale and strongly affects biodiversity. How urbanization affects the biodiversity of soil microbial communities, especially in large cities, however, is poorly known. We investigated soil microbial communities from 258 sites covering a variety of environmental gradients in the megacity of Shanghai, China, to determine the impact of urbanization on soil microbial biodiversity. Using the distance to city centre, urbanized land cover, and road density as three proxies to characterize the levels of urbanization, we revealed that increased urbanization was associated with slightly homogenized communities of prokaryotes, total fungi, and arbuscular mycorrhizal fungi but not ectomycorrhizal fungi. The richness of soil prokaryotes and total fungi was weakly but positively related to urbanization as well. For the abundance of microbial phylotypes along urban gradients, we observed synchronous increases and decreases of many phylotypes at relatively high and low urbanization levels, respectively. Further, urbanization explained an independent part of microbial variances in richness and community composition, although the contribution of soil properties in explaining the variances was generally larger than that of urbanization. Together, this work provides evidence for the influences of urbanization on the biodiversity of soil microbes and highlights the importance of considering taxa and the level of urbanization to assess the impacts of urbanization on biodiversity.

KEYWORDS
indicator microbe, microbial biogeography, plant–microbial linkages, urban biodiversity, urbanization

1 | INTRODUCTION

Urban spaces are expanding at an unprecedented rate, and the transition of human populations from rural to urban areas increases anthropogenic modification of the urban environment, including land use change, development of transportation networks, and management of urban soils and plants (Antrop, 2004; Hoyt, 1939; McDonnell & Pickett, 1990). The impact of urbanization on species distributions and community composition is expected to cause diversity loss and biotic homogenization based on studies on macroorganisms (e.g., plants (Aronson et al., 2014), birds (Sol et al., 2017), and arthropods (Merckx & Van Dyck, 2019). However, how soil microbial diversity and community composition respond to urbanization remains poorly understood and controversial (Schmidt et al., 2017; Wang et al., 2018), despite the critical role that soil microbes play in biogeochemical cycling (Falkowski et al., 2008), pollutant detoxification (Boetius, 2019), urban air quality (Bowers et al., 2011), and human health (Mills et al., 2017).

Quantifying changes in diversity and community composition of soil microbes in relation to urban gradients provide an approach for understanding the ecosystem consequences of urbanization (McDonnell & Pickett, 1990). Using land use types to represent a range of human disturbances and management impacts, a few studies have reported similar or converged microbial communities or functional guilds (e.g., mycorrhizal fungi) in response to the intensive human-impacted land use (e.g., residential land) (Schmidt et al., 2017; Wang et al., 2018). Using rural–urban gradient to reflect the differences of population density and its induced environmental change, some studies have documented higher soil microbial diversity in the urban area (Wang et al., 2017; Yan et al., 2016), while others reported little or no difference between urban and rural soils (Boeraeve et al., 2019; Docherty et al., 2018; Huot et al., 2017). Although those analyses are important in understanding the highly complex human-modified ecosystem, categorical or quantitative metrics of urbanization may carry different meanings in different studies or regions (Theobald, 2004), hindering a synthesis of results. Meanwhile, the same land use type in city centre and rural areas may have different impacts due to the modulating effects of the surrounding environment. For instance, land use and road density of surrounding areas may influence soil microbes by affecting habitat fragmentation and microbial dispersal limitation (Ramalho & Hobbs, 2012; Reese et al., 2016). In addition, urban environments could have more significant influences on some microbial phylotypes (so-called indicators), leading to significant changes in their abundance and frequency along urban gradients. Recent studies in stream ecosystems have documented the loss of microbial indicators with increased urbanization (Martin et al., 2018; Simonin et al., 2019). However, most studies on soil microbes have involved a small number of sampling sites (e.g., Hu et al., 2018; Lumini et al., 2010), limiting our understanding of how soil microbes respond to the complex urban environment.

Extensive physical, chemical, and biotic changes in urban areas can have confounding effects on soil microbes. Human effects can be both intentional (e.g., fertilization and irrigation) and unintentional (e.g., atmospheric deposition of organic chemicals), which could result in highly variable soil physical and chemical properties in urban (Wall et al., 2015). Soil physicochemical properties can directly regulate the diversity and community composition of soil microbes (Fierer & Jackson, 2006; Lauber et al., 2008; Wang et al., 2018). Moreover, recent studies suggest that soil management enriches copiotrophic microorganisms (e.g., *Proteobacteria* in bacteria and *Ascomycotabacteria* in fungi) adapted to higher resource availability (Thompson & Kao-Kniffin, 2019). Urban plant communities are characterized by relatively homogeneous composition, with a high proportion of exotic species (Aronson et al., 2014). Urban plants could affect belowground microbes by direct interaction with microbes (e.g., plant-mycorrhizal fungi) and by indirect mediation of soil physicochemical properties via litterfall and root exudates (Hooper et al., 2000; Liu et al., 2020). However, these effects have been largely unexplored in urban ecosystems.

As the economic capital in China and one of the most populated megacities in the world, Shanghai has witnessed rapid urbanization on an unprecedented scale in the last four decades, which dramatically changed its landscape and soil environment (Li et al., 2013). With a residential population of over 24 million and a land area of 6340.5 km^2, urbanization-associated changes have placed important and diverse effects on urban ecosystems and the organisms living therein (Cui & Shi, 2012). To evaluate whether or not and how urbanization influences the diversity and community composition of soil microbes, we conducted a large sampling effort across 258 sites in Shanghai City (~5200 km^2, Figure 1). Meanwhile, we quantified the level of urbanization using three metrics, including the distance between each site and the city centre, urbanized land coverage, and road density surrounding each site. The detailed information on soil properties and vascular plant diversity at each site was collected. The main objectives of this study were: (a) characterize the variations in genetic diversity and composition of soil microbes in relation to the three urbanization indices, which reflected different aspects of anthropogenic modification of the urban environments; (b) identify how soil microbial indicators respond to urbanization; and (c) evaluate the direct and indirect effects of urbanization on diversity and composition of soil microbial communities.

