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Abstract

Microbiota in urban green spaces underpin ecosystem services that are essential to environmental health and human wellbeing. However, the factors shaping the microbial communities in urban green spaces, especially those associated with turf grass phyllosphere, remain poorly understood. The lack of this knowledge greatly limits our ability to assess ecological, social and recreational benefits of urban green spaces in the context of global urbanization. In this study, we used amplicon sequencing to characterize soil and grass phyllosphere bacterial communities in 40 urban green spaces and three minimally disturbed national parks in Victoria, Australia. The results indicated that urbanization might have shown different impacts on soil and grass phyllosphere microbial communities. The bacterial diversity in soil but not in grass phyllosphere was significantly higher in urban green spaces than in national parks. Principal coordinate analysis revealed significant differences in the overall patterns of bacterial community composition between urban green spaces and national parks for both soil and grass phyllosphere. Industrial development, as represented by the number of industries in the region, was identified as a key driver shaping the bacterial community profiles in urban green spaces. Variation partitioning analysis suggested that industrial factors together with their interaction with other factors explained 20% and 28% of the variances in soil and grass phyllosphere bacterial communities, respectively. The findings highlight the importance of industrial development in driving the spatial patterns of urban microbiomes, and have important implication for the management of microbiomes in urban green spaces.

Keywords	green spaces; urban microbiome; human health; urbanization; phyllosphere
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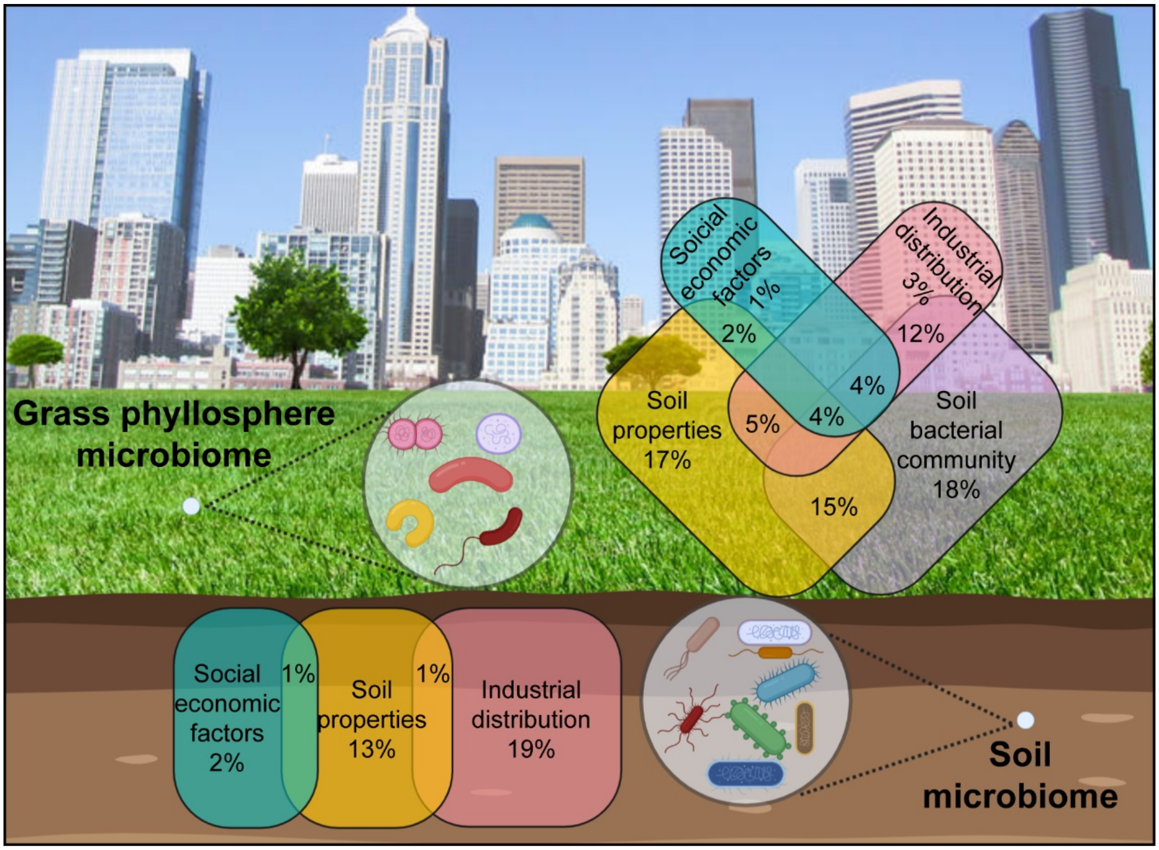
Highlights

- Urbanization differently impacts soil and grass phyllosphere microbiota.
- Soil bacterial diversity is higher in urban green spaces than national parks.
- Industrial development was a key driver shaping the bacterial communities.

Graphic abstract



Industrial development as a key factor explaining variances in soil and grass phyllosphere microbiomes in urban green spaces

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Abstract

Microbiota in urban green spaces underpin ecosystem services that are essential to environmental health and human wellbeing. However, the factors shaping the microbial communities in urban green spaces, especially those associated with turf grass phyllosphere, remain poorly understood. The lack of this knowledge greatly limits our ability to assess ecological, social and recreational benefits of urban green spaces in the context of global urbanization. In this study, we used amplicon sequencing to characterize soil and grass phyllosphere bacterial communities in 40 urban green spaces and three minimally disturbed national parks in Victoria, Australia. The results indicated that urbanization might have shown different impacts on soil and grass phyllosphere microbial communities. The bacterial diversity in soil but not in grass phyllosphere was significantly higher in urban green spaces than in national parks. Principal coordinate analysis revealed significant differences in the overall patterns of bacterial community composition between urban green spaces and national parks for both soil and grass phyllosphere. Industrial development, as represented by the number of industries in the region, was identified as a key driver shaping the bacterial community profiles in urban green spaces. Variation partitioning analysis suggested that industrial factors together with their interaction with other factors explained 20% and 28% of the variances in soil and grass phyllosphere bacterial communities, respectively. The findings highlight the importance of industrial development in driving the spatial patterns of urban microbiomes, and have important implication for the management of microbiomes in urban green spaces.

Main findings

- Urbanization might differently impact soil and grass phyllosphere microbial communities in urban green spaces.

- Industrial development was a key driver shaping the bacterial community profiles in urban green spaces.
- The impact of urbanization on the bacterial communities might derive from increased environmental pollution.

Key words: green spaces, urban microbiome, human health, urbanization, phyllosphere

1. Introduction

Microorganisms, as a vital component of the urban ecosystem, play a critical role in regulating ecosystem services (Eldridge et al., 2019, Hui et al., 2017, Kabisch, 2015), such as pollutant remediation, nutrient cycling and genetic diversity preservation (Escobedo et al., 2011, Groffman et al., 2009, Ramirez et al., 2014, Strohbach and Haase, 2012). These ecosystem functions are important in reducing the growing global burden of urban-associated chronic diseases and maintaining the wellbeing of urban environment and city dwellers. There has been evidence that exposure of humans to microorganisms associated with soil and plants in urban green spaces could reduce the dysfunction of immune system and supress inflammation (Flies et al., 2017, Flies et al., 2018). During the past several decades, the massive transition of human population from rural to urban living has resulted in dramatic alternations of land use types which may consequently change the environmental habitats for microorganisms (Grimm et al., 2008, Li et al., 2018, Zhu et al., 2011). A better understanding of how anthropogenic disturbance impacts microbial community profiles in the urban environment is essential to prediction and mitigation of the influence of urbanization on ecosystem functioning (Falkowski et al., 2008, Kaye et al., 2005, Reese et al., 2016).

Soil and the aerial part (phyllosphere) of turf grass are the two largest habitats for microorganisms in urban green spaces (Crowther et al., 2019, Lindow and Brandl, 2003, Peñuelas and Terradas, 2014, Vorholt, 2012). Urban ecosystem is subject to intensive management practices, and therefore the microbial communities in urban green spaces are not only influenced by environmental factors (e.g. soil properties and climate factors) (Hui et al., 2017, Wang et al., 2018a) but also by anthropogenic activities. Urban environment is considered as a social economic ecosystem (Cadenasso et al., 2007, Pickett et al., 2011) characterized by high human population density and rapid economic growth (Wang et al., 2018a, Zhu et al., 2017). The increasing demand for material by a growing population is usually associated with urban expansion, resulting in an increasing number of industries in the region, which is considered as another important measurement of urbanization (Xu et al., 2014). To date, a few studies have reported that urbanization can substantially change the microbiota in urban green spaces (Francini et al., 2018, Hui et al., 2017, Ramirez et al., 2014, Wang et al., 2018a, Xu et al., 2014), but it remains largely unknown which aspects of urbanization account for the major variance in microbial community profiles in urban green spaces. Additionally, previous studies have mostly focused on the impact of urbanization on soil microbiota but overlooked the plant phyllosphere microbiota despite the significance of phyllosphere as an important environmental habitat for microorganisms. As a vast environment with an estimated area of >1 billion km^2 across the globe (Vorholt, 2012, Woodward and Lomas, 2004), phyllosphere is described as a formidable playground for testing fundamental ecological principles in microbiology (Meyer and Leveau, 2012). In addition to promoting plant growth and production (Canto and Herrera, 2012, Grady et al., 2019, Taghavi et al., 2009), phyllosphere microbiomes have other important ecosystem functions, for example participating in Earth's biogeochemical cycles through moderating plant ethanol emission and contributing to nitrogen fixation (Galbally and Kirstine, 2002, Fürnkranz et al., 2008). In addition, phyllosphere microbiomes are closely linked to human health as phyllosphere is the potential bridge between environmental microbiome and human microbiome (Chen et al., 2018).

In this study, we used Illumina Miseq sequencing to analyse microbial communities in soil and grass phyllosphere samples collected from 40 urban green spaces across the Greater Melbourne metropolitan. As the capital of Australian state Victoria and the second most populous city in Oceania (ABS, 2019), Melbourne is a highly urbanized city with a long legacy of public green space development. In addition, samples from three national parks from remote Victoria were studied as representative of environments with minimal anthropogenic disturbance. Multiple factors including soil properties and anthropogenic parameters were collected to interpret their impacts on the microbial community profiles in urban green spaces. The main aims of this study were to (i) determine the potential differences and links between soil and grass phyllosphere bacterial communities; (ii) compare the bacterial community profiles in highly urbanized environment with those in minimally disturbed natural environment; and (iii) determine the most important factors shaping the bacterial community profiles in urban green spaces.

