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Urbanization significantly impacts the connectivity of soil microbes involved in nitrogen dynamics at a watershed scale☆

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ABSTRACT

As one of the most dominant ecosystems of urban green space, turfgrasses provide a wide range of ecosystem services. However, little is known about the interactions of microbial communities in turfgrass soils and how these interactions respond to expanding development of impervious surfaces during watershed urbanization. In this study, we analyzed bacterial communities and their co-occurrence patterns in turfgrass soils along an urbanization gradient as measured by the proportion of impervious surfaces in Jiulong River watershed in Fujian, China. Results show that the diversity and network size of bacterial communities negatively associated with impervious surfaces. The bacterial communities showed non-random co-occurrence patterns, with more intra-module connections observed for urbanized networks. The co-occurrence network with distinct modules of soil samples with contrasting land cover imperviousness suggested different functional organizations with altered microbial nitrogen processes. Structural equation modelling revealed that watershed impervious surfaces had indirect impacts on microbial connectivity by altering soil properties, including pH, temperature, moisture, C/N and nitrate (NO_3^-). Moreover, impervious surfaces affected microbial connectivity far more than human population density. Our study highlights the significance of human disturbances in affecting microbial interactions and assemblies in turfgrass ecosystems through impervious surfaces and provides benefits for sustainable urban planning and management at a watershed scale.

1. Introduction

Urbanization has disturbed many natural ecosystems through the expansion of impervious surfaces (Wigginton et al., 2016). Development of impervious surfaces has considerably altered microbial communities through affecting dispersal processes and modifying environmental conditions at local scales (Rodrigues et al., 2013; Hosen et al., 2017; Tin et al., 2018). As one of the most dominant ecosystems of urban green space (Wang et al., 2017), turfgrass is mainly maintained for aesthetic and recreational purposes under intense management practices such as mowing and fertilization. Turfgrass could also provide other crucial ecosystem services including urban heat island mitigation and carbon sequestration (Jenerette et al., 2011). While turfgrass and its benefits have garnered increased attention since the latter 20th century, there are also reports of disservices through, for example, losses of inorganic nitrogen (Frank et al., 2006) and releases of nitrous oxide (N_2O) (Kaye et al., 2004), a potent greenhouse gas. A variety of biogeochemical processes relating to nitrogen (N) fluxes are mediated by diverse microbial communities (Fierer, 2017). However, our knowledge of soil microbiomes in turfgrass ecosystems and the extent to which shifts in microbial communities respond to environmental disturbances remains rudimentary.

Soil environment provides heterogenous conditions for different microbial communities, which interact with each other in ways that affect their growth and metabolic processes (Montoya et al., 2006). Cooperative interaction may lead to synergistic growth and co-occurrence patterns of interacting communities, whereas competition for the same resource may lead to limited growth and metabolism (Newman, 2006). To a certain extent, microbial connectivity which refers to the inter-taxa associations in

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this study reveals patterns of niche occupancy for different microbial communities and offers insights into their functional attributes and adaptation strategies in response to environmental disturbances (Williams et al., 2014). The positive and negative feedbacks of microbial communities contribute to the complex structure and functions of terrestrial ecosystems. Grouping community data into a network, which is comprised of nodes and edges representing different species and their correlations, allow ecologists to better understand the interactions of community members and thereby infer consequent ecological functions (Röttjers and Faust, 2018). Although network analysis of microbial interactions has been found insightful in recent years, with studies from a wide range of ecosystems including grassland (Zhou et al., 2011; Barberán et al., 2012; Khan et al., 2018). However, studies linking microbial interactions to external drivers such as environmental gradients are limited, which hampers our holistic view of complex microbial communities.

Linking geographic patterns of microbial community features (e.g., composition, diversity and interactions) to environmental drivers is a central goal of microbial ecology (Faust and Raes, 2012). Changes in land cover imperviousness (e.g., impervious surface) during watershed urbanization can exert significant impacts on soil physicochemical properties across the landscape (Gregg et al., 2003; Huang et al., 2013; Becker et al., 2017; Delden et al., 2018). These impacts may ultimately cascade to changes in the structure of microbial communities, as well as the underlying ecological processes and functions. For example, the development of impervious surfaces impedes exchanges in gases, water and nutrients at the interface between soil and the atmosphere, thereby affecting microbial enzymatic activity and the size of terrestrial C and N pools (Raciti et al., 2012; Wei et al., 2013). Recent research indicated that impervious surfaces induce changes in bacterial community composition and decrease bacterial diversity (Hu et al., 2018). Although associations between impervious surfaces and microbial communities have been proposed, the impacts of impervious surfaces on microbial connectivity in turfgrass ecosystems remain unclear. Furthermore, fluctuations in demographic and soil physicochemical characteristics due to human activities have significant associations with microbial communities (Yan et al., 2016; Hu et al., 2018; Wang et al., 2018), which increases the complexity of imperviousness influences. Identifying determinants of microbial communities and functions requires detailed information on land cover imperviousness, demographic and soil physicochemical properties. However, to date, the causal relationships between these factors and microbial connectivity in turfgrass ecosystems have not been well understood.