2 | MATERIALS AND METHODS

2.1 | Study sites

Shanghai City (120°52′–122°12′ E, 30° 40′–31°53′ N) is located in eastern China at the southern estuary of the Yangtze River. As the central city of the Yangtze River Delta urban agglomeration, Shanghai has experienced a dramatic urban expansion in the past four decades. The urbanized land area increased more than threefold from 1984 to 2014, with a nearly 11% expansion rates annually on average (Zhao et al., 2016). The sprawling of the urban area is characterized by increasing major roads and urban land uses (Gao & Wu, 2005).

To better represent the spatial and environmental heterogeneity, a dual-density, tessellation-stratified random sampling design was

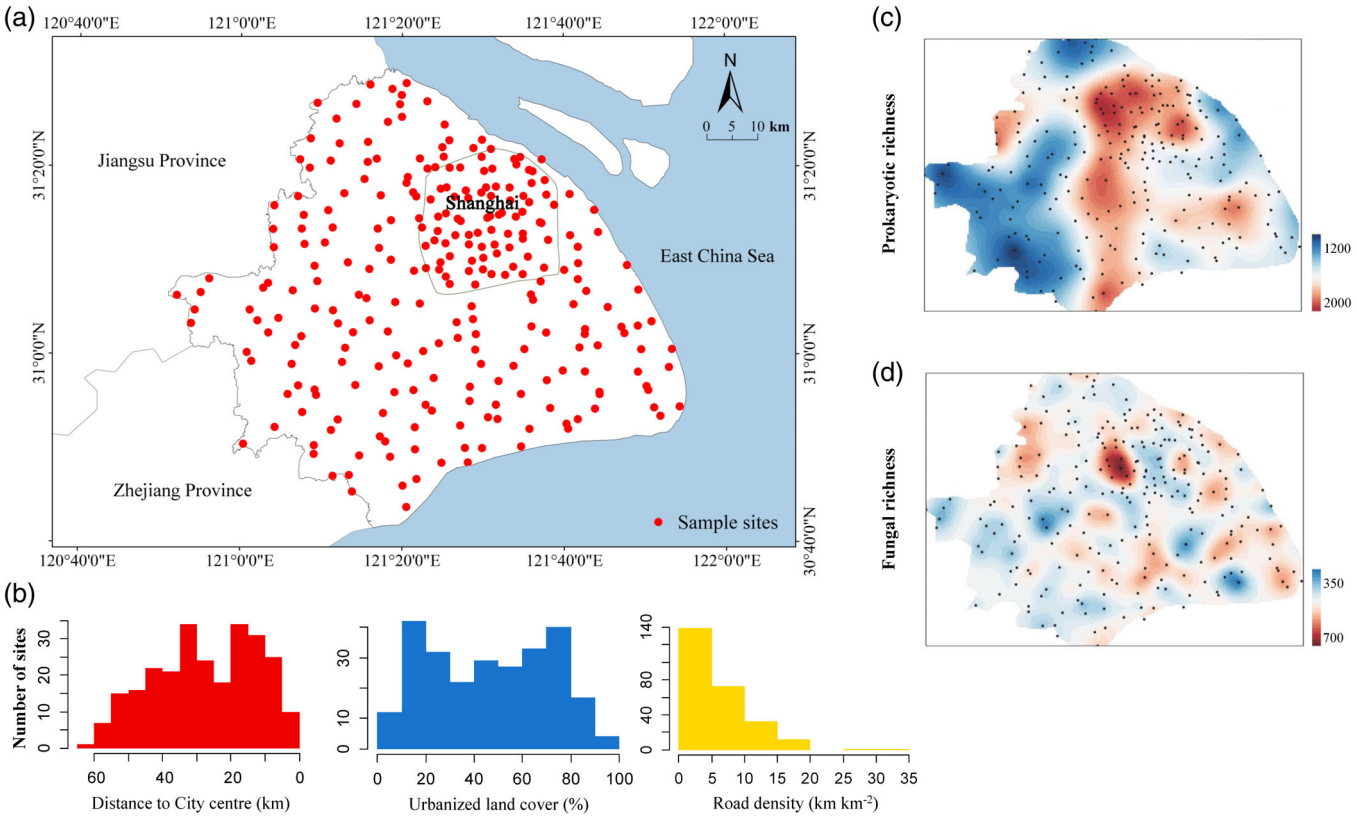

FIGURE 1 Distribution of sample sites and richness of soil microbes. (a) The geographic distribution of 258 sampling sites across Shanghai City (excluding Chongming Islands). (b) Histograms representing the distribution of the 258 sites along three urbanization metrics. Distance to the City centre, urbanized land cover, and road density were used as proxies to characterize the level of urbanization. (c-d) Maps showing richness distribution of prokaryotic and total fungal communities. The richness is the number of different phylotypes found in each sample at the ASV level. Ordinary kriging interpolation was used for microbial richness mapping [Colour figure can be viewed at wileyonlinelibrary.com]

adopted (Hope et al., 2003). We divided the Shanghai city (except for Chongming Islands) into 861 3 km × 3 km grids. A sampling density of 3:1 was used to sample the grids inside and outside the highly urbanized areas (Figure 1a; < 16 km radius from the Shanghai People's Square). Within each selected grid, a modified Whittaker sample site with the size of 20 m × 50 m was settled on a randomly selected greenspace (Wang et al., 2020). In total, 258 sampling sites were established, ranging from 45 km in latitude and 63 km in longitude (Figure 1a). The geographic locations of all sites were recorded using a centimetre-level differential GPS (Trimble Geo 7x).