2. Material and methods

2.1. Sampling

Soil and grass samples were collected during May 2018 from 40 urban parks across Greater Melbourne metropolitan and three national parks in remote Victoria, Australia. All samples were collected on sunny or cloudy days with no rainfall event. The daytime temperature during the sample collection period ranged from 12.6 °C to 19.5 °C. At each park, three independent samples for each sample type (i.e. soil and grass) were collected. The surface 5 cm of soil and aerial parts of grass were collected. All samples were immediately transported in sterilized plastic bags on ice to the laboratory after collection. In total, for each sample type, 120 samples were collected from urban green spaces and nine samples were collected from national parks, resulting in a total of 258 soil and grass samples. Detailed information of sampling sites is provided in Table S1. A portion of soil samples were air dried and gently crumbed to pass through a 2 mm sieve for characterization of soil properties. The rest of the samples were stored at -80 °C prior to DNA extraction.

2.2. Soil properties characterization

Soil pH and electronic conductivity (EC) were measured in 1:5 soil water suspension using Orion Star A215 pH/Conductivity Benchtop Multiparameter Meter (Thermo Scientific

Inc., Waltham, MA, USA). Soil cation change capacity (CEC) was determined by the concentration of exchangeable bases (Ca^{2+}, Mg^{2+}, Na^{+}, and K^{+}) in 1 M ammonium acetate at pH 7 measured using an Optima 8300 ICO-OES spectrometer (PerkinElmer, Waltham, MA, USA). Soil total carbon (TC) and total nitrogen (TN) were measured by Dumas combustion method using LECO FP628 analyser (LECO Corp, Michigan, USA). Soil mineral nitrogen including extractable ammonium and nitrate were measured in 1 M KCl solution using Skalar San^{++} continuous flow analyser (Skalar Analytical B.V. Tinstraat, Bread, Netherland).

2.3. Anthropogenic parameters collection

Anthropogenic parameters of the Statistical Area Level 2 (SA2), in which the investigated urban green spaces are located, were collected from Australia Bureau of Statistic (ABS) website (https://itt.abs.gov.au/itt/r.jsp?databyregion#/). SA2s are medium sized statistical area that represent socially and economically interacted communities and significant urban areas in the Australia Statistical Geography Standards. SA2s are primary output regions of ABS non-census and intercensal data (ABS, 2016). In this study, the 40 urban green spaces investigated are in 40 SA2s across Greater Melbourne metropolitan without overlap. Data of five anthropogenic parameters were collected, and these parameters were divided into two categories: social economic factors including population density (people km^{-2}) and median equivalised total household income (weekly AU$); and industrial factors including total number of industries, number of construction and manufacturing industries (Table S2).

2.4. DNA extraction and sequencing

Soil and grass phyllosphere DNA were extracted using the DNeasy PowerSoil Kit (QIAGEN Pty Ltd., Hilden, Germany). Soil DNA was extracted from 0.25 g soil according to the supplier’s manual. Grass phyllosphere DNA was extracted using a previous method

(Chen et al., 2017, Zhang et al., 2019) with some modifications. Briefly, 5 g grass was weighted into a 50 ml sterilized centrifuge tube containing 45 ml autoclaved 0.01 M phosphate buffered saline with 0.02% Tween 20. The tubes were shaken at 30 °C and 200 rpm for 2 h using a shaking incubator. After shaking, the solution was filtered through a 40 µm nylon cell strainer into a new sterilized 50 ml centrifuge tube, and centrifuged at 4500 rpm for 30 min. After centrifugation, the supernatant was discarded, and the obtained pellets were transferred into the PowerBead tubes provided by the DNA extraction kit. The quality and concentration of the extracted DNA were assessed by agarose gel electrophoresis and spectrophotometric analysis using a NanoDrop ND2000c spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA).

The V3 – V4 hypervariable region of the 16S rRNA gene was amplified using the primer pair 341F and 806R for sequencing (Klindworth et al., 2013). Sequencing was performed on the Illumina Miseq platform at Australian Genome Research Facility. The PCR amplification protocol for the Illumina library generation was as follows: an initial enzyme activation and denaturation at 94 °C for 3 min, followed by 32 cycles of 94 °C for 45 s, 55 °C for 45 s and 72 °C for 90 s with a final extension at 72 °C for 10 min. After sequencing, the barcode and adaptor sequences were removed, and ambiguous nucleotides and low-quality reads were filtered. The cleaned raw pair-ended sequencing reads were merged using FLASH (Magoč and Salzberg, 2011) and demultiplexed using Quantitative Insight into Microbial Ecology (QIIME) (Caporaso et al., 2010). Operational taxonomic unit (OTU) picking was conducted based on the threshold of 97% similarity level using UCLUST (Edgar, 2010). Chimeric and singleton OTUs, as well as chloroplast and mitochondrial OTUs were removed from the final OTU data set. Taxonomic classification and OTU quantification were performed against Greengenes 16S rRNA database version 13.8 (McDonald et al., 2012).

2.5. Statistical analysis

All statistical tests were considered significant at $P < 0.05$ level. Bacteria community compositions at the phylum level were visualized using the 'ggplot2' (Wickham, 2016) package in R (R Core Team, 2015). Log-transformed relative abundances of dominant genera identified in samples from different parks were present in heatmaps using the 'pheatmap' package (Kolde, 2012). Venn diagram was used to illustrate the shared and unique bacterial taxa in soil and grass phyllosphere samples using the 'VennDiagram' package (Chen and Boutros, 2011). Differences in bacterial α-diversity between soil and grass phyllosphere and samples from urban green spaces and national parks were explored using Mann-Whitney-Wilcoxon test and visualized using the 'ggplot2' package (Wickham, 2016). Principal coordinate analysis (PCoA) based on the Bray-Curtis distance was conducted to evaluate the separation of microbial communities using the 'labdsv' package (Roberts and Roberts, 2016). Adonis test and variation partitioning analysis (VPA) were conducted using the 'vegan' package (Oksanen et al., 2013). Significant differences in specific bacterial taxa between urban green spaces and national parks were identified by liner discriminant analysis (LDA) effect size (LEfSe) according to the instructions (http://huttenhower.sph.harvard.edu/galaxy/root). The threshold on the LDA logarithmic was set at 2.0 and $\alpha = 0.01$ (Chen et al., 2017). The correlations between microbial community composition at the genus level (first axis values of PCoA analysis) and soil properties and anthropogenic parameters were explored by Spearman's ranked correlation analysis using the 'psych' package (Revelle and Revelle, 2015). P values of the Spearman's correlation analyses were corrected using Benjamini-Hochberg procedure. Ordinary least square (OLS) regression models were used to determine the linear relationships between microbial community composition and anthropogenic parameters and soil properties, and were visualized using the 'ggplot2' package (Wickham, 2016).

2.6 Availability of data and materials

Raw sequencing data of 16S rRNA gene of the soil and grass phyllosphere samples were deposited in the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) under the accession number of PRJNA549108.

3. Results

3.1. Soil properties

Three independent soil samples were collected from each of the 40 urban green spaces. In total, 120 soil samples were characterized for physiochemical properties. The soil properties, including soil pH, EC (ds m^{-1}), CEC (com kg^{-1}), TC (%), TN (%), ammonium (mg L^{-1}) and nitrate (mg L^{-1}) contents are summarised in Table S3. Soil pH ranged from 5.56 to 7.98, and more than 80% of the soils had pH values below 7. Only 23 soil samples were alkalescent or alkaline. Soil EC ranged from 0.06 (ds m^{-1}) to 0.91 (ds m^{-1}). In general, nutrients in the soil samples were low with total C and N ranging from 1.70-9.71% and 0.16-0.91%, respectively.

3.2. Characteristics of bacterial communities in soil and grass phyllosphere

Bacterial community profiles in soil and grass phyllosphere at different taxonomic levels from phylum to genus were characterised. After assembling and quality filtering, 8,732,777 and 9,783,785 high quality sequences were obtained for soil and grass phyllosphere samples, respectively. The sequences were clustered into OTUs at 97% similarity level.

For soil samples, 1,071,713 OTUs were assembled into 50 phyla, 174 classes, 374 orders, 633 families, and 1,246 genera. At the phylum level, Actinobacteria (accounting for 40.74% of sequences) were the most abundant phylum detected in soil samples from urban green spaces, followed by Proteobacteria (31.97%) and Acidobacteria (10.48%). (Fig. S1). Soils from national parks were dominated by the phylum Proteobacteria (66.71%) followed by the second and third most abundant phyla of Actinobacteria (13.71%) and Verrucomicrobia (8.44%) (Fig. S1). There was no significant difference between the bacterial community compositions among samples from different urban parks. Soils from all 40 urban parks were dominated by Actinobacteria and Proteobacteria, which accounted for 18.21-56.39% and 22.48-41.68% of the sequences of samples from different parks, respectively (Fig. S2A). Compared with soils from urban parks, the dominance of the phylum Proteobacteria was more apparent for soils from national parks. For soil samples from all three national parks, Proteobacteria accounted for more than half of the sequences (Fig. S2A). At the genus level, 374 genera were detected in all investigated parks. According to the heatmap showing the abundances of the 48 dominant genera (defined as those accounting for > 5% sequences in each sample (Wang et al., 2018b)), the bacterial communities were dominated by a few genera of the phyla Actinobacteria and Proteobacteria in soil samples from most of the investigated parks (Fig. S3A).

For grass phyllosphere samples, 389,809 OTUs were assembled into 40 phyla, 131 classes, 260 orders, 456 families and 946 genera. At the phylum level, Proteobacteria (accounting for 77.17% and 72.54% of sequences for samples from urban parks and national parks, respectively), Bacteroidetes (17.99% and 8.77%), and Actinobacteria (2.95% and 8.49%) were the three most abundant phyla for samples from both urban green spaces and national parks (Fig. S1). Only seven phyla were detected in grass phyllosphere from all investigated parks (Fig. S2B). For samples from both urban and national parks, microbial communities were dominated by the phylum Proteobacteria, which accounted for 42.18-95.31% and 63.35-79.45% of sequences, respectively (Fig. S2B). At the genus level, 81 genera were detected in all investigated parks. The abundances of all these genera are presented by heatmap (Fig. S3B). There was no significant difference in the bacterial communities between urban and national parks and among different urban parks. Bacterial communities of grass phyllosphere samples from all the investigated parks were dominated by a few bacterial genera belonging to the phyla Proteobacteria and Bacteroides (Fig. S3B).