Watersheds with varying types of dominant land cover have different potential for microbial transformation, which, in turn, affect the distribution of sources and sinks for different contaminants. Understanding microbial connectivity and how they respond to environmental changes can guide sustainable urban planning and management at the watershed scale. In this study, based on soil data gathered from the high throughput sequencing of 16S rRNA genes from turfgrass soil microbes, we constructed a microbial network and characterized the spatial variation of network topology, as well as microbial composition and diversity along an urbanization gradient as measured by the proportion of impervious surfaces in a watershed. We aimed to: (1) identify how microbial communities change and connect in response to varying urbanization levels; (2) quantify the causal relationships among impervious surface, population density, soil physicochemical properties and microbial connectivity at a watershed scale; and (3) identify the factors that explain the most variations in the microbial connectivity.

2. Materials and methods

2.1. Site description and sample collection

Sampling was conducted during 5–27 May 2018 in the Jiulong River watershed in Fujian, China (Fig. S1). As the second largest watershed in Fujiang province, the Jiulong River watershed covers an area of about 14,000 km^2 and hosts two regional cities (Longyan, Zhangzhou). This region is characterized by a subtropical marine climate with an annual average precipitation of 1200 mm and an annual average temperature of 21 °C. The watershed is experiencing rapid urbanization, with a significantly spatial pattern of land cover imperviousness (Fig. S2). Since 1995, the average growth rate of urban population has more than doubled and future expansion of urban areas will continue, which has imposed great pressure on the local environments (Zhang et al., 2018; Huang et al., 2019).

Sampling sites were chosen from a total of 15 subwatersheds. Based on the distribution of the proportion of impervious surface, the subwatersheds were categorized into quartiles to represent natural (lower 25%: 0.7–5.5%), suburban (central 50%: 5.6–44.1%) and urban (upper 25%: 44.2–53.4%) subwatersheds (Table S1). Five turfgrass sites were randomly selected within each subwatershed. At each site, top soils (0–20 cm) were collected, and a total of 4 replicates were conducted for each site. In total, we collected 300 soil samples and grouped them into natural (80), suburban (120) and urban (100) categories based on the sampling sites. Following the removal of litter, the soil samples were transported to the laboratory on ice. Each sample was divided into two subsamples, with one subsample sieved (2.0 mm) for analysis of physicochemical properties and the other subsample stored at −80 °C for DNA extractions and molecular analysis.

2.2. Soil physicochemical property measurement

Soil moisture was measured gravimetrically by oven-drying the samples at 105 °C for 24 h. Soil pH was determined in a 1:2.5 soil/water suspension using a pH meter (Orion Versa Star Multiparameter, Thermo Scientific, USA) (Gillman and Sumpter, 1986). In situ temperature of topsoil was determined using an RTD Thermometer (HH370, Omega, USA). Total organic carbon (TOC) was determined with a TOC-V CPH analyzer (Shimadzu, Duisburg, Germany). Total nitrogen (TN) was measured using dry combustion in a CNS analyzer (Vario MAX, Elementar, Germany). For ammonium (NH_4^+) and nitrate (NO_3^-), 10 g of soil samples were added to 100 mL of 2 M potassium chloride and the slurry was cultivated for 1 h in a shaker; the suspension was passed through a quantitative filter paper with a pore size of 0.45 μm before analyses of NH_4^+ and NO_3^- in a flow-injection analyzer (Hach Co, Loverland, CO, USA). All analyses were performed in triplicate for each sample.

2.3. DNA extraction, sequencing and taxonomic assignment

Total genomic DNA of each sample was extracted from 0.5 g of soil samples using the Fast DNA SPIN Kit for Soil (MP Biomedicals, Santa Ana, CA, USA) in accordance with the manufacturer's instruction. The V4 region of bacterial 16S rRNA was amplified using 515F (5′-GTGYCAGCMGCCGCGGTAA-3′) and 806R (5′-GGACTACNVGGGTWTCTAAT-3′) prokaryotic primers (Claesson et al., 2010). Amplifications were performed in a 25 μL reaction mixture containing 12.5 μL of Premix Ex Taq (Takara), 1 μL of each primer (5 μM), 1 μL of DNA template, 9.5 μL of PCR water. The amplifications were run with the following protocol: 94 °C for 3 min; 35 cycles of 94 °C for 45 s, 50 °C for 60 s and 72 °C for 90 s; and 72 °C

for 10 min. PCR products were verified via electrophoresis on a 1% agarose gel and expected fragments were excised and purified with the DNA purification Kit (TIANGEN, China). The concentrations of the purified fragments were then quantified using a QuantiFluor dsDNA System (Promega, CA, USA). The barcoded PCR products were pooled in an equimolar concentration and were subsequently sent to Personal Biotechnology Co., Ltd. Shanghai, China, for sequencing on an Illumina MiSeq platform.