The modified Whittaker nested sampling method was used for plant surveys (Stohlgren et al., 1995). Each sampling site was separated into 14 subplots, including two 50 m^2, two 10 m^2, and ten 1 m^2 subplots. All vascular plants in the subplots were identified from July to September in 2014–2017 (Wang et al., 2020). Overall, we recorded 214 woody species and 439 herbaceous species, accounting for 10% and 26% of the current plant records of Shanghai City (Ma, 2014).

2.2 | The metrics of anthropogenic impacts

We used ArcGIS with land use maps derived from 1 m spatial resolution aerial images (http://www.shanghai-map.net) to measure three urbanization indices. The distance to City centre (Euclidian distance between each site and Shanghai People's Square) was used to represent the roughly increased population density approaching City centre in Shanghai (Cui & Shi, 2012). Urbanized land cover (%) and road density (km km^{-2}) reflected variation in urban land use (Luck & Wu, 2002) and transportation networks (Hawbaker et al., 2006) surround each site. Within a 1 km radius of each site, we identified four major urban land use types, including industrial land, traffic road, public infrastructure, and residential land (Li et al., 2013). Urbanized land cover was calculated as the proportion of the summed area of four land use types of the total area of each circle. Road density was calculated as the total road length within the same radius for each site.

2.3 | Soil sampling and soil physicochemical properties

We performed soil sampling in September 2017. In each site, we selected three subplots (2 m × 2 m) along the diagonal line from southeast to northwest to obtain the representative soil samples.

Three soil replicates were obtained by equally mixing five equally distributed soil cores (2.5 cm in radius and 15 cm in depth) in each subplot. Soil sampling tools were surface sterilized between sampling

sites. All samples were transported to the laboratory on ice within 12 h after collection and separately stored at −20°C until DNA extractions or at 4°C until physiochemical analysis. Eight soil physicochemical properties, including pH, total organic carbon (TOC), total nitrogen (TN), total phosphorus (TP), total potassium (TK), soil water content (SW), and inorganic nitrogen (IN: N-NH_4^+ and N-NO_3^-) were measured as described previously (Liu et al., 2021).

2.4 | Molecular analysis and sequence processing

Total DNA was extracted from 0.5 g soil mixed from each site using the Mag-Bind Soil DNA Kit (Omega Bio-Tek, Norcross, Georgia, USA). Primers 338F and 806R and ITS5F and ITS2R combined with Illumina adaptors were used to amplify the 16S rRNA gene in prokaryotes and internal transcribed spacer (ITS1) regions in fungi (Lee et al., 2012; Tedersoo et al., 2011). Each sample was amplified in triplicates. Positive PCR products were confirmed by electrophoresis. Amplicons from triplicate reactions were purified with GeneJET Gel Extraction Kit (Thermo Scientific) and mixed in equal density ratios. Sequencing was performed using Illumina Miseq (2 × 300 bp paired-end reads) platform at Personal Biotechnology Company (Shanghai, China).

The QIIME2 pipeline (version 2019.10) and the DADA2 plugin with default settings were used to process raw reads (Bolyen et al., 2019). Taxonomy was assigned to representative sequences using the SILVA 132 (Quast et al., 2012) and UNITE v8.0 database (Abarenkov et al., 2010) for prokaryotes and fungi, respectively. AVS (phylotype) abundance tables were normalized to the smallest sample size (9146 and 21,584, respectively) after singletons were excluded. Fungal phylotypes were further assigned to ectomycorrhizal fungi (ECM) using FUNGuild (Nguyen et al., 2016) by including highly probable confidence score guild assignments. Phylotypes belong to subphylum Glomeromycotina were assigned to arbuscular mycorrhizal fungi (AM) (Spatafora et al., 2016). There were 51 and 59 sites omitted from AM and ECM community analysis, respectively, because they lacked any AM or ECM fungi.

2.5 | Statistical analysis

Subsequent analyses were carried out in R 3.5.0 (R CoreTeam, 2018). Microbial diversity and community composition were calculated as the number of different phylotypes (richness) and abundance-based Bray–Curtis dissimilarity. Soil microbial diversity and spatial variations across the city were mapped by a geostatistical analysis. We calculated the semivariogram from the observations and predicted the interested property at unsampled locations using semivariogram models by ordinary kriging. For each diversity index, we applied quantile transformation to guarantee an approximated normal distribution and eliminate the effects of outliers (Song et al., 2015). We fitted two semivariogram models (ordinary least squares and restricted maximum likelihood methods) and retained the one that minimized the effect of random variability on total semivariance. The goodness of the adopted models was then evaluated in terms of the adjusted coefficient of determination (R^2_{adj}) and the root mean square error (RMSE) using cross-validation. The R package 'gstat' was used for these analyses (Pebesma, 2004). The relations between microbial diversity and community composition and urbanization were examined by ordinary least squares regression. For visual simplicity, we computed the mean dissimilarity of each sample to all others, which produced generally similar outcomes as all pairwise comparisons of Bray–Curtis dissimilarity (data not shown). Significant differences in linear regression slope between taxonomic groups were compared using R package lsmeans (Lenth & Lenth, 2018).

To identify urbanization indicators, we applied threshold indicator taxa analysis using R package TITAN v2.1 (Baker et al., 2016). This analysis detected the phylotype abundance and frequency changes via calculating the phylotype-specific indicator value (IndVal) (Dufrêne & Legendre, 1997). TITAN distinguishes negative and positive changes in abundance and frequency and tracks cumulative responses of those phylotypes in the community. Large cumulative change within a narrow range of urbanization values is evidence of a community threshold. The 95% confidence interval was assessed from 1000 bootstraps (Baker & King, 2010). All potential indicators were identified by performing TITAN along three urbanization metrics separately. Indicators in three metrics were also identified to investigate whether urbanization selected for phylotypes in a particular phylum or order.