The shared and and unique bacterial genera in soil and grass phyllosphere sample were explored using the Venn diagram. A total of 855 bacterial genera were detected in both soil and grass phyllosphere samples, 400 genera were detected only in soil samples, and 91 genera only in grass phyllosphere samples (Fig. 1A). The α diversity as revealed by the Shannon index was significantly higher (Mann-Whitney-Wilcoxon test, $P < 0.001$) in the soil than that in the grass phyllosphere (Fig. 1B). PCoA analysis based on the Bray-Curtis distance revealed a significant difference in microbial communities at the genus level between soil and phyllosphere (Adonis test, $P < 0.001$), which were well separated along the PCo1 axis (Fig. 1C).

3.3. Soil and grass phyllosphere bacterial communities in urban green spaces and national parks

The soil from urban green spaces had significantly higher α diversity than those from national parks (Mann-Whitney-Wilcoxon test, $P < 0.001$) (Fig. S4A). There was no significant difference between the α diversity of grass phyllosphere from urban green spaces and national parks (Fig. S4A). The β diversity analysis indicated that the overall compositions of bacterial communities in urban green spaces were distinct from those in national parks for both soil and grass phyllosphere at the genus level (Adonis test, $P < 0.001$) (Fig. S4B).

Bacterial taxa that were significantly different between the urban green spaces and national parks were identified by LEfSe analysis and visualized in cladogram (Fig. 2). A total of 29 bacterial taxa were significantly enriched in the soil from urban green spaces ($P < 0.05$), for example, the phyla Actinobacteria, Chloroflexi, Firmicutes, Gemmatimonadetes, and Nitrospira were significantly enriched in soil from urban green spaces (Fig. 2). In contrast, only two bacterial taxa were significantly enriched in soil from national parks (Fig. 2). As for grass phyllosphere, only six bacterial taxa had higher relative abundances in samples from urban green spaces, whereas 19 bacterial taxa belonging to the phyla of Acidobacteria, Chloroflexi, and Verrucomicrobia, as well as Alphaproteobacteria, and Deltaproteobacteria of the phylum Proteobacteria were significantly enriched in samples from national parks (Fig. 2).

3.4. Factors shaping the bacterial community compositions in urban green spaces

Spearman's ranked correlation analysis revealed that bacterial community compositions in soil and grass phyllosphere were significantly correlated with a range of anthropogenic parameters including population density (people km^{-2}), median equivalised total household income (weekly AU$), and total number of industries ($P < 0.01$) (Table 1). However, only one soil property, soil EC (ds m^{-1}), was significantly correlated with soil bacterial community composition ($P < 0.01$) (Table 1). Similarly, grass phyllosphere bacterial community composition was significantly correlated with various anthropogenic parameters but had no significant correlation with any soil property analysed (Table 1). Similar to the results of correlation analysis, OLS regression model revealed that soil EC was the only soil property significantly linearly correlated with the soil bacterial community composition (Fig. 3). Soil properties including soil EC, CEC, TN, and TC were significantly linearly correlated with the bacterial community composition of grass phyllosphere samples (Fig. 3). According to the OLS regression analysis results, all analysed anthropogenic parameters except the number of construction industries were significantly related to soil bacterial community composition (Fig. 4). Similarly, three out of the five analysed anthropogenic parameters including median equivalised total household income, total number of industries, and the number of construction industries were significantly related to the bacterial community composition in grass phyllosphere (weekly AU$), (Fig. 4).

VPA analysis was conducted to decipher the key contributors to the variance in soil and grass phyllosphere bacterial communities in urban green spaces. For soil, the selected variables explained 36% of the variance in bacterial community (Fig. 5A). Soil properties and social economic factors accounted for 13% and 2% of the variance, respectively (Fig. 5A). The interaction between soil properties and social economic factors and industrial factors both explained 1% of the variance (Fig. 5A). Notably, industrial factors individually contributed 19% to the variance, and were the most important factors shaping the soil bacterial community composition (Fig. 5A). For grass phyllosphere, the selected variables explained 89% of the variance in bacterial community (Fig. 5B). Compared to soil, the contribution of anthropogenic parameters to the variance in bacterial community was less apparent for grass phyllosphere. Social economic factors and industrial factors individually explained only 1% and 3% of the variances, respectively (Fig. 5B). Soil related biotic and abiotic factors, including soil microbial community and soil properties, as well as their interactions, explained 50% of the variance in grass phyllosphere bacterial community (Fig. 5B). However, it should be noted that industrial factors still played an unneglectable role in shaping grass phyllosphere bacterial community through interacting with other factors, which explained 25% of the variance (Fig. 5B).

4. Discussion

4.1. Distinct responses of soil and grass phyllosphere bacterial communities to urbanization

The impact of urbanization on microbial diversity has received substantial attention, because loss of microbial diversity could potentially lead to serious environmental and human health problems (Philippot et al., 2013, Rook, 2009, Wang et al., 2018b). The significantly higher soil bacterial α diversity in urban green spaces than in national parks (Fig. S4A) indicated that urbanization might lead to an increase in soil bacterial diversity. Other studies of soil microbial diversity in parks of highly urbanized cities have reported similar results. A prior study reported that as a proxy of urbanization, the increase in human population density is associated with the increase in soil bacterial α diversity in urban green spaces in Chicago, USA (Wang et al., 2018a). Another study reported that urban park soils from Shanghai, China had a high bacterial diversity (Wang et al., 2018b). One potential explanation for the higher soil bacterial diversity in urban green spaces could be that, different from soils in national parks with minimal disturbance, soils in urban green spaces are commonly under intensive management. For example, fertilization and irrigation, which may increase the environmental heterogeneity and provide diverse ecological niches for soil microbes, contribute to a higher soil bacterial α diversity. Another key element that could contribute to the elevated level of bacterial diversity in urban environment is environmental pollution. The increase in population density and excessive use of urban land are associated with increased level of various organic and inorganic pollutants (Liang and Yang, 2019), which may also increase the habitat heterogeneity and diversify the nutrient sources, resulting in the elevated bacterial diversity in urban green spaces. However, Xu et al. (2014) suggested that soil bacterial diversity in urban parks in China was not associated with urbanization factors. Multiple factors could lead to the inconsistency in results across different studies, such as differences in laboratory methods, sampling strategies, and sample handling practices. In this case, the range of study area could be one of the main reasons for the discrepancies among study results. This study, Wang et al. (2018a) and Wang et al. (2018b) investigated urban green spaces in only one metropolitan area. However, Xu et al. (2014) collected samples from 16 cities across China. As the largest developing country, the urbanization rate of China is rapid and has increased greatly during the last decade (Liang and Yang, 2019). Therefore, the disagreement among studies could also derive from the different levels of urbanization. The inconsistency in results also indicated the complexity of factors influencing microbial diversity in the urban environment. Therefore, it is necessary to identify the key factors shaping the soil microbiome in the context of urbanization.

Different from previous studies focusing on soil microbiome only, this study also investigated the impact of urbanization on the grass phyllosphere microbiome. Phyllosphere is an enormous environment for ecosystem functions that is densely populated by diverse microorganisms (Vorholt, 2012, Woodward and Lomas, 2004). Bacteria, the most dominant group of microorganisms in the phyllosphere, can reach a surprisingly dense population of 10^5 bacterial cells per mm^{-2} leaf surface (Remus-Emsermann and Schlechter, 2018). The huge bacterial population in phyllosphere is composed mostly by commensals and beneficial symbionts, but also can include plant pathogens and even human pathogens (Dees et al., 2015, Delmotte et al., 2009, Lindow and Brandl, 2003; Yan et al., 2019). As turf grass is the direct intermedia between human skin microbiome and environmental microbiome, the microbial community in grass phyllosphere in urban green spaces could closely link to the health of urban residents (Chen et al., 2019). Our results suggested that compared to soil microbiome, the impact of urbanization on grass phyllosphere microbiome might be less apparent as there was no significant difference in phyllosphere bacterial α diversity between urban green spaces and remote national parks (Fig. S4A).

The significant differences in the bacterial community compositions (β diversity) between urban green spaces and national parks of both soil and grass phyllosphere revealed by PCoA analysis (Fig. S4B) indicated that urbanization could have altered both soil and grass phyllosphere microbial community profiles. However, the responses of soil and grass phyllosphere microbiomes to urbanization are different. According to the LEfSe analysis (Fig. 2), more bacterial taxa, including those associated with Actinobacteria, Chloroflexi, and Verrucomicrobia, were significantly enriched in soils from urban green spaces but significantly enriched in grass phyllosphere from national parks.

The potential different impacts of urbanization on soil and grass phyllosphere microbiomes could be attributed to the differences between the soil and phyllosphere environments. As the aerial part of plants, phyllosphere is a highly dynamic environment which is subject to frequent fluctuations of various environmental conditions, such as changes in temperature, moisture and UV radiation (Cordovez et al., 2019). All these factors could minimize the impact of urbanization on grass phyllosphere microbiota. Particularly, due to the ephemeral nature of grass, environmental pollutants cannot persist on grass phyllosphere for a long time. These factors could lead to no significant difference in the bacterial diversity between urban green spaces and national parks, and not many bacterial taxa enriched in grass phyllosphere. In contrast, soil is a relatively stable underground environment that is not easily impacted by environmental fluctuations but has a tight dependency on human regulation. Human activities could both directly shift the soil microbial community, for example bring adventitious microorganisms to the soil ecosystem, and indirectly influence soil microbiota by changing soil properties and thus modifying the habitats of microorganisms (Geisen et al., 2019). More importantly, it has been widely reported that environmental organic pollutants are persistent in soil (Jones and De Voogt, 1999, Klánová et al., 2008, Manz et al., 2001, Reid et al., 2000) and could have profound impact on soil microbial community.

4.2. Industrial development as an important factor shaping the bacterial communities in urban green spaces

We characterized the relationships between soil and grass phyllosphere bacterial community compositions in urban green spaces with a variety of factors including soil properties, social economic factors and industrial factors. Although several soil properties have been identified as key drivers shaping soil microbial community (Wang et al., 2018a, Xu et al., 2014, Yan et al., 2016), in the present study, surprisingly, soil properties were not among the most important factors shaping either soil or grass phyllosphere bacterial community profile. Particularly, soil pH, which has been widely reported to be a primary factor shaping soil microbial community profile (Hu et al., 2013, Xu et al., 2014, Yan et al., 2016), had no significant relationship with bacterial community composition of either sample type as suggested by the correlation (Table 1) and regression (Fig. 3) analyses. In this study, both the soil and phyllosphere bacterial communities in urban green spaces were dominated by Actinobacteria and Proteobacteria. These two bacterial taxa accounted for 72.71 % and 80.12% of bacterial sequences for soil and grass phyllosphere, respectively (Fig. S1). It has been reported that the abundances of these two taxa were not sensitive to soil pH (Xu et al., 2014, Yan et al., 2016). The weak correlation between soil pH and the bacterial community compositions could be attributed to the insensitivity of the dominant bacterial taxa to soil pH. In addition, because of the relatively uniform environmental condition of our sampling range, there was no great variance in soil properties from different sampling sites (Table S3). This suggests that compared with the differences in environmental factors among the sampling sites, the variance in the urbanization degrees of the regions might play a more significant role in shaping the bacterial community profiles. Based on the correlation and regression analyses, we found that anthropogenic changes, including changes in social economic factors and industrial factors, were important drivers shaping both soil and grass phyllosphere bacterial community profiles in urban green spaces.