Analyses of the sequencing data were carried out using QIIME version 1.9.1 (Caporaso et al., 2010). Briefly, raw sequences were demultiplexed, after which ambiguous bases and primer sequences were removed. The trimmed sequences were clustered into operational taxonomic units (OTUs) at a 97% similarity level and then taxonomically assigned based on the Greengenes database. Singleton OTUs and those assigned with mitochondria and chloroplast were filtered and the resulting OTUs were rarefied for each sample. Samples with <13,000 sequences were discarded, resulting in a total of 279 samples that were processed for further analyses.

2.4. Network construction and analyses

Since the number of individual OTUs varied significantly across different samples, we used the relative abundances of the OTUs to construct a molecular network using the method described by Zhou et al. (2010). In summary, Spearman correlation coefficients were calculated for all possible pairs of OTUs and the correlation matrix was then converted to a similarity matrix that measures the degree of concordance between the abundance profiles of OTUs across the samples. Following the determination of an appropriate threshold for defining network structure based on the random matrix theory-based method (Luo et al., 2006; Zhou et al., 2010), the similarity matrix was used to construct an adjacency matrix which encodes the strength of the connection between each pair of nodes. We considered a co-occurrence pattern between bacterial taxa to be robust if the absolute correlation coefficient was greater than 0.8 with a *P*-value < 0.01 (Zhou et al., 2010). To reduce possible false positive results, we employed a Benjamini and Hochberg false-discovery-rate adjustment for the *P* values using the *multtest* package in R (Benjamini and Hochberg, 1995; Pollard et al., 2005). To improve the representativeness of the OTUs and reduce the network complexity, only significant pairwise correlations were used to construct the network, with each node representing an OUT and each edge representing a significant association between the OTUs.

The topologic features of the resulting network were described based on indices including clustering coefficient, diameter, path length, closeness, degree and betweenness centrality which were estimated according to the method reported by Zhou et al. (2010). The microbial connectivity was calculated with scripts in the *igraph* package in R (Zhou et al., 2010). Nodes with higher betweenness centrality were considered as playing a core role in the network and those with lower values had a more peripheral role. The distribution of the nodes and their group attributes were visualized with Cytoscape version 3.7.1 (Shannon et al., 2003).

2.5. Statistical analyses

All statistical analyses were performed using R version 3.5.0 (R Foundation for Statistical Computing, Vienna, Austria). Differences in the soil physicochemical properties and species richness across the urbanization gradient were determined using the Kruskal-Wallis test, followed by the Tukey post hoc test if a significant difference ($P < 0.05$) was observed. Species richness was used to calculate alpha diversity, and Bray-Curtis dissimilarity was used to calculate beta diversity and perform principle coordinate analysis (PCoA). Analysis of similarity (ANOSIM) was performed to assess differences of microbial taxonomy across the urbanization gradient. To determine the environmental factors that shape the microbial communities, we fitted soil factors into the PCoA plot using the *ape* and *vegan* packages in R (Paradis et al., 2004; Oksanen et al., 2013). Spearman's rank correlation analysis was employed to examine the relationships of environmental factors with the abundances and diversity of microbial communities as well as the network topological features. To infer the relative importance of environmental factors for the microbial connectivity, we fitted a structural equation model (SEM) using impervious surfaces, population density, geographic distance and soil physicochemical factors as predictors. The SEM was fitted using the *sem* package in R. We fitted the SEM as a linear model and reported a standardized coefficient for each path. The overall fit of the SEM was evaluated using Shipley's test of d-separation (Shipley, 2000).

3. Results

3.1. Soil physicochemical characteristics

Significant differences were found for all the measured soil physicochemical factors among the samples, except TN, which showed no significant difference (Fig. S3). The pH was significantly higher for urban soils as compared to the suburban ($P < 0.05$) and natural ($P < 0.01$) soils, which showed no significant difference in this factor. The temperature was significantly higher for urban ($P < 0.01$) and suburban ($P < 0.05$) soils than the natural soils. The moisture and chemical properties including TOC, C/N, NO_3^- and NH_4^+ were significantly lower ($P < 0.05$) for urban and suburban soils and significantly higher ($P < 0.05$) for natural soils.

3.2. Relative abundances of microbial taxa

Significant differences in the distribution of microbial taxa were observed at the phylum (ANOSIM: $R = 0.98$, $P < 0.05$) and genus (ANOSIM: $R = 0.97$, $P < 0.05$) levels along the urbanization gradient. Among the microbial phyla comprising the network, *Alphaproteobacteria* and *Firmicutes* exhibited the greatest variation in their relative abundances, with the former taxa decreasing from 27.62% for natural soils to 11.72% for urban soils and the latter taxa increasing from 10.41% for natural soils to 24.82% for urban soils (Fig. 1a). Additionally, as the urbanization level increased, the relative abundances of *Acidobacteria* and *Cyanobacteria* decreased from 26.21% to 11.24% and from 2.96% to 0.04%, respectively, while the relative abundance of *Betaproteobacteria* increased from 0.83% to 12.76%.