Canonical variance partitioning was first used to estimate the relative contribution of urbanization (the distance to city centre, urbanized land cover, and road density), soil properties (pH, SW, TOC, TP, TK, IN, C/N), plant diversity [woody and herbaceous richness, evenness, and Shannon diversity (PWri, PWev, PHri, PHev, PWsh, and PHsh)], and geographic locations (latitude and longitude) in explaining the microbial diversity and community composition (Table S1). All variables were standardized to guarantee approximated Gaussian and homoskedastic 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 applied to select significant variables. The explained variance (independent and interactions between the significant variables) in microbial diversity and composition 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 (Karimi et al., 2018).

Structural equation modeling (SEM) (Grace et al., 2009) adopted to build a system-level understanding of the direct and indirect effects of urbanization on the diversity and composition of prokaryotic and total fungal communities (Figure S1). The variables identified as significant predictors from canonical variance partitioning were included. Three urbanization metrics were included considering that the importance might be underestimated in canonical variance partitioning due to high correlations with some environmental variables (Table S1).

Latitude and longitude were included to account for spatial autocorrelation and other unexamined variables that may covary with latitude and longitude (Delgado-Baquerizo et al., 2018). The SEM analyses were conducted using R package 'lavaan' (Rosseel, 2012). Model performance was indicated by a nonsignificant χ^2, high comparative fit index (CFI), high Tucker-Lewis Index (TLI), low root square mean error of approximation (RMSEA), and standardized root mean square residual (SRMR) (Fan et al., 2016).

3 | RESULTS

3.1 | Urbanization gradient and microbial diversity

The level of urbanization described by the distance to City centre, urbanized land cover, and road density varied across 258 sites (Figure 1b). Although three metrics were correlated (Table S1), no single variable could characterize urbanization alone. In areas >50 km from city centre, over 13% (3 in 23 sites) and more than 21% (5 in 23 sites) sites were associated with >60% urbanized land cover and > 6 km/km^2 road density, respectively. In highly urbanized areas (Figure1a; < 16 km radius from the City centre), only eight sites (about 11%) were associated with >80% urbanized land use and > 10 km/km^2 road density.

Across 258 soil samples, we detected a total of 143,931 and 33,295 different prokaryotic and fungal phylotypes (Figure 1c,d). The average soil sample harboured 1442 (±245) and 436 (±132) prokaryotic and fungal phylotypes, respectively. Soil prokaryotes were dominated by the phyla Proteobacteria (29.37% of 2,359,668 total sequences), Acidobacteria (18.44%), Chloroflexi (16.03%), Actinobacteria (13.94%), Gemmatimonadetes (5.59%), and Latesciprokaryotes (3.87%), while fungi were dominated by phyla of Ascomycota (52.12% of 5,568,672 total sequences), Basidiomycota (9.47%), and Mortierellomycota (4.47%) (Figure S2). The richness of prokaryotes and fungi, as well as the most dominant phyla, exhibited a clustered or patchy distribution (Figure S3).

3.2 | Relationships between urbanization and microbial diversity and community composition

Prokaryotic and total fungal richness were weakly but positively associated with three urbanization metrics, except for total fungal richness along the distance to City centre (Figure 2a–c). Compositional dissimilarity in prokaryotic and total fungal communities was weakly but negatively correlated to three urbanization metrics, except for the total fungal dissimilarity along road density (Figure 2d–f). The richness and relative abundance of AM and ECM fungi did not relate to urbanization metrics, although the relative abundance of AM fungi clearly showed a clustered distribution (Figure 3a,b). However, AM fungal compositional dissimilarity showed a similar pattern to that of total fungi where increased urbanization was associated with more similar AM fungal communities (Figure 3c–e).

In general, more indicators showed an increased (positive indicators) rather than a decreased (negative indicators) occurrence with increased urbanization (1.39%–0.74% vs. 0.93%–0.48% in prokaryotes and 1.26–0.80% vs. 0.56%–0.44% in total fungi; Table S2). The highest numbers of indicators were associated with road density. We revealed two thresholds for each gradient at which a synchronous increase and decrease occurred for many phylotypes at relatively high and low urbanization levels, respectively. For instance, along the distance to City centre, prokaryotic and fungal thresholds were detected around the distance of 15.94 and 44.87 km and 18.41 and 48.03 km from the City centre, respectively (Figure 4). Identifying the indicators that responded to the three urbanization metrics, we found no indication that urbanization selected certain phyla, but indicators belonging to prokaryotes of the order *Betaproteobacteriate* and fungi of the order *Trichosporonales* and *Diversisporales* were more likely to increase their abundance with urbanization (Figure S4).

3.3 | Predictors of microbial diversity and community composition

The SEM and canonical variance partitioning results showed that the explained variances in prokaryotic and total fungal richness ranged from 9.55% to 18.20% (Figure 5; Figure S5a). Both approaches showed that the independent effects of soil properties on prokaryotic and fungal richness were 1.5–9 times higher than urbanization, plant diversity, and geographic distance. The effects of soil properties predominated the variances explaining ECM fungal richness and contributed largely to AM fungal richness as well. Among edaphic variables, effect sizes of soil pH on prokaryotic and total fungal richness were about 1.5 times stronger than other variables. TP was correlated with AM fungal richness, while TOC was correlated with ECM richness (Figure S5b). Urbanization had independent effects on prokaryotic, fungal, and AM fungal richness but not ECM fungal richness. Urbanization was also indirectly related to the richness patterns via its effects on soil properties like pH, TK, and plant diversity like PWri and PWev. PWri and PWev had direct effects on prokaryotic richness, and PWri was negatively correlated to soil IN. Geographic distance directly affected prokaryotic and AM fungal richness and was indirectly related to microbial distribution via strong effects on soil properties.