Among the urbanization related anthropogenic changes, industrial development was proposed to be one of the key drivers shaping bacterial community profiles in urban green spaces. For soil, VPA analysis revealed that industrial factors (i.e. the number of industries in the region), which independently explained 19% of the total variance in soil bacterial community (Fig. 5A), was the dominant predictor among all selected variables. For grass phyllosphere, although industrial factors individually explained only 3% of the variance, the interaction between industrial factors and other factors, including social economic factors, soil properties, and soil bacterial community, together explained 25% of the variance in grass phyllosphere bacterial community (Fig. 5B). Such results indicate that industrial development might be a dominant factor in shaping both soil and grass phyllosphere bacterial community profiles. The crucial effect of industrial development on the urban might mainly derive from environmental pollution, which is a major consequence of industrialization (Cherniwchan, 2012, Liang and Yang, 2019). Air pollution due to industrial emission is a crucial problem being faced by regions with an increased number of industries (Chauhan and Pawar, 2010, Han et al., 2014), which could have subtle and overt impact on airborne microbial community (Babich and Lighthart, 1974). The replacement of natural land cover with impervious surface, such as cement roads, parking lots, and other constructions, in industrialized area could further contribute to the alternation of airborne bacterial community (Docherty et al., 2018, Mhuireach et al., 2016). The deposition of air particles is highly likely to result in changes in the bacterial community compositions of both surface soil and grass phyllosphere microbiotas in urban green spaces. In addition, heavy metal pollution in the surface soils resulting from industrial wastewater discharge is another key problem faced by regions undergoing industrialization (Dolar et al., 2016, Fiorentino et al., 2019, Hu et al., 2013) which could greatly impact soil microbial communities.

5. Conclusions

In this study, we comprehensively characterized the soil and grass phyllosphere bacterial communities in urban green spaces. Our findings indicated that urbanization could have altered bacterial community profiles, and the alternations were different for below ground (soil) and above ground (grass phyllosphere) microbiomes. Most importantly, we found that compared with soil properties, anthropogenic changes, particularly industrial development may play a more significant role in shaping bacterial community profiles in urban settings. These findings expended our knowledge of the microbiota in urban green spaces and could have important implication for achieving better urban planning and urban green space management practices toward maximising the benefits of urban infrastructure.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

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Appendix A. Supporting data

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Table 1. Spearman's ranked correlation coefficient between soil properties and anthropogenic parameters and microbial community compositions (the first axis values of PCoA analysis) in the soil and grass phyllosphere samples from urban green spaces.

	Soil properties							Anthropogenic parameters				
								Socio-economic factors		Industrial distribution		
	pH	EC (ds m^{-1})	CEC (cmol kg^{-1})	Total carbon (%)	Total nitrogen (%)	Ammonium (mg L^{-1})	Nitrate (mg L^{-1})	Population density (people km^{-2})	Median equivalised total household income (weekly AU$)	Total number of industries	Number of construction industries	Number of manufacturing industries
Soil bacterial community	-0.051	**-0.242****	-0.107	0.001	-0.023	0.104	0.155	**0.307****	**0.266****	**0.411***	0.151	0.010
Grass phyllosphere microbial community	0.108	-0.091	0.150	0.105	0.068	-0.011	0.141	0.118	**0.238****	**0.401****	**0.267****	0.172

** indicates significant level of $P < 0.01$. Significant correlations are shown in bold text

Legends

Fig. 1. Venn diagram showing the numbers of unique and shared genera in soil and grass phyllosphere samples (A). Boxplots showing the Shannon index (α diversity in soil and grass phyllosphere samples (B). *** indicates $P < 0.001$ determined by Mann-Whitney-Wilcoxon test. The white squares indicate the mean values. Principal coordinate analysis (PCoA) based on the Bray-Curtis distance showing the distribution patterns of microbiome in soil and grass phyllosphere (β diversity) (C).

Fig. 2. Cladograms of the LEfSe analysis results indicating differences of taxa between soil and grass phyllosphere in urban green spaces and national parks. The red and blue denote the taxon with significantly higher abundance ($\alpha > 0.01$, LDA score > 2.0) in urban green spaces and national parks, respectively. Yellow denotes that taxon not significantly different between urban green spaces and national parks.

Fig. 3. Ordinary least square (OLS) regression model showing the relationships between soil properties and microbial community compositions (the first axis values of PCoA analysis) in soil and grass phyllosphere in urban green spaces.

Fig. 4. Ordinary least square (OLS) regression model showing the relationships between anthropogenic parameters and microbial community compositions (the first axis values of PCoA analysis) in soil and grass phyllosphere in urban green spaces.

Fig. 5. Variation partitioning analysis (VPA) showing the effects of soil properties, soil bacterial communities, social economic factors and industrial factors on the alternation of soil (A) and grass phyllosphere microbial communities(B).

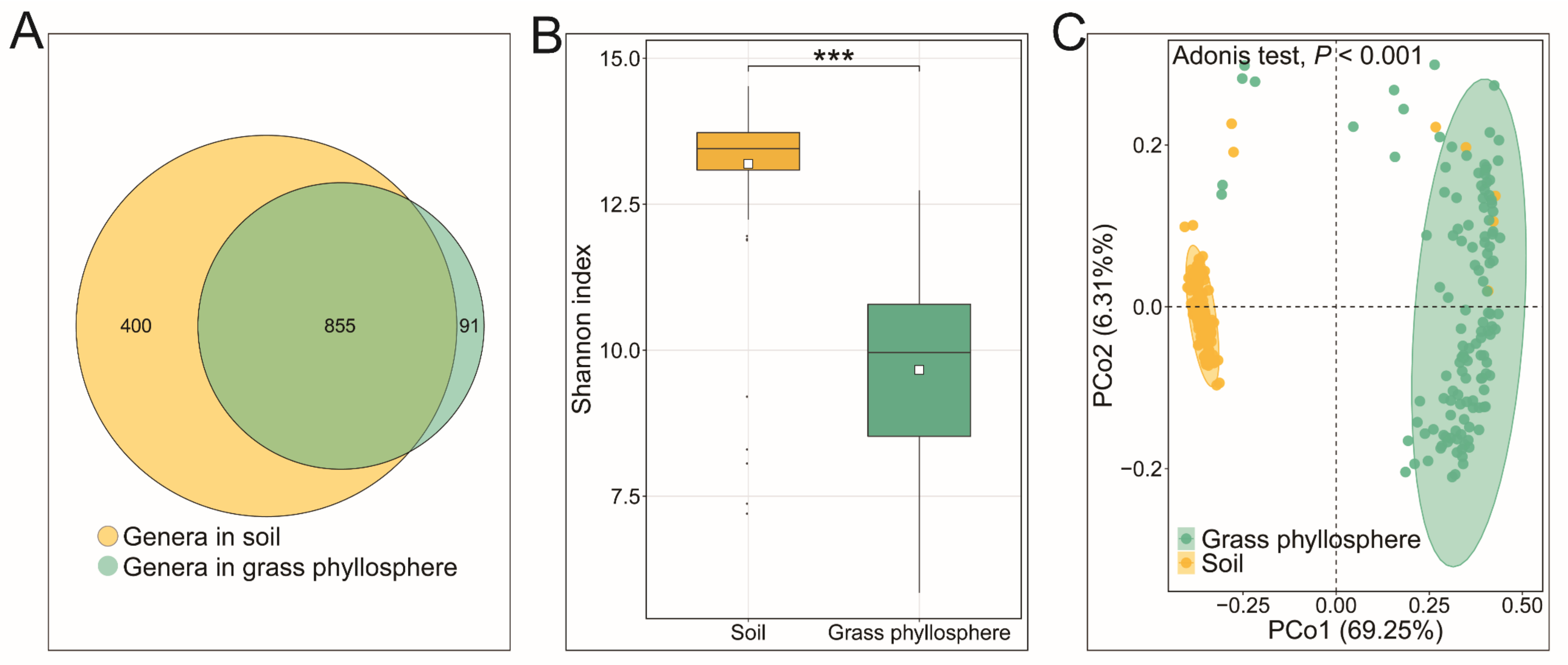


Fig. 1. Venn diagram showing the numbers of unique and shared genera in soil and grass phyllosphere samples (A), boxplots showing the Shannon index (α diversity in soil and grass phyllosphere samples. *** indicates $P < 0.001$ determined by Mann-Whitney-Wilcoxon test. The white squares indicate the mean values (B). and principal coordinate analysis (PCoA) based on the Bray-Curtis distance showing the distribution patterns of microbiome in soil and grass phyllosphere (β diversity) (C).