The top 15 genera accounted for 35.81%, 32.56% and 34.52% of the microbial communities in natural, suburban and urban samples, respectively. Among these genera, the greatest decrease in response to increasing urbanization was found for *Rhodoplanes*, with a relative abundance of 12.66% for natural soils and 3.30% for urban soils (Fig. 1b). In contrast, *Sporosarcina* showed the greatest increase in its relative abundance, with 2.94% and 10.95% for the natural and urban soils, respectively. Significant variations were found for *Sphingomonas* and *Acidothermus*, which had higher relative abundances in natural soils, and for *Actibacterium* and *Nitrososphaera*, which had higher relative abundances in urban soils.

3.3. Network topology and modularity characteristics

The microbial network comprised a total of 279 nodes and 1208 edges, with positive correlations for 1123 edges and negative correlations for 85 edges. We detected a total of 17 modules, with 13

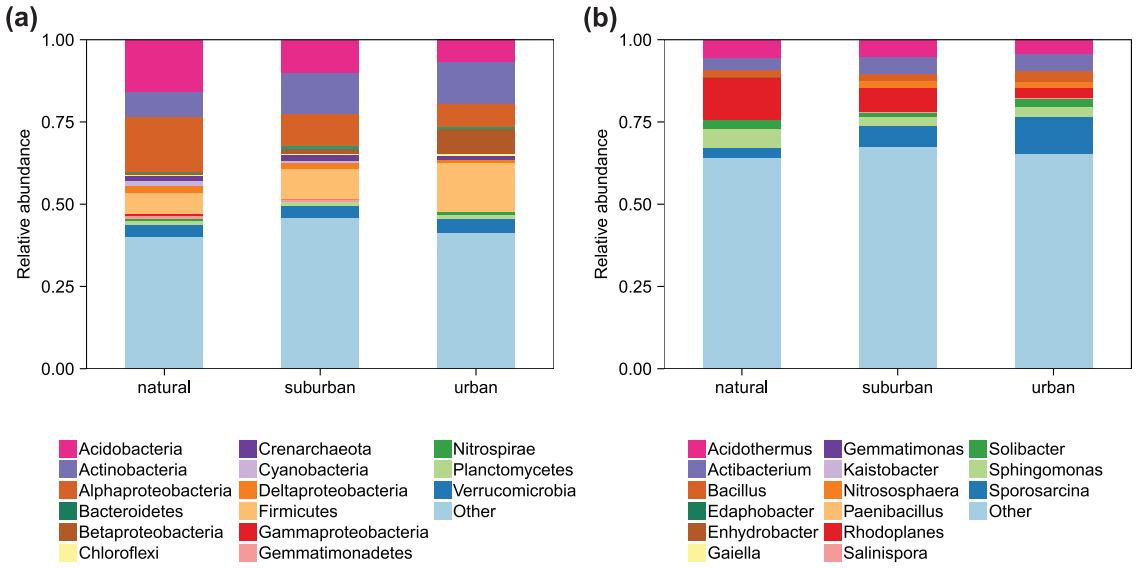

Fig. 1. Average relative abundances of (a) major microbial phyla and (b) genera in urban, suburban and natural soils.

modules consisting of only nodes associated with individual sample type (natural, suburban or urban), 3 modules consisting of two types of nodes and 1 module consisting of all three types of nodes (Fig. 2a). A lower number of nodes (40) and edges (191) was observed for the urban-associated modules as compared to those of the suburban- (61 nodes and 257 edges) and natural-associated (178 nodes and 760 edges) modules. Comparison of the topological features of the three node types showed that urban nodes had higher scores on closeness, but lower scores on betweenness centrality, degree, diameter, path length and transitivity metrics as compared to the suburban and natural nodes (Fig. S4).

Among the modules identified, three modules with corresponding average connectivity scores of 1.60, 2.09 and 2.27 were found to be the most connected among microbial taxa associated with natural, suburban and urban sites, respectively (Fig. 2b). The urban module had 23 nodes, where majority of the nodes belonged to *Firmicutes* (39.1%) and *Candidatus* (21.7%). The suburban module also had 23 nodes, with 31.8% belonging to *Firmicutes*, followed by *Acidobacteria* (18.2%) and *Candidatus* (17.3%). As for the natural module, *Alpharoteobacteria* and *Acidobacteria* comprised 23.3% and 16.7% of the total nodes, respectively. Among the classified taxa with known functional attributes, *Canditatus*, *Cenarchaeales* and *Rhodoplances* were identified in the natural module, with average betweenness centrality scores of 0.029, 0.026 and 0.041, respectively. Major taxa including *Nitrospira*, *Rhodoplanes*, *Candidatus* and *Ammoniphilus*, with average betweenness centrality scores of 0.033, 0.010, 0.009 and 0.002, respectively, were identified in the suburban module. Major taxa identified in the urban module were *Nitrospira*, *Canditatus*, *Cenarchaeales* and *Rhodoplanes*, with average betweenness centrality scores of 0.079, 0.046, 0.037 and 0.033, respectively.