The explained variances in microbial composition ranged from 10.48% in ECM fungi to 37.14% in prokaryotes (Figure 4c,d; Figure S5a). Similar to the results of richness, independent effects of soil properties on microbial composition were stronger than urbanization, plant diversity, and geographic distance, except for ECM fungal composition. Higher soil pH and/or nutrients were associated with similar microbial communities, except for the positive relationship between C/N ratio and fungal composition. The effect sizes of soil pH on prokaryotic and total fungal composition were at least 1.5-times stronger than other edaphic variables. Urbanization showed independent effects on prokaryotes, AM and ECM fungi, and indirectly operated on the microbial composition via its effects on soil properties like pH, TOC, C/N ratio, and plant diversity. PWri and PWev explained

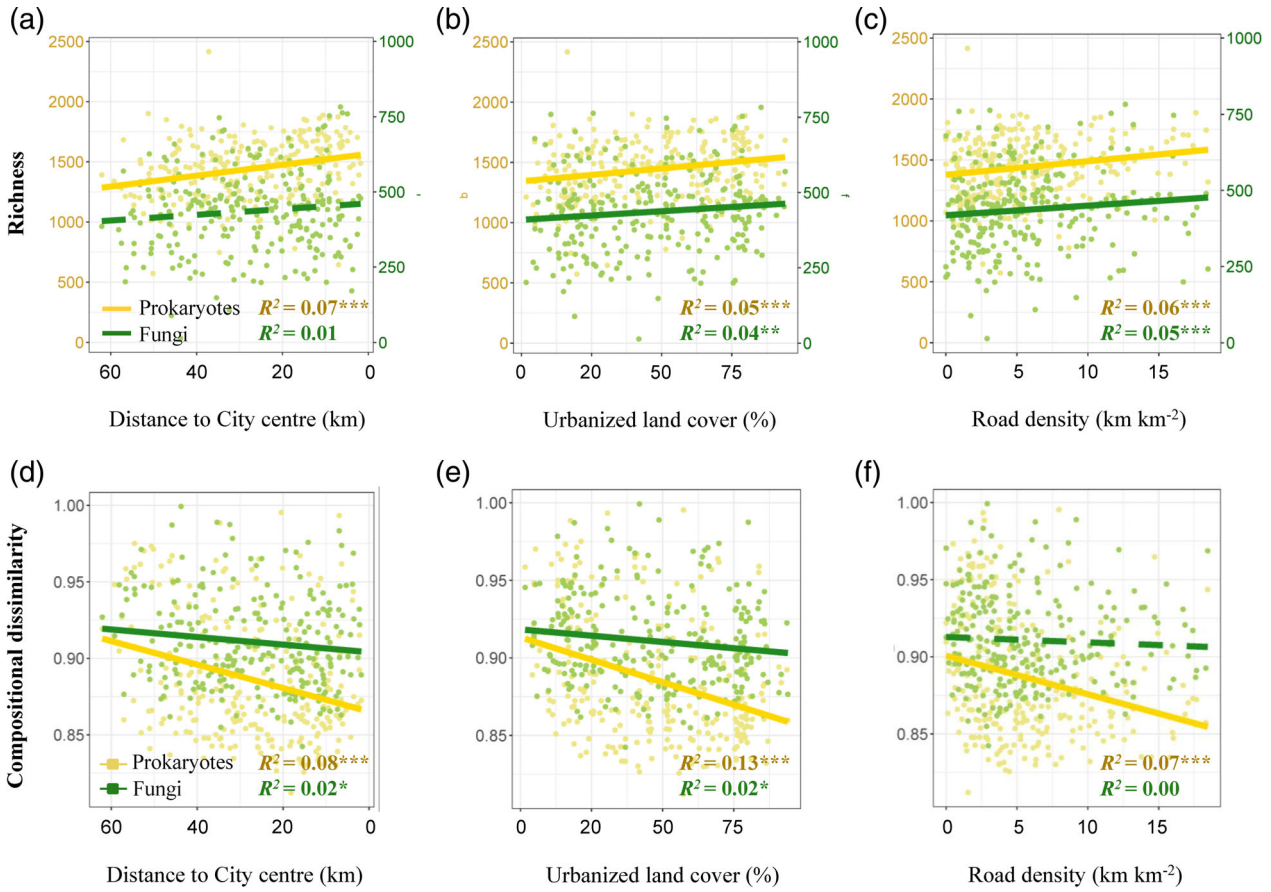

FIGURE 2 The relationships between urbanization and richness and compositional dissimilarity of soil prokaryotes and total fungi. Compositional dissimilarity was calculated as mean Bray–Curtis dissimilarity of each sample to all others. The slopes were significantly different between prokaryotic and total fungal communities in all cases ($p < 0.001$). Two sites with >25 km km^{-2} road density were treated as outliers and excluded in (c) and (f). The asterisk indicates a significant association between microbial diversity or composition with urbanization examined by ordinary least squares regressions: $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ [Colour figure can be viewed at wileyonlinelibrary.com]

prokaryotic composition but showed opposite trends. PHsh was positively associated with AM and ECM fungal composition. PWev was negatively related to ECM fungal composition. For urbanization indicators, soil properties independently explained large portions of their variations in soil (6.81%–19.72%; Figure S5a). Plant diversity explained some variations in positive indicators in prokaryotic and total fungal communities, which were associated with high PWri and low PGri.