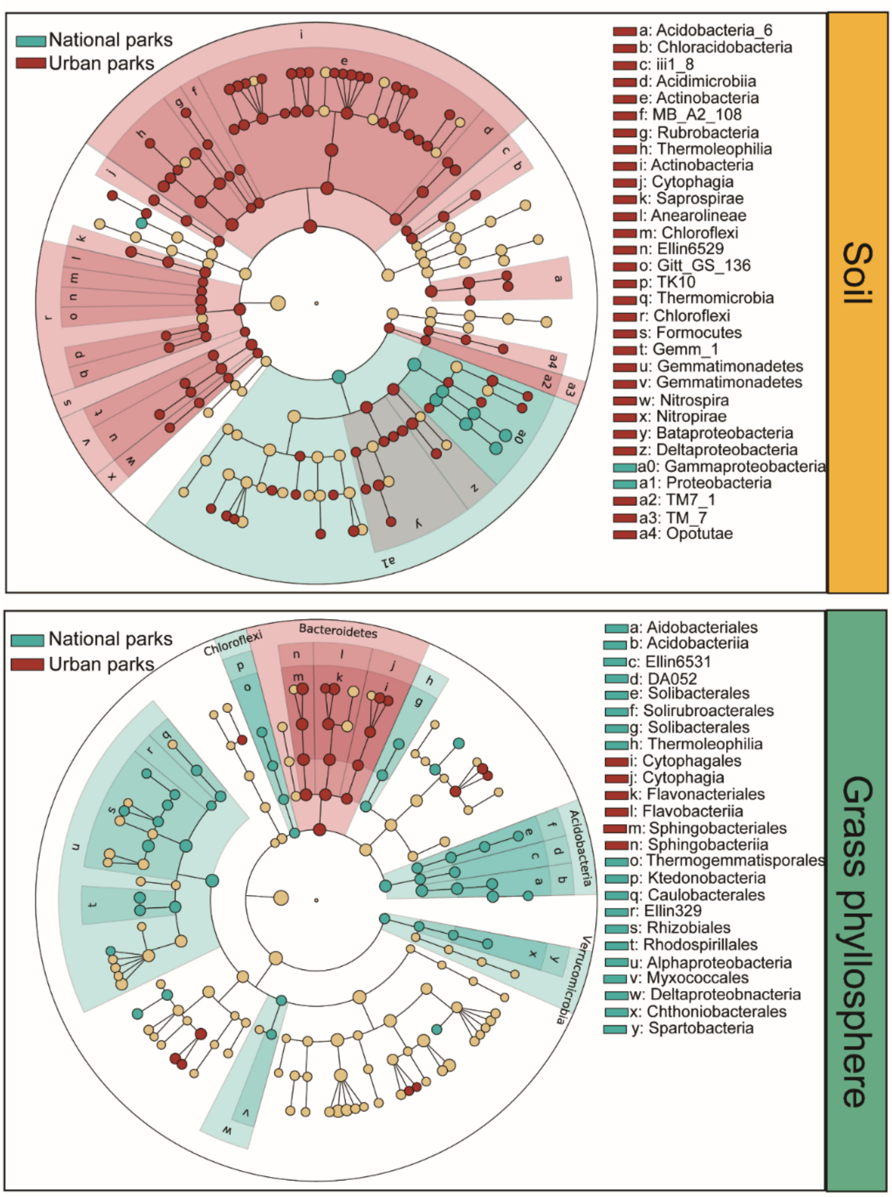


Fig. 2. Cladograms of the LEfSe analysis results indicating differences of taxa between soil and grass phyllosphere in urban green spaces and national parks. For each taxon, the red and blue denote the significantly higher abundance (α > 0.01, LDA score > 2.0) in urban green spaces and national parks, respectively. Yellow denotes that taxon not significantly different between urban green spaces and national parks.

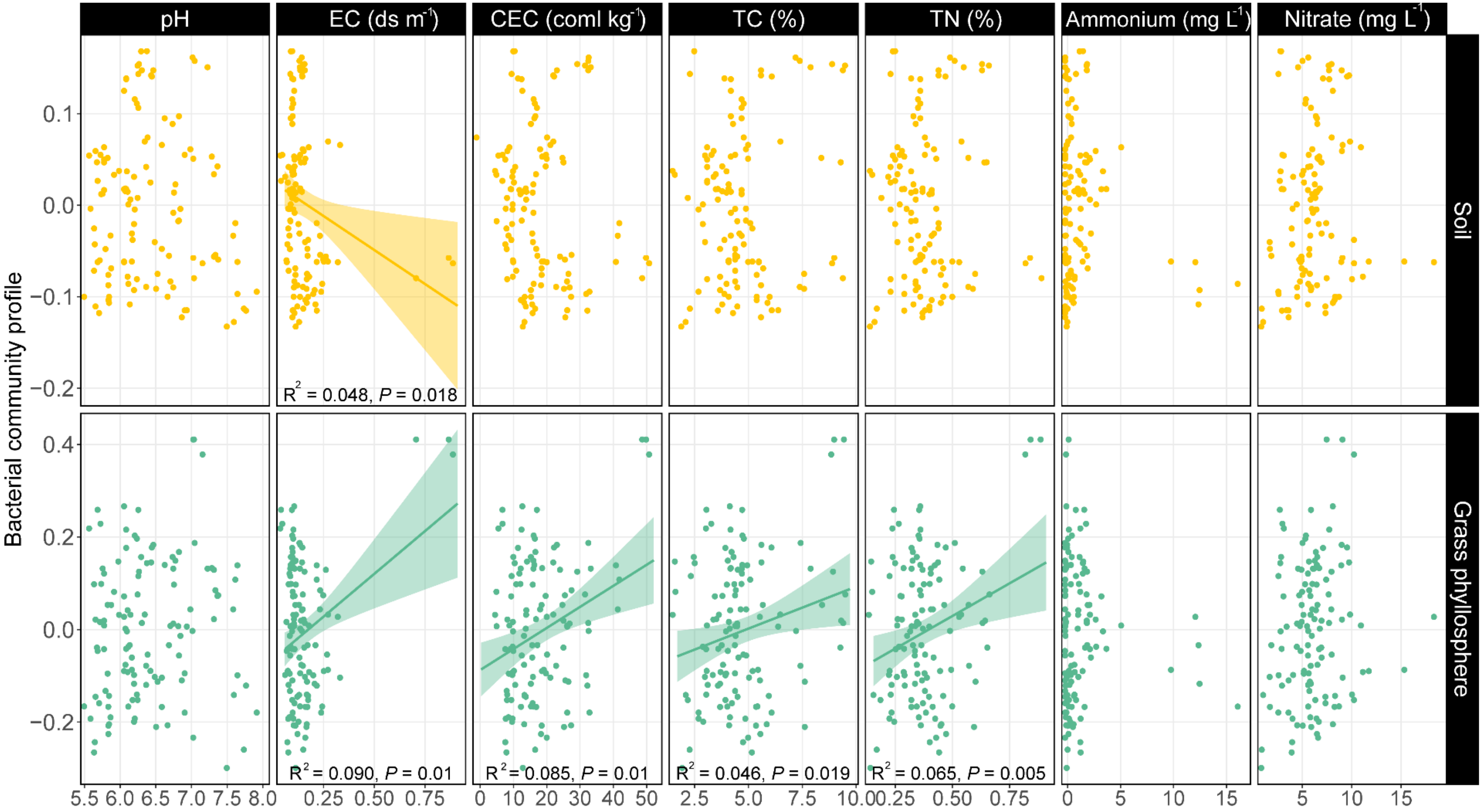
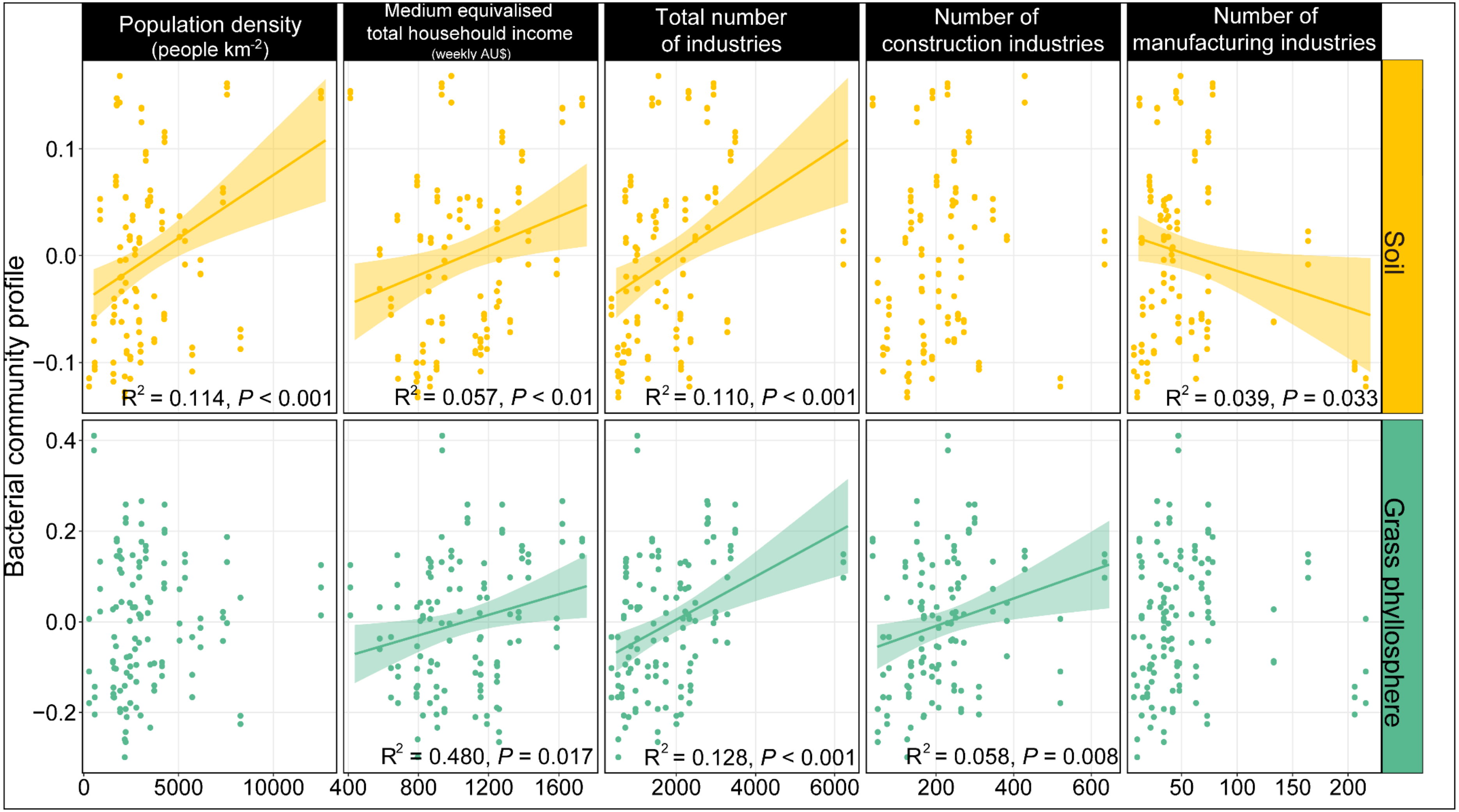


Fig. 3. Ordinary least square (OLS) regression model showing the relationships between soil properties and microbial community compositions (the first axis values of PCoA analysis) in soil and grass phyllosphere in urban green spaces.