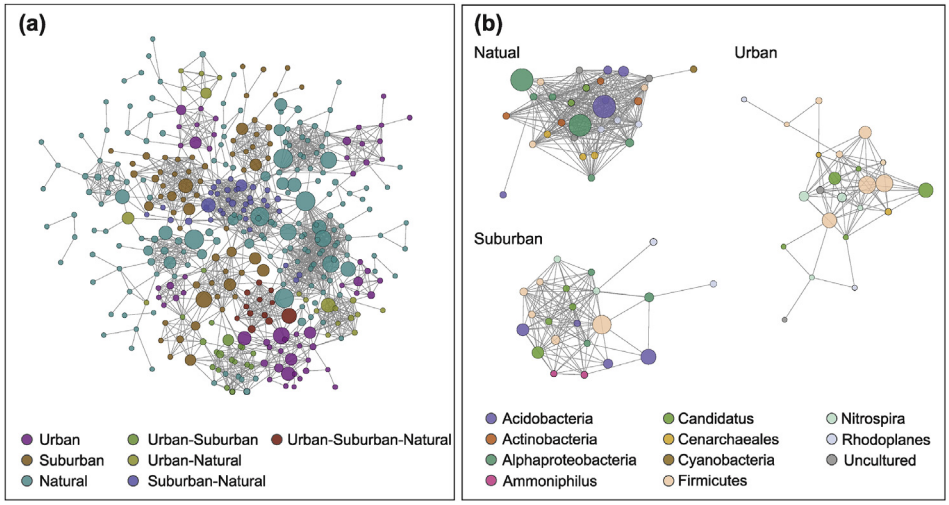

Fig. 2. Network of co-occurring OTU pairs based on significant Spearman correlations ($r \leq -0.8$ or $r \geq 0.8$) with a significance of adjusted *P*-value < 0.01. (a) Network for all nodes across the three urbanization levels. (b) Representative modules with the most connection among community members for urban, suburban and natural samples. The sizes of the nodes were scaled to their betweenness centrality scores to indicate their relative importance in each module.

3.4. Drivers of microbial diversity and connectivity

The PCoA results revealed that the distribution of microbial communities was significantly explained by impervious surface, population density and soil factors including moisture, pH, temperature, C/N and NO_3^- (Fig. S5). Among the environmental factors investigated, we found that species richness and community dissimilarity were positively corelated with soil moisture and C/N (Fig. S6), but negatively correlated with impervious surface, population density, soil temperature and pH. Compared to other soil factors measured, the relative abundances of most of the major taxa were found to be influenced by soil pH, temperature, moisture and C/N across the sampling sites (Fig. 3). Specifically, soil pH and temperature had strong positive correlations with *Firmicutes*, *Betaproteobacteria*, *Actinobacteria*, *Verrucomicrobia*, *Crenarchaeota* and *Nitrospirae*, but negative correlations with *Acidobacteria*, *Alphaproteobacteria*, *Deltaproteobacteria*, *Gammaproteobacteria*, *Cyannobacteria* and *Chloroflexi*. In contrast, soil moisture and C/N ratio had reverse correlations with the above taxa, except for *Gammaproteobacteria* and *Chloroflexi*, which had weak correlations with the above two factors. Impervious surface showed positive correlations with *Firmicutes*, *Nitrospirae* and *Verrucomicrobia*, but negative correlations with *Alphaproteobacteria*, *Deltaproteobacteria*, *Gammaproteobacteria* and *Chloroflexi*. Population density and geographic distance had positive correlations with *Actinobacteria* and *Firmicutes*, but negative correlations with *Acidobacteria*. Moreover, we found that the investigated soil factors had no significant correlations with *Planctomycetes* or *Gemmatimonadetes*.

Based on the OTUs from each site, we constructed a subnetwork for each site and examined the impacts of environmental factors on the network topology. We found that soil pH was positively correlated with closeness and connectivity, but negatively correlated with degree, betweenness centrality, path length, transitivity and edge number (Fig. S6). The C/N was positively correlated with transitivity and edge number, but negatively correlated with closeness and connectivity. Impervious surface and human population density were positively correlated with connectivity, but negatively correlated with closeness, betweenness centrality, transitivity and edge number. Other significant correlations were found between network connectivity and soil factors including temperature (0.672), moisture (−0.653) and NO_3^- (−0.702).