4 | DISCUSSION

In the megacity Shanghai, we found that urbanization was associated with homogenized community composition in soil prokaryotes and total fungi. The compositional dissimilarity of soil microbes decreased with urbanization, despite the increased richness in more urbanized areas (Figure 2). Among examined environmental factors, soil properties had the major direct impacts (Figure 5). Particularly, soil pH was negatively related to community composition and positively to the richness of prokaryotes and total fungi. Consistent with observations in natural ecosystems (Fierer & Jackson, 2006), the results suggest that soil pH regulates microbial survival and fitness in urban ecosystems. Our results also suggest that urbanization could directly contribute to compositional homogeneity, particularly in prokaryotic communities (Figure 5c), by increasing urbanized land cover. Mechanisms underlying the direct effects of urbanized land cover on soil microbes may be related to habitat fragmentation and habitat loss (Reese et al., 2016; Seto et al., 2012) but need further confirmation. Urbanization also has indirect impacts on soil microbes by the changes in soil properties. For instance, soil management practices (e.g., the application of fertilizer, irrigation for salt washing, and the use of amendments in urban land) could lead to higher or neutralized soil pH (Pouyat et al., 2007), supporting a high survival rate and fitness of microbes, thus inducing high soil microbial richness but less varied communities among localities. Similarly, generally negative relationships between examined soil chemical properties and microbial composition suggest that high soil nutrients enable similar community composition, potentially by reducing resource competition and providing more diverse resources to less abundant microbes. The hyphal network that facilitates nutrient uptake of fungi may cause them to

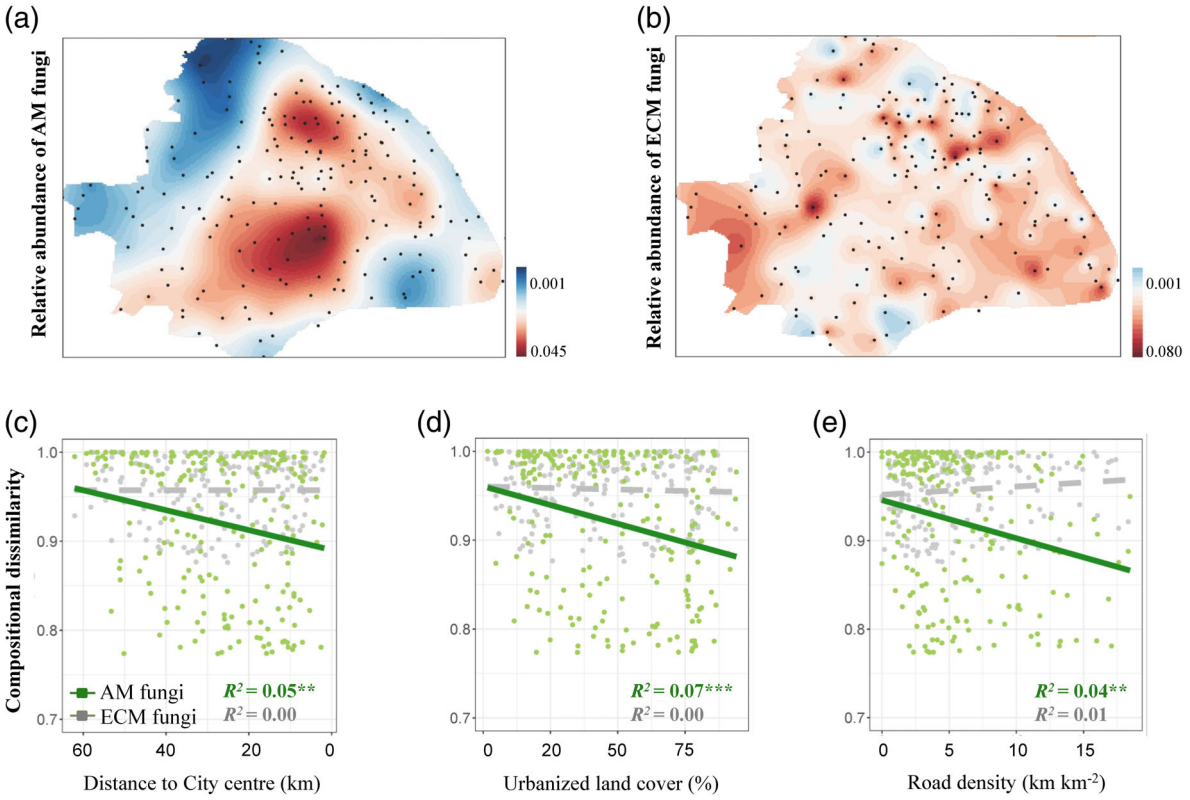

FIGURE 3 Maps of relative abundance and the relationships between urbanization and compositional dissimilarity for arbuscular mycorrhizal (AM) and ectomycorrhizal fungal (ECM) communities. Ordinary Kriging interpolation was used for microbial mapping in (a-b). Compositional dissimilarity in (c-e) was calculated as mean Bray–Curtis dissimilarity of each sample to all others. Statistical analysis was performed using ordinary least squares regressions in (c-e): $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ [Colour figure can be viewed at wileyonlinelibrary.com]