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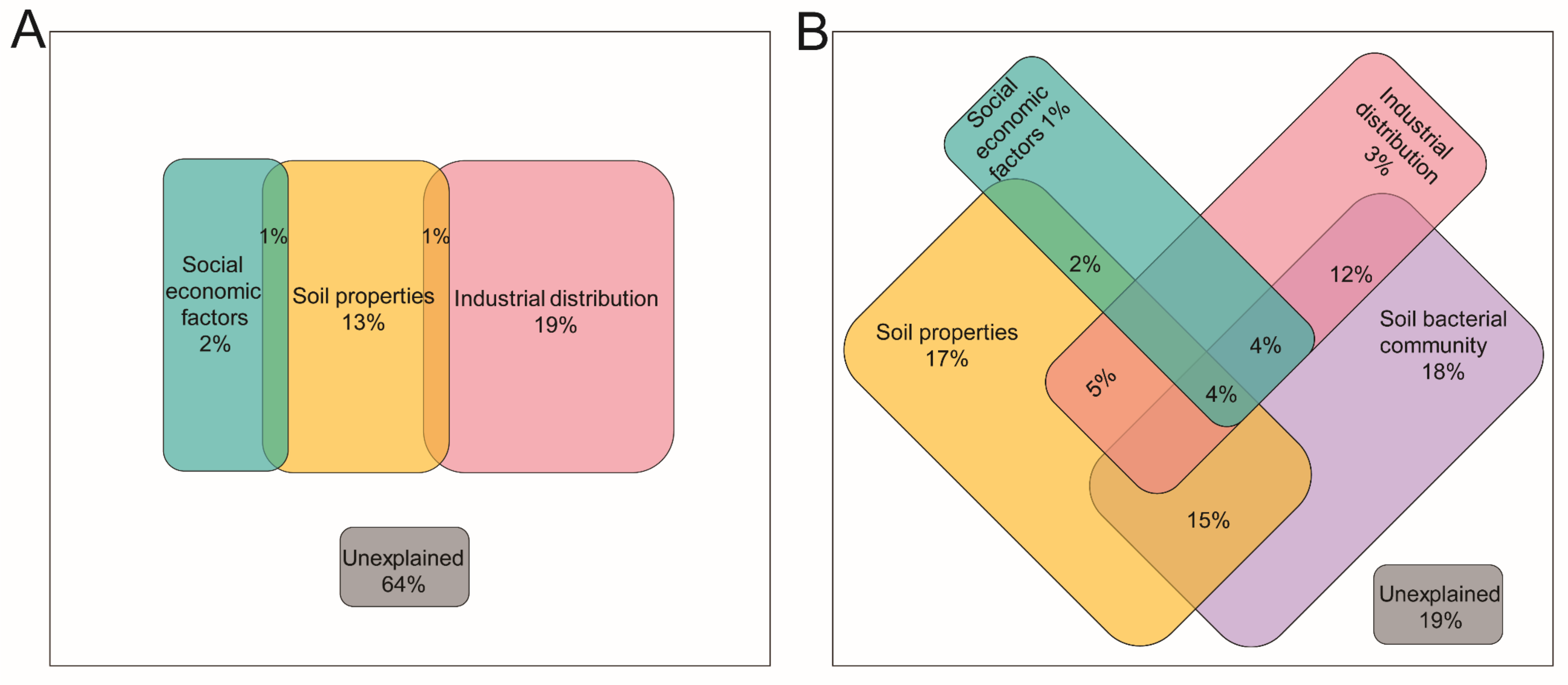

Fig. 5. Variation partitioning analysis (VPA) showing the effects of soil properties, soil bacterial communities, social economic factors and industrial factors on the alternation of soil (A) and grass phyllosphere microbial communities(B).

The authors declare no conflict of interest.

Author contributions: Zhen-Zhen Yan and Qing-Lin Chen designed research, Zhen-Zhen Yan performed the experiments and analyzed the data. Zhen-Zhen Yan wrote original draft. Qing-Lin Chen, Yu-Jing Zhang, Ji-Zheng He and Hang-Wei Hu contributed to manuscript revision. Hang-Wei Hu and Ji-Zheng He, Funding acquisition, Project administration.

Supporting data

Industrial development as a key factor explaining variances in soil and grass phyllosphere microbiomes in urban green spaces

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Contents

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Table S2 Anthropogenic parameters of Statistical Area Level 2 (SA2) regions where the urban green spaces locate in.

Table S3 List of analysed soil properties of the 120 soil samples from urban green spaces

Fig. S1 Bacterial community compositions in soil and grass phyllosphere samples across sampling sites at phylum level.

Fig. S2. Distribution bar-plot of bacterial community compositions in soil (A) and grass phyllosphere (B) samples across different urban green spaces and national parks at phylum level.

Fig. S3. Heatmap of bacterial community compositions in soil (A) and grass phyllosphere (B) samples across the investigated urban green spaces and national parks at genera level.

Fig. S4. Boxplots showing the Shannon index (α diversity) in soil and grass phyllosphere samples in the 40 urban green spaces and three national parks. *** indicates $P < 0.001$ determined by paired t-test(A); and principal coordinate analysis (PCoA) based on the Bray-Curtis distance showing the distribution patterns of microbiome in soil and grass phyllosphere samples (β diversity) (B).

Table S1 Detailed information of sampling sites.

Sample site ID	Green space Name	Statistic Area Level 2 (SA2) name	Suburb/Region name	Post code	Latitude	Longitude
U1	Parker Reserve	Coburg North	Coburg North	3058	37°43'20.30"S	144°58'2.56"E
U2	Parker Reserve	Coburg North	Coburg North	3058	37°43'22.53"S	144°58'3.08"E
U3	Parker Reserve	Coburg North	Coburg North	3058	37°43'17.40"S	144°58'1.07"E
U4	Charles Mutton Reserve	Fawkner	Fawkner	3060	37°42'49.99"S	144°58'12.46"E
U5	Charles Mutton Reserve	Fawkner	Fawkner	3060	37°42'50.12"S	144°58'9.37"E
U6	Charles Mutton Reserve	Fawkner	Fawkner	3060	37°42'49.86"S	144°58'16.41"E
U7	Greensborough Park	Greensborough	Greensborough	3088	37°42'0.76"S	145° 6'23.72"E
U8	Greensborough Park	Greensborough	Greensborough	3088	37°42'2.45"S	145° 6'22.59"E
U9	Greensborough Park	Greensborough	Greensborough	3088	37°41'59.92"S	145° 6'16.54"E
U10	Hibiscus Park	Bundoora-North	Bundoora	3083	37°41'2.01"S	145° 3'43.57"E
U11	Hibiscus Park	Bundoora-North	Bundoora	3083	37°41'3.11"S	145° 3'43.71"E
U12	Hibiscus Park	Bundoora-North	Bundoora	3083	37°41'1.97"S	145° 3'45.35"E
U13	Bricker Reserve	Moorabbin-Heatherton	Moorabbin	3189	37°56'27.67"S	145° 3'6.42"E

U14	Bricker Reserve	Moorabbin-Heatherton	Moorabbin	3189	37°56'29.98"S	145° 3'4.88"E
U15	Bricker Reserve	Moorabbin-Heatherton	Moorabbin	3189	37°56'31.77"S	145° 3'3.48"E
U16	Banksia Reserve Playground	Beaumaris	Beaumaris	3193	37°59'12.17"S	145° 2'31.58"E
U17	Banksia Reserve Playground	Beaumaris	Beaumaris	3193	37°59'9.90"S	145° 2'32.34"E
U18	Banksia Reserve Playground	Beaumaris	Beaumaris	3193	37°59'10.69"S	145° 2'29.98"E
U19	Dendy Park	Brighton East	Brighton East	3187	37°55'31.45"S	145° 1'23.98"E
U20	Dendy Park	Brighton East	Brighton East	3187	37°55'32.89"S	145° 1'20.83"E
U21	Dendy Park	Brighton East	Brighton East	3187	37°55'34.22"S	145° 1'25.40"E
U22	Carrum Pirate Ship Park	Carrum - Patterson Lakes	Carrum	3197	38° 4'35.75"S	145° 8'2.39"E
U23	Carrum Pirate Ship Park	Carrum - Patterson Lakes	Carrum	3197	38° 4'35.50"S	145° 7'59.92"E
U24	Carrum Pirate Ship Park	Carrum - Patterson Lakes	Carrum	3197	38° 4'38.22"S	145° 7'55.86"E
U25	Princess Park	Caulfied - South	Caulfied South	3162	37°53'37.50"S	145° 1'28.37"E
U26	Princess Park	Caulfied - South	Caulfied South	3162	37°53'39.11"S	145° 1'30.42"E
U27	Princess Park	Caulfied - South	Caulfied South	3162	37°53'36.81"S	145° 1'35.46"E

U28	Harleston Park	Elsternwick	Elsternwick	3185	37°52'52.10"S	145° 0'35.06"E
U29	Harleston Park	Elsternwick	Elsternwick	3185	37°52'53.00"S	145° 0'32.84"E
U30	Harleston Park	Elsternwick	Elsternwick	3185	37°52'53.50"S	145° 0'35.63"E
U31	Lord Reserve	Carnegie	Carnegie	3163	37°53'39.68"S	145° 2'56.80"E
U32	Lord Reserve	Carnegie	Carnegie	3163	37°53'38.23"S	37°53'38.23"S
U33	Lord Reserve	Carnegie	Carnegie	3163	37°53'41.83"S	145° 3'1.45"E
U34	Victoria Gardens	Prahran-Windsor	Prahran	3181	37°51'6.75"S	145° 0'3.68"E
U35	Victoria Gardens	Prahran-Windsor	Prahran	3181	37°51'7.04"S	145° 0'3.03"E
U36	Victoria Gardens	Prahran-Windsor	Prahran	3181	37° 51'7.03"E	145° 0'3.01"E
U37	Brookville Gardens	Toorak	Toorak	3142	37°50'43.81"S	37°50'43.81"S
U38	Brookville Gardens	Toorak	Toorak	3142	37°50'43.49"S	145° 0'32.10"E
U39	Brookville Gardens	Toorak	Toorak	3142	37°50'43.07"S	145° 0'35.02"E
U40	Citizens Park	Richmond (Vic.)	Richmond	3121	37°49'0.32"S	145° 0'2.53"E
U41	Citizens Park	Richmond (Vic.)	Richmond	3121	37°48'59.93"S	37°48'59.93"S
U42	Citizens Park	Richmond (Vic.)	Richmond	3121	37°49'1.69"S	145° 0'4.04"E
U43	Garden City Reserve	Port Melbourne	Port Melbourne	3207	37°50'11.08"S	144°55'47.35"E