To determine the causal relationships of environmental factors and their relative importance in affecting the microbial connectivity directly and indirectly through other factors, we fitted a SEM using data on impervious surfaces, human population density, geographic distance and soil physicochemical factors. The SEM with an explanation of 83.7% in total variance fitted the observed data well (Fisher'$C = 7.223$, $P = 0.903$, AIC = 27.604). The results

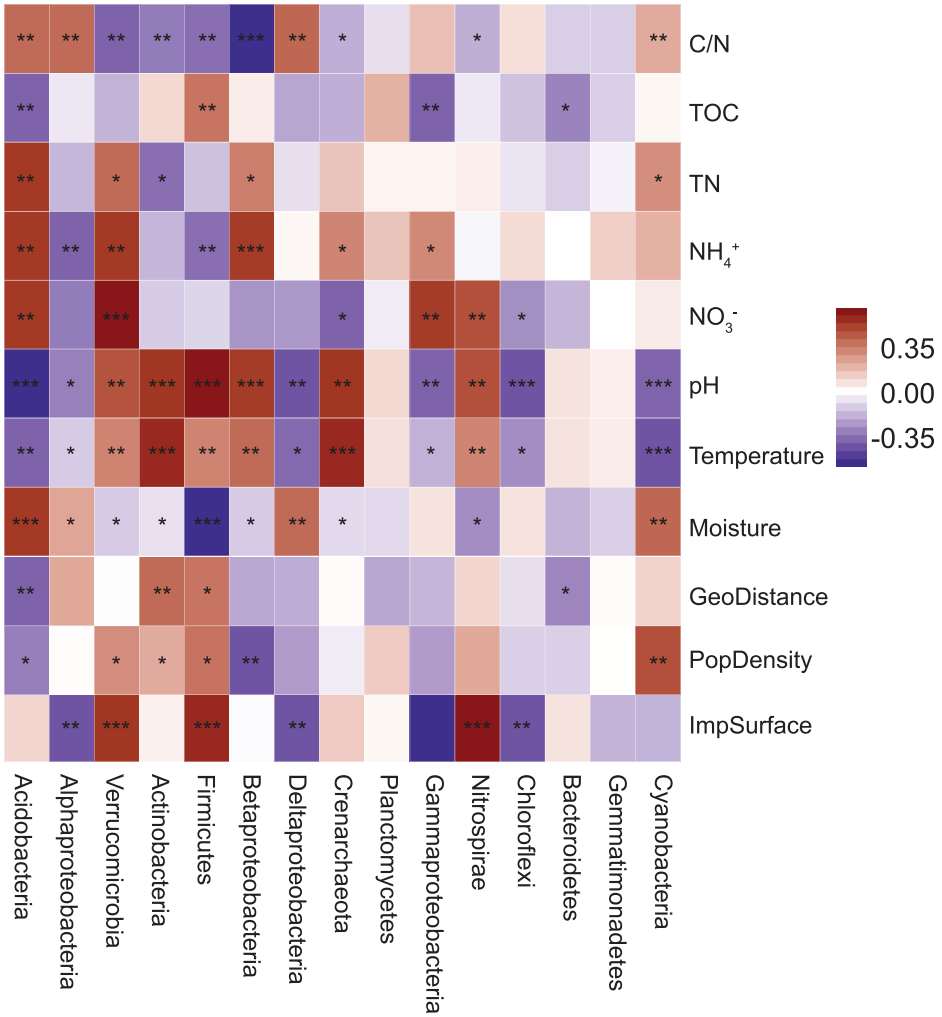

Fig. 3. Pearson correlations between soil physicochemical properties and the relative abundances of microbial taxa. The color gradient represents the strength of correlations. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

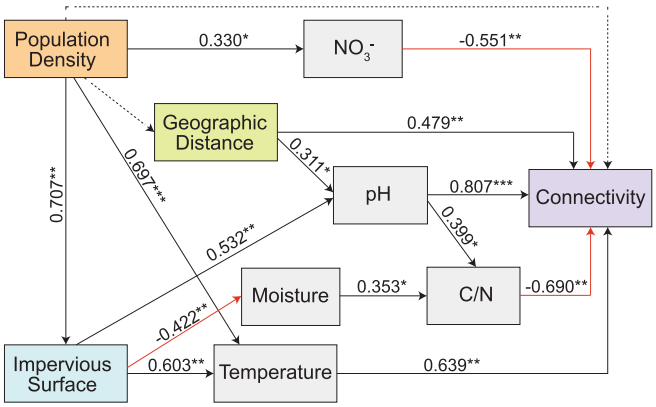

Fig. 4. Structural equation model (SEM) of impervious surface, population density, geographic distance and soil physicochemical factors as indicators of microbial network connectivity. Solid black arrows represent positive paths, solid red arrows represent negative paths, dashed arrows indicate nonsignificant paths. Path coefficients are standardized. $^{*}P < 0.05$. $^{**}P < 0.01$. $^{***}P < 0.001$. A nonsignificant P - value ($P > 0.05$) for the statistic indicates that there was no significant difference in the covariance pattern predicted by the SEM and from the observed covariance, indicating a good fit with the data. AIC, akaike information criterion. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