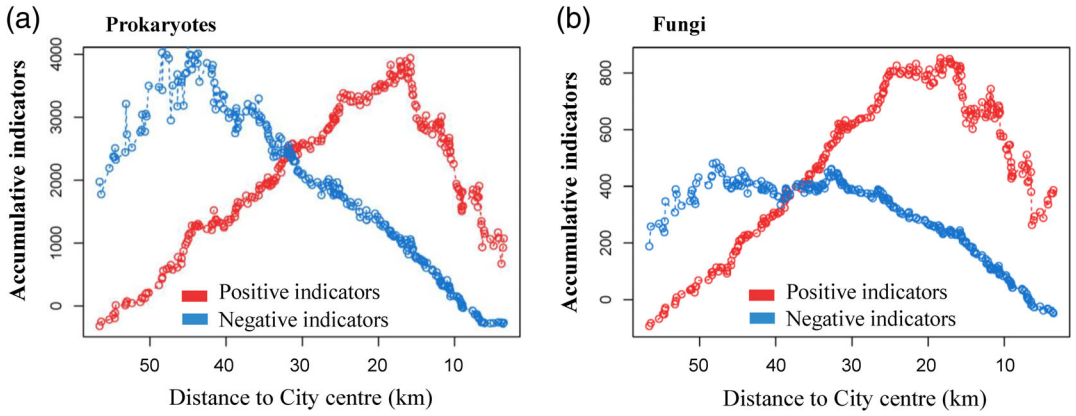

FIGURE 4 Accumulation of urbanization significantly influenced indicators for prokaryotic and fungal communities. The peaks indicate thresholds of urbanization value at which increased (positive) or decreased (negative) abundance and frequency of many indicators were observed. Thresholds for prokaryotic and fungal communities along urbanized land cover and road density can be found in Table S2 [Colour figure can be viewed at wileyonlinelibrary.com]

be less sensitive to environmental changes than prokaryotes (Guhr et al., 2015). However, DNA amplicon sequencing–based taxonomic assays may lead to biased estimates of prokaryotic: fungal ratio due to differences in inherent primer activity for prokaryotes and fungi. Further work applying methods such as quantitative PCR-based analysis and sequencing of total extracted DNA without amplifying specific gene region can be used to evaluate how urbanization alters the prokaryotic:fungal ratio (Fierer et al., 2005; Malik et al., 2016).

Urbanization was associated with similar AM fungal communities, but we observed no impacts on ECM fungal richness and community dissimilarity. The underlying mechanisms may be, in part, related to the differential effect of urbanization on AM versus ECM plant hosts