U44	Garden City Reserve	Port Melbourne	Port Melbourne	3207	37°50'13.37"S	144°55'50.80"E
U45	Garden City Reserve	Port Melbourne	Port Melbourne	3207	37°50'10.95"S	37°50'10.95"S
U46	Templestowe Reserve	Templestowe	Templestowe	3106	37°45'14.52"S	145° 8'14.12"E
U47	Templestowe Reserve	Templestowe	Templestowe	3106	37°45'14.42"S	145° 8'15.63"E
U48	Templestowe Reserve	Templestowe	Templestowe	3106	37°45'16.54"S	145° 8'11.76"E
U49	Macleay Park	Balwyn North	Balwyn North	3104	37°48'4.88"S	145° 4'31.23"E
U50	Macleay Park	Balwyn North	Balwyn North	3104	37°48'3.62"S	145° 4'32.60"E
U51	Macleay Park	Balwyn North	Balwyn North	3104	37°48'5.91"S	145° 4'32.47"E
U52	Schramms Reserve	Doncaster	Doncaster	3108	37°46'58.83"S	145° 8'1.69"E
U53	Schramms Reserve	Doncaster	Doncaster	3108	37°47'1.10"S	145° 7'59.80"E
U54	Schramms Reserve	Doncaster	Doncaster	3108	37°47'0.73"S	145° 8'2.23"E
U55	Bennettswood Reserve	Burwood	Burwood	3125	37°50'54.44"S	145° 6'43.46"E
U56	Bennettswood Reserve	Burwood	Burwood	3125	37°50'51.51"S	145° 6'45.15"E
U57	Bennettswood Reserve	Burwood	Burwood	3125	37°50'57.05"S	145° 6'45.34"E
U58	Lynden Park	Camberwell	Camberwell	3124	37°50'36.34"S	145° 5'11.72"E
U59	Lynden Park	Camberwell	Camberwell	3124	37°50'38.81"S	145° 5'12.65"E

U60	Lynden Park	Camberwell	Camberwell	3124	37°50'38.04"S	145° 5'6.31"E
U61	Eley Park	Blackburn South	Blackburn South	3130	37°50'45.23"S	145° 8'34.33"E
U62	Eley Park	Blackburn South	Blackburn South	3130	37°50'44.83"S	145° 8'36.44"E
U63	Eley Park	Blackburn South	Blackburn South	3130	37°50'43.73"S	145° 8'33.14"E
U64	Central Gardens	Hawthorn	Hawthorn	3122	37°49'16.06"S	145° 2'29.33"E
U65	Central Gardens	Hawthorn	Hawthorn	3122	37°49'18.00"S	145° 2'27.44"E
U66	Central Gardens	Hawthorn	Hawthorn	3122	37°49'14.96"S	145° 2'31.59"E
U67	Malvern Public Gardens	Malvern - Glen Iris	Malvern	3144	37°51'25.03"S	145° 2'7.30"E
U68	Malvern Public Gardens	Malvern - Glen Iris	Malvern	3144	37°51'23.82"S	145° 2'8.77"E
U69	Malvern Public Gardens	Malvern - Glen Iris	Malvern	3144	37°51'20.57"S	145° 2'8.56"E
U70	Fitzroy Gardens	East Melbourne	East Melbourne	3002	37°48'45.94"S	144°58'51.36"E
U71	Fitzroy Gardens	East Melbourne	East Melbourne	3002	37°48'50.47"S	144°58'45.74"E
U72	Fitzroy Gardens	East Melbourne	East Melbourne	3002	37°48'42.46"S	144°58'57.72"E
U73	Queens Park	Moonee Ponds	Moonee Ponds	3039	37°45'43.70"S	37°45'43.70"S
U74	Queens Park	Moonee Ponds	Moonee Ponds	3039	37°45'43.95"S	144°55'27.56"E
U75	Queens Park	Moonee Ponds	Moonee Ponds	3039	37°45'42.11"S	144°55'24.19"E

U76	Batman Park	Northcote	Northcote	3070	37°49'18.34"S	144°57'26.08"E
U77	Batman Park	Northcote	Northcote	3070	37°49'18.80"S	144°57'23.70"E
U78	Batman Park	Northcote	Northcote	3070	37°49'18.94"S	144°57'21.71"E
U79	Flagstaff Gardens	North Melbourne	West Melbourne	3003	37°48'38.46"S	144°57'15.65"E
U80	Flagstaff Gardens	North Melbourne	West Melbourne	3003	37°48'35.44"S	144°57'18.43"E
U81	Flagstaff Gardens	North Melbourne	West Melbourne	3003	37°48'33.59"S	37°48'33.59"S
U82	Atherton Reserve	Fitzroy	Fitzroy	3065	37°48'11.98"S	144°58'45.28"E
U83	Atherton Reserve	Fitzroy	Fitzroy	3065	37°48'12.93"S	144°58'46.01"E
U84	Atherton Reserve	Fitzroy	Fitzroy	3065	37°48'12.81"S	144°58'44.11"E
U85	Carlton Gardens	Carlton	Carlton	3053	37°48'19.51"S	144°58'17.39"E
U86	Carlton Gardens	Carlton	Carlton	3053	37°48'20.30"S	144°58'14.83"E
U87	Carlton Gardens	Carlton	Carlton	3053	37°48'20.15"S	144°58'12.44"E
U88	H.P. Zwar Park	Preston-West	Preston	3072	37°44'27.80"S	144°59'46.74"E
U89	H.P. Zwar Park	Preston-West	Preston	3072	37°44'31.45"S	144°59'47.78"E
U90	H.P. Zwar Park	Preston-West	Preston	3072	37°44'31.10"S	144°59'44.17"E
U91	Brimbank Park	Keilor	Keilor East	3033	37°44'2.40"S	144°50'13.22"E

U92	Brimbank Park	Keilor	Keilor East	3033	37°44'1.94"S	144°49'58.45"E
U93	Brimbank Park	Keilor	Keilor East	3033	37°43'54.11"S	144°50'17.52"E
U94	A J Davis Reserve	Airport West	Airport West	3042	37°43'41.99"S	144°52'26.40"E
U95	A J Davis Reserve	Airport West	Airport West	3042	37°43'42.86"S	144°52'23.65"E
U96	A J Davis Reserve	Airport West	Airport West	3042	37°43'45.54"S	144°52'24.35"E
U97	Napier Park	Strathmore	Strathmore	3041	37°44'28.28"S	144°55'7.90"E
U98	Napier Park	Strathmore	Strathmore	3041	37°44'28.15"S	144°55'10.18"E
U99	Napier Park	Strathmore	Strathmore	3041	37°44'26.36"S	144°55'4.91"E
U100	Selwyn Park	Ardeer - Albion	Albion	3020	37°46'51.27"S	144°48'51.66"E
U101	Selwyn Park	Ardeer - Albion	Albion	3020	37°46'53.14"S	144°48'53.11"E
U102	Selwyn Park	Ardeer - Albion	Albion	3020	37°46'50.00"S	144°48'53.65"E
U103	Cairnlea Park	Cairnlea	Cairnlea	3023	37°45'35.68"S	144°47'44.24"E
U104	Cairnlea Park	Cairnlea	Cairnlea	3023	37°45'35.98"S	144°47'42.99"E
U105	Cairnlea Park	Cairnlea	Cairnlea	3023	37°45'36.77"S	144°47'44.79"E
U106	Braybrook Park	Braybrook	Braybrook	3019	37°47'13.78"S	144°51'17.84"E
U107	Braybrook Park	Braybrook	Braybrook	3019	37°47'12.17"S	144°51'19.15"E

U108	Braybrook Park	Braybrook	Braybrook	3019	37°47'15.04"S	144°51'20.62"E
U109	Pioneer Park	Sydenham	Sydenham	3037	37°41'13.06"S	37°41'13.06"S
U110	Pioneer Park	Sydenham	Sydenham	3037	37°41'15.28"S	144°45'31.88"E
U111	Pioneer Park	Sydenham	Sydenham	3037	37°41'13.56"S	144°45'42.77"E
U112	Mappin Reserve	Seddo - Kingsville	Seddon	3011	37°48'33.67"S	144°53'50.51"E
U113	Mappin Reserve	Seddo - Kingsville	Seddon	3011	37°48'33.04"S	144°53'51.48"E
U114	Mappin Reserve	Seddo - Kingsville	Seddon	3011	37°48'35.68"S	144°53'49.61"E
U115	Logan Reserve	Altona	Altona	3018	37°52'12.15"S	144°49'46.08"E
U116	Logan Reserve	Altona	Altona	3018	37°52'12.13"S	144°49'45.07"E
U117	Logan Reserve	Altona	Altona	3018	37°52'11.30"S	144°49'47.39"E
U118	Bruce Comben Reserve	Altona - Meadows	Altona Meadows	3028	37°52'19.19"S	144°46'1.12"E
U119	Bruce Comben Reserve	Altona - Meadows	Altona Meadows	3028	37°52'20.45"S	144°45'59.82"E
U120	Bruce Comben Reserve	Altona - Meadows	Altona Meadows	3028	37°52'17.88"S	144°46'3.48"E
N1	Yarra ranges national park	NA	Reefton	3799	37˚38'11"S	145˚54'23"E
N2	Yarra ranges national park	NA	Reefton	3799	37˚37'53"S	145˚54'28"E

N3	Yarra ranges national park	NA	Reefton	3799	37˚37'52"S	145˚54'31"E
N4	Lake Eildon national park	NA	Central highlands (Victoria)	3660	37˚14'33"S	145˚57'14"E
N5	Lake Eildon national park	NA	Central highlands (Victoria)	3660	37˚14'2"S	145˚57'7"E
N6	Lake Eildon national park	NA	Central highlands (Victoria)	3660	37˚13'52"S	145˚54'10"E
N7	Kinglake national park	NA	Kinglake	3763	37˚25'28"S	145˚15'48"E
N8	Kinglake national park	NA	Kinglake	3763	37˚27'33"S	145˚14'29"E
N9	Kinglake national park	NA	Kinglake	3763	37˚27'37"S	145˚14'19"E

Table S2 Anthropogenic parameters of Statistical Area Level 2 (SA2) regions where the urban green spaces locate in.