indicated that human population density affected microbial connectivity indirectly through impervious surface and soil factors including pH, temperature, moisture, C/N and NO_3^- (Fig. 4). The most important factor that affected microbial connectivity was pH, followed by C/N (Table S2). Impervious surface had a stronger impact on microbial connectivity as compared to human population density (Table S2), and showed indirect impacts through soil temperature, moisture, pH and C/N. In addition, geographic distance affected microbial connectivity both directly and indirectly through pH and C/N.

4. Discussion

Microbial communities with diverse compositions and variable abundances in soils function as ecological associations and respond to environmental drivers at different spatial and temporal scales (Lidicker, 1979). Given the importance of microbial communities in biogeochemical processes and ecosystem functioning, understanding microbial interactions is vital to develop ecosystem models to predict how communities assemble and respond to environmental changes driven by urbanization. Our results suggested that soil microbial taxa were not randomly distributed but showed module patterns along the urbanization gradient. The high dissimilarity scores for the distinct modules comprised of urban, suburban and natural nodes revealed that the microbial network was organized with different functional groups in response to variations in impervious surface, demographic, geographic and soil physicochemical characteristics. Differences in the topological features of urban-, suburban- and natural-associated modules indicated distinct traits of microbial communities from respective soil samples, and thus likely different ecological functions.

Based on the sequencing data, we found significant differences in the abundances of microbial taxa along the urbanization gradient. The variations in the abundances of *Alphaproteobacteria* and *Firmicutes* among different samples with contrasting levels of land cover imperviousness agreed with previous studies that reported great changes of spatial patterns in their abundances during land-use changes in the Amazon rainforest (Navarrete et al., 2015; Khan et al., 2018). In this study, the variations in the abundances of major taxa were mostly due to the selective pressure of soil pH, temperature, moisture and C/N (Figs. 3 and 4). The strong correlations of soil pH and temperature with microbial communities were in line with previous studies where similar correlation patterns were observed in alkaline permafrost-affected soils and tropical forest soils (Zhang et al., 2014; Navarrete et al., 2015). The urban soils were characterized by low moisture and C/N and surrounded by more impervious surfaces compared to the suburban and natural soils, which explained the low abundances of *Acidobacteria* and *Alphaproteobacteria*, which were highly correlated with soil moisture and C/N in this study. The high abundance of *Verrucomicrobia* in the urban soils was probably due to its high level of adaptation to urban soil environments, which is a function of its slow-growing and oligotrophic nature (Senechkin et al., 2010). The relationship of soil C/N with microbial composition might be related to the covariation of soil physicochemical characteristics, because variations in temperature and pH might change biochemical processes (e.g., degradation of organic matters, denitrification and nitrification) by affecting microbial activities, leading to fluctuations in nutrient composition (Khan et al., 2018).

The smaller amounts of nodes and edges in urban modules than in suburban and natural modules might be due to increased human disturbances in urban areas. Previous studies examining the impacts of environmental factors on microbial network demonstrated increase in network modularity as environmental disturbances increased (Parter et al., 2007; Hosen et al., 2017). Accordingly, we found lower betweenness centrality and transitivity scores for the urban network, which suggested a reduction of microbial taxa that played a core role in highly urbanized areas, as some previously well-adapted taxa might become inactive and/or less functional under urbanized conditions (Stewart and Ulloa, 2012). In addition, the lower betweenness centrality for the urban modules implied that the microbial communities at urban sites seemed to reside more in peripheral areas, where soil biotic and abiotic factors had greater impacts on microbial communities. A high urbanization level with large impervious surfaces exacerbated the deterioration of soil physicochemical conditions, which required multiple microbial taxa to interact synergistically to protect against external disturbances (Khan et al., 2018). The closer relationships among urban nodes could also be evidenced by the higher closeness and intra-connectivity for the urban modules. In this study, we found that differences in the network topology were mostly explained by soil pH and C/N. To a certain extent, this result indicated that the oligotrophic conditions (high pH and low C/N) in urban soils contributed to relatively higher microbial heterogeneity and larger niche differentiation (Faust and Raes, 2012).