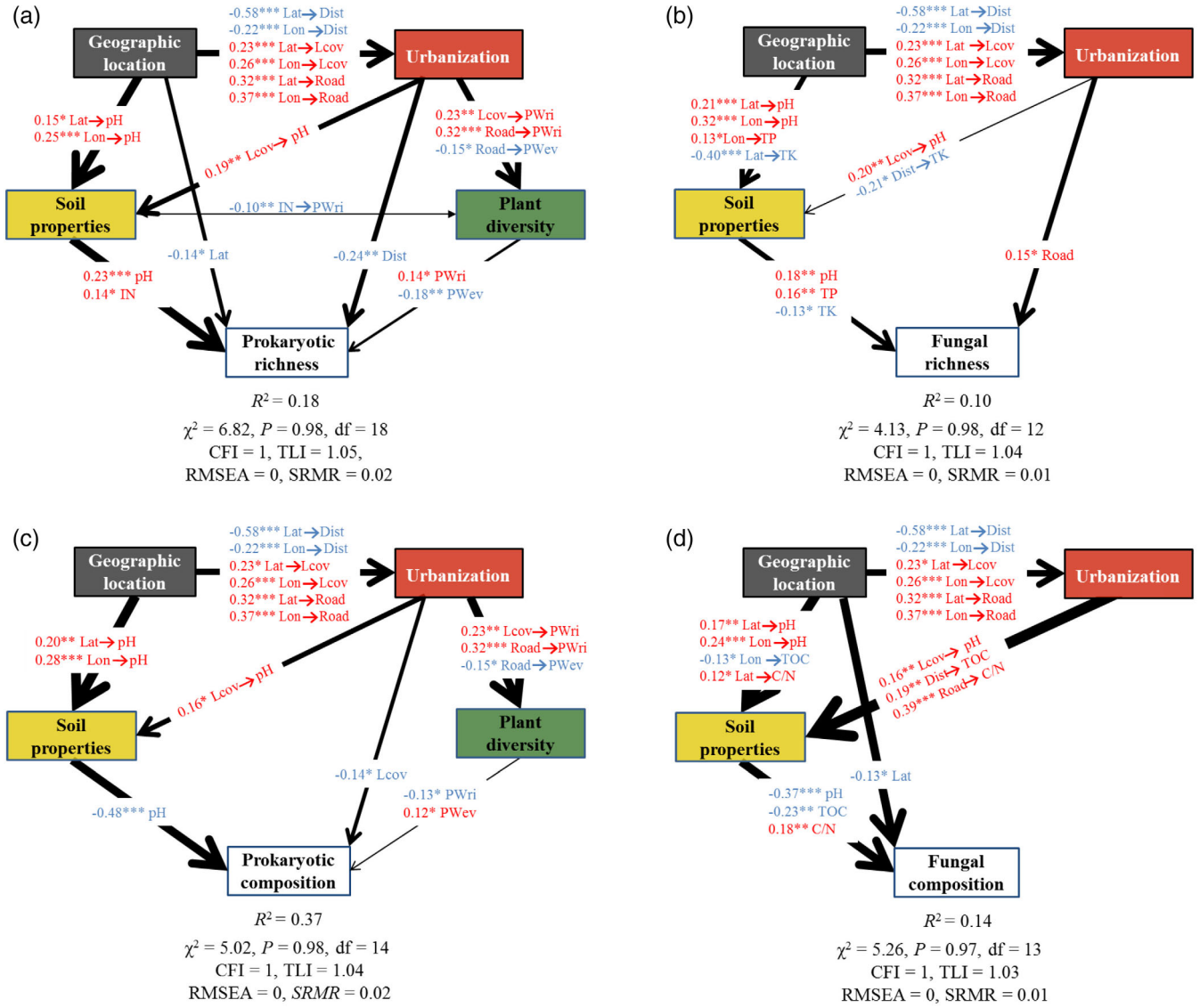

FIGURE 5 Structural equation model describing environmental and spatial drivers structuring richness and community composition of prokaryotes and total fungi. We grouped these predictors in the same boxes for graphical simplicity and only displayed significant relationships. The values adjacent to arrows show the effect size of each relationship. Model performance was indicated by a nonsignificant χ^2, high comparative fit index (CFI), high Tucker–Lewis index (TLI), low root square mean error of approximation (RMSEA), and standardized root mean square residual (SRMR). Significance levels are: $^*p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$. Abbreviations of variables are as follows: Lat, latitude; Lon, longitude; Dist, distance to City centre; Lcov, urbanized land cover; Road, road density; TP, total phosphorus; TK, total potassium; IN, inorganic nitrogen; C/N, carbon/nitrogen ratio; PWri, woody plant richness; PWev, woody plant evenness [Colour figure can be viewed at wileyonlinelibrary.com]

and factors driving the composition of their mycorrhizal fungal communities. As showed in our results, the community composition of AM fungi was best explained by soil properties, plant diversity, urbanization, and their interactions, while ECM fungal composition was mainly explained by plant diversity and urbanization (Figure S5a). Thus, AM fungi followed the general pattern of total fungi such that urbanization was associated with homogenized AM fungal communities. This might be explained by the declining richness and evenness of herbaceous plant species with urbanization, as most herbaceous species associate with AM fungi (Table S1) (Smith & Read, 2008). For ECM fungi, previous work documented decreased diversity but increased homogenization in more urbanized areas, particularly in the temperate zone where they are most diverse (Schmidt et al., 2017; Tedersoo et al., 2012). Removal of original ectomycorrhizal trees could lead to ECM fungal losses in temperate cities (Schmidt et al., 2017). However, our findings suggest that even though woody plant richness increased with urbanization (Table S1), ECM fungal richness did not. And woody plant evenness, which was unaffected by urbanization, explained variance in the ECM fungal community composition (Table S1; Figure S5b). Patterns in ECM fungal community composition may, therefore, be better explained by analyzing the abundance and diversity of ECM host plants.

The complex effects of urbanization we revealed in the megacity offer potential explanations for the current inconsistencies in the literature on how soil microbial biodiversity responds to urbanization (Docherty et al., 2018; Reese et al., 2016; Schmidt et al., 2017; Wang et al., 2018). Despite the relatively low R^2, three urbanization metrics were related to soil microbial diversity and community composition. Such a finding suggests that environmental filtering associated with human population density, urbanized land use, and road density may create a heterogeneous environment for soil microbes (Pouyat et al., 2007; Vasenev et al., 2013), meaning that a low number of soil samples may bias the estimation of urbanization effects. Our sampling intensity across the megacity of Shanghai has provided explicit evidence supporting threshold responses of microbes to different types of urban gradients. At a relatively low level of urbanization (e.g., > 15.94 km from city centre, < 72.28% urbanized land cover, or <6.73 km km^{-2} road density), urbanization and its induced environmental changes might cause loss of microbial diversity, while increased diversity is expected at the relatively high level of urbanization (Figure 4). Meanwhile, urbanization indicators seemed more sensitive to shifts in road density than in human population density and urbanized land cover (Table S2), suggesting microbial dispersal through public transportation or commuting may be especially important on soil microbes. In addition, large residual variances for both microbial richness and composition indicate the importance of unexamined factors or other processes (e.g., stochasticity) in understanding urbanization effects on soil microbes (Yang et al., 2021).

We revealed that woody plant diversity directly explained a part of the total variances in soil prokaryotic richness and composition. Particularly, woody plant richness had positive effects on the positive indicators in prokaryotes and fungi. These results suggest specific microbial establishment or accumulation around plants in urban areas (Van Der Heijden et al., 2008). For instance, symbiotic interactions between plants and AM fungi (e.g., *Diversisporales*), or the accumulation of pathogens (e.g., *Betaproteoprokaryotices*), could contribute to the increased microbes around host plants (Brenner et al., 2005). Significant changes in these phylotypes might be due to the presence of certain host plants and/or the direct human management of urban plants. In Shanghai, most ornamental plants are transplanted from other regions, and over 70% of the trees are nonnative species in sites within 11 km from the City centre (Wang et al., 2020). The positive relationships between woody plant richness and the positive indicators suggest soil microbes have been introduced with transplanted or nonnative trees (Lekberg et al., 2013). These results merit further investigation to determine how much the pattern in soil microbial diversity and composition can be explained by nonnative soil microbes.

5 | CONCLUSIONS

Rapid urban sprawling has raised large concerns that urbanization reduces biodiversity, alters ecosystem functioning, and affects human well-being. Our results from the megacity of Shanghai provide evidence for the effects of urbanization on soil microbial biodiversity. Particularly, urbanization explained an independent proportion of soil microbial richness and composition that could not be explained by factors such as soil physicochemical properties. We also showed that some microbial phylotypes sensitively responded to urbanization and its induced environmental changes and displayed threshold effects on their abundance and frequency. Such information is critical to improve our understanding of how soil biodiversity responds to anthropogenic modification of natural and semi-natural ecosystems and provides guidelines for effective management of urban ecosystems.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

AUTHOR'S CONTRIBUTIONS

Lan Liu and Jian Zhang conceived the idea. Zhaochen Zhang, Xin Wang, and Ran Zhang collected field sampling. Meng Wang and Junxiang Li performed the plant survey. Lan Liu, Xin Wang, and Ran Zhang performed laboratory work. Lan Liu led the writing of this manuscript; Albert Barberán, Cheng Gao, Nina Wurzburger, Jian Zhang, and Junxiang Li contributed critically to the data analysis and writing the manuscript. All authors contributed critically to the drafts.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study 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.

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