To better understand microbial taxa with similar habitat-adaptation strategies or ecological functions, three most connected modules with the highest connectivity scores for urban, suburban and natural nodes were chosen as representatives of soil samples from contrasting urbanization levels (Lv et al., 2017a). **(1) Urban module.** We found that several nodes of the urban module were associated with nitrification performed by *Nitrospira* and *Cenarchaeales* (Leininger et al., 2006; Daims et al., 2015), and denitrification performed by *Rhodoplanes* and *Canditatus* (Hiraishi and Imhoff, 2005; Lv et al., 2017b). That the betweenness centrality of *Nitrospira* was the highest in the urban module indicated its important role in the network, which is in line with previous studies that identified *Nitrospira* as playing a key role in urban areas with extensive human disturbances (Wang et al., 2017; Khan et al., 2018). In contrast, *Rhodoplanes* categorized as peripheral nodes with the lowest betweenness centrality in the urban module, was

mainly involved in the production of N_2O during denitrification (Hiraishi and Imhoff, 2005). **(2) Suburban module.** Similarly, the higher betweenness centrality of nodes associated with *Nitrosphira* than those associated with *Rhodoplanes* and *Canditatus* in the suburban modules indicated relatively high nitrification potential with a transitional spectrum of the urbanization gradient. Two nodes with lower betweenness centrality scores in the suburban module were associated with *Ammoniphilus*, which has genetic dependence on ammonia (Zaitsev et al., 1998). **(3) Natural module.** Two of the natural nodes with relatively low betweenness centrality were found to be related to *Cenarchaeales*, which is known for ammonium oxidation (Leininger et al., 2006). The highest betweenness centrality in the natural module was found for a node associated with *Canditatus Solibacter*, which is involved in the reduction of nitrate and nitrite (Pearce et al., 2012). Several nodes with relatively high betweenness centrality scores in the natural modules were associated with *Rhodoplanes*, suggesting potential N loss through N_2O emissions from surface soils in lands towards the natural spectrum of the urbanization gradient. The succession of microbial processes within the N cycle along the urbanization gradient revealed the impacts of human disturbances on N_2O flux from the surface soils investigated. With increased urbanization, changes in the microbial taxa with high betweenness centrality in the network suggested that these key microbes were susceptible to alterations in land cover imperviousness, and hence provided space for other microbes to dominate in an altered community (Sole and Montoya, 2001). In addition, we found that some taxa, such as *Nitrospira*, had a high network centrality score, but was less abundant in urban soils, while some taxa including *Firmicutes* and *Canditatus* had low network centrality scores but had high abundances. Differences in the patterns of network centrality and microbial abundance have also been found for microbial taxa in oil palm soils (Wood et al., 2017) and plant ecosystems (Jain et al., 2014), which might be due to the fraction of the life strategies of microbial communities from their functional traits (Martiny et al., 2015).

In the context of urbanization, understanding the patterns and determinants of microbial connectivity is important for the prediction of their ecological processes and functions (Barberán et al., 2012). Our results provide an evidence that impervious surfaces coupled with human population density could affect microbial connectivity indirectly by changing soil physicochemical characteristics. Microbial connectivity was found to be positively correlated with these two factors, indicating that tight interactions among microbial community members could be expected in highly urbanized watersheds with both large impervious surfaces and high human population density. Watershed urbanization with more populations and building constructions feature a range of human activities, including settlements, traffic and industry, which result in soil physicochemical heterogeneity (Yan et al., 2016). These associations contributed to the indirect impacts of human population density on microbial connectivity through impervious surfaces in the watershed as shown in our results (Fig. 4). That impervious surfaces had a stronger impact than human population density indicated that microbial connectivity seems to be more affected by land cover imperviousness in watersheds. Although the impacts of geographic distance on microbial community structure have been reported at continental and city scales (Yuan et al., 2008; Wang et al., 2018), there is limited support on its impacts at a watershed scale. In this study, the close relationship of microbial connectivity with geographic distance in the watershed suggests that land scale was an important factor that affects microbial biogeography and should be considered in synergistic understandings of community structure and function patterns.

5. Conclusions

This study provides insights into the causal relationships among impervious surface, demographic, geographic and soil physicochemical characteristics and microbial connectivity at a watershed scale. Increased impervious surfaces significantly changed microbial composition with a decreased microbial diversity in soils. Network analyses suggested losses of key taxa but increased intramodular connections with increased impervious surfaces, which had profound impacts on microbial N processes like nitrification and denitrification. Impervious surfaces had indirect impacts on microbial connectivity by altering soil factors including pH, temperature, moisture, C/N and NO_3^- and influenced microbial connectivity far more than human population density. The results could inform urban planning and land management, thereby improving sustainability of urban development. This study is the first to evaluate the complex direct and indirect associations at a watershed scale. Given that urban spaces tend to expand with extensive development of impervious surfaces and increasing population density, revealing the causal relationships of these factors and their impacts on microbial connectivity is important for synthetically understanding the ecological-functional consequences of human disturbances.

CRediT authorship contribution statement

Yan Zhang: Conceptualization, Methodology, Investigation, Writing - original draft. **Guodong Ji:** Writing - review & editing, Visualization. **Tong Wu:** Writing - review & editing. **Jiangxiao Qiu:** Writing - review & editing.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2019.113708.

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