	Socio-economic parameters		**Industrial distribution**		
SA2 name	Population density (people km^{-2})	Median equivalised total household income (weekly AU$)	Total number of industries	Number of construction industries	Number of manufacturing industries
Coburg North	1777	898	1130	174	137
Fawkner	2943	605	1126	174	45
Greensborough	2136	1013	1661	440	53
Bundoora-North	1801	815	735	141	23
Moorabbin-Heatherton	817	929	2231	322	210
Beaumaris	2447	1285	1644	61	38
Brighton East	3010	1273	1846	218	27
Carrum - Patterson Lakes	2155	974	1084	276	46
Caulfied - South	3955	1179	2472	258	52
Elsternwick	4366	1274	1597	147	50
Carnegie	5291	1003	1539	250	42
Prahran-Windsor	7582	1397	3102	262	78
Toorak	3281	1646	2892	162	32
Richmond (Vic.)	5576	1452	6331	646	168

Port Melbourne	6395	1614	2282	218	38
Templestowe	1100	1061	2339	358	40
Balwyn North	2451	1105	2910	311	43
Doncaster	2667	836	2591	394	38
Burwood	2814	705	1186	171	35
Camberwell	3156	1348	3402	283	63
Blackburn South	3168	896	840	132	18
Hawthorn	4498	1303	3599	296	78
Malvern - Glen Iris	3510	1416	3485	258	66
East Melbourne	1979	1759	1502	48	16
Moonee Ponds	3611	1175	1853	254	38
Northcote	4478	1200	2216	268	72
North Melbourne	7797	958	3054	241	82
Fitzroy	8505	1216	2116	85	77
Carlton	12753	437	2421	202	49
Preston-West	3745	933	826	146	26
Keilor	518	891	2443	532	220
Airport West	2226	884	848	175	78
Strathmore	2503	1150	908	179	29

Ardeer - Albion	1843	669	477	90	19
Cairnlea	2399	821	648	137	14
Braybrook	2690	709	1396	201	67
Sydenham	3232	851	786	180	18
Seddo - Kingsville	5959	1180	636	75	11
Altona	775	961	1129	242	51
Altona - Meadows	1936	818	961	213	25

Table S3 List of analysed soil properties of the 120 soil samples from urban green spaces

Sample ID	pH	EC (ds m^{-1})	CEC (coml kg^{-1})	Total carbon (%)	Total nitrogen (%)	Ammonium (mg L^{-1})	Nitrate (mg L^{-1})
SU1	6.12	0.27	19.95	5.41	0.47	1.86	12.20
SU2	6.12	0.34	21.39	5.75	0.55	12.50	18.80
SU3	6.23	0.29	20.52	5.66	0.52	10.20	15.80
SU4	6.18	0.11	11.16	3.10	0.29	3.14	4.90
SU5	6.21	0.14	15.20	3.85	0.36	2.55	6.30
SU6	6.23	0.15	17.04	4.75	0.44	2.64	7.42
SU7	6.36	0.11	11.35	2.68	0.26	1.57	3.31
SU8	6.44	0.12	11.76	2.72	0.27	1.89	3.23
SU9	6.32	0.11	10.84	2.54	0.25	1.23	3.04
SU10	5.56	0.16	16.67	5.63	0.52	0.54	9.18
SU11	5.77	0.13	17.17	4.45	0.39	0.20	7.81
SU12	5.72	0.14	15.12	4.71	0.42	0.20	7.80
SU13	5.90	0.18	13.65	5.57	0.43	0.83	8.73
SU14	5.91	0.19	14.62	6.06	0.47	1.00	8.56
SU15	5.91	0.20	14.77	6.16	0.48	1.03	8.78
SU16	5.71	0.09	8.95	4.97	0.42	1.64	4.43
SU17	5.70	0.10	9.29	5.38	0.46	1.67	4.38
SU18	5.65	0.10	9.08	5.07	0.45	1.67	4.47
SU19	5.86	0.11	9.36	4.60	0.40	0.20	6.18
SU20	5.93	0.10	9.90	4.36	0.37	0.27	6.27
SU21	5.89	0.11	9.99	4.18	0.37	0.71	5.62
SU22	6.31	0.12	9.46	2.40	0.21	0.56	6.78
SU23	6.27	0.12	11.11	2.90	0.25	0.56	6.90
SU24	6.26	0.15	10.98	3.12	0.27	0.68	7.13
SU25	6.24	0.20	10.76	4.15	0.34	1.24	10.70
SU26	6.17	0.20	11.21	3.92	0.31	1.11	11.60
SU27	6.30	0.19	9.52	3.26	0.25	1.09	10.50
SU28	6.26	0.08	11.06	3.56	0.24	0.74	4.53
SU29	6.43	0.10	11.93	3.33	0.25	0.62	6.04
SU30	6.49	0.10	11.54	3.28	0.25	0.55	5.63
SU31	6.15	0.12	10.56	3.82	0.3	4.10	3.37

SU32	6.16	0.12	11.25	4.175	0.34	3.78	3.26
SU33	6.12	0.12	10.71	3.66	0.30	3.63	3.55
SU34	5.84	0.13	8.93	3.25	0.27	2.55	7.16
SU35	5.72	0.17	9.57	3.59	0.30	3.09	9.48
SU36	5.84	0.20	10.19	3.298	0.28	5.50	11.4
SU37	6.15	0.12	13.82	4.38	0.38	0.56	8.19
SU38	6.15	0.12	12.76	4.14	0.34	0.39	7.56
SU39	6.12	0.13	14.06	4.35	0.38	0.32	8.55
SU40	6.81	0.13	17.41	4.38	0.34	0.27	6.54
SU41	6.82	0.14	17.19	4.22	0.34	0.20	6.91
SU42	6.84	0.14	17.37	4.26	0.35	0.20	6.73
SU43	6.89	0.11	13.89	5.11	0.36	0.20	6.03
SU44	6.91	0.11	14.57	4.62	0.32	0.24	6.42
SU45	6.88	0.11	6.33	4.69	0.34	0.20	5.94
SU46	7.38	0.13	18.16	3.72	0.281	0.20	6.69
SU47	7.35	0.13	20.02	4.40	0.31	0.20	7.17
SU48	7.43	0.13	21.07	4.01	0.29	0.20	6.28
SU49	5.63	0.06	6.88	3.24	0.26	1.64	3.59
SU50	5.75	0.06	8.07	3.26	0.27	2.01	3.18
SU51	5.79	0.07	8.11	3.49	0.29	2.18	3.44
SU52	6.17	0.17	15.26	4.37	0.39	1.45	6.84
SU53	6.14	0.16	15.08	4.35	0.39	1.39	7.24
SU54	6.14	0.16	14.58	4.04	0.36	1.08	7.00
SU55	5.98	0.12	6.27	1.83	0.17	1.63	5.44
SU56	6.04	0.13	15.47	1.80	0.17	1.91	6.14
SU57	6.05	0.12	5.89	1.70	0.16	1.43	5.58
SU58	5.70	0.10	12.23	4.56	0.40	0.75	5.74
SU59	5.75	0.09	11.33	4.26	0.36	0.20	5.29
SU60	5.78	0.09	11.23	4.31	0.37	0.29	5.40
SU61	5.80	0.11	13.48	4.89	0.42	1.51	6.48
SU62	5.82	0.11	13.58	4.91	0.43	2.04	6.66
SU63	5.84	0.10	13.18	4.92	0.43	1.88	6.03
SU64	6.30	0.12	17.83	5.00	0.38	0.49	5.77
SU65	6.32	0.12	18.44	4.93	0.37	1.21	6.34
SU66	6.27	0.12	17.46	4.94	0.37	0.84	5.82

SU67	6.69	0.11	17.67	4.93	0.38	0.75	6.94
SU68	6.89	0.12	17.98	4.43	0.35	0.52	6.83
SU69	6.80	0.12	16.64	4.52	0.36	0.83	6.98
SU70	6.51	0.17	23.59	6.29	0.49	0.20	10.00
SU71	6.50	0.17	23.35	5.82	0.46	0.24	10.20
SU72	6.53	0.158	24.45	5.82	0.46	0.40	9.45
SU73	5.74	0.18	26.42	9.48	0.67	2.08	7.38
SU74	5.89	0.15	26.10	8.64	0.59	2.50	6.99
SU75	5.84	0.16	26.43	9.52	0.66	2.25	7.41
SU76	7.44	0.27	26.85	4.60	0.38	0.23	5.15
SU77	7.38	0.28	25.38	4.72	0.39	0.20	5.37
SU78	7.40	0.26	28.84	4.63	0.38	0.21	6.16
SU79	7.29	0.156	34.61	8.10	0.57	0.20	5.03
SU80	7.11	0.15	33.90	7.64	0.53	0.20	5.48
SU81	7.08	0.17	33.98	7.43	0.51	0.20	6.06
SU82	5.94	0.13	19.83	5.89	0.48	2.34	5.40
SU83	5.90	0.13	19.87	5.57	047	0.49	5.50
SU84	5.90	0.14	19.68	5.77	0.49	0.53	6.95
SU85	6.37	0.18	33.87	9.62	0.652	2.26	8.17
U86	6.31	0.17	32.91	9.712	0.682	2.23	8.13
U87	6.33	0.18	30.56	9.14	0.65	2.28	8.42
U88	6.97	0.16	21.17	4.59	0.40	0.20	6.11
U89	7.05	0.17	19.41	5.11	0.44	0.20	6.47
U90	7.09	0.18	21.40	5.20	0.45	0.20	7.06
U91	7.00	0.12	27.57	6.60	0.42	0.20	3.87
U92	6.93	0.12	26.98	5.75	0.39	0.20	4.07
U93	6.97	0.12	27.55	6.29	0.40	0.20	4.06
U94	7.65	0.26	42.73	4.85	0.43	0.20	8.40
U95	7.68	0.24	43.19	5.30	0.46	0.20	7.88
U96	7.71	0.24	42.17	5.16	0.44	0.48	9.41
U97	6.59	0.19	27.91	7.83	0.62	0.78	6.23
U98	6.43	0.17	24.20	7.63	0.59	0.79	6.31
U99	6.57	0.19	26.59	7.83	0.61	0.94	6.37
U100	6.75	0.19	18.60	4.59	0.38	2.29	2.32
U101	6.55	0.18	17.24	3.20	0.28	1.46	2.15

U102	6.65	0.19	18.23	3.90	0.34	2.04	2.18
U103	7.56	0.14	14.21	2.14	0.16	0.35	1.30
U104	7.66	0.15	14.70	2.31	0.18	0.42	1.49
U105	7.80	0.17	17.91	2.49	0.19	0.44	1.35
U106	7.83	0.24	33.61	3.80	0.31	0.24	3.40
U107	7.98	0.26	34.23	2.90	0.25	0.33	3.03
U108	7.71	0.22	33.24	4.15	0.31	0.38	3.06
U109	6.81	0.13	28.26	4.69	0.37	0.25	4.93
U110	6.77	0.13	27.49	4.60	0.36	0.24	5.23
U111	6.73	0.13	28.71	4.32	0.38	0.30	4.94
U112	6.18	0.27	17.47	4.24	0.364	16.50	6.06
U113	6.20	0.23	17.01	3.98	0.35	12.80	6.03
U114	6.26	0.23	16.63	3.90	0.36	12.90	6.33
U115	7.09	0.89	51.23	9.20	0.86	0.54	9.53
U116	7.10	0.73	50.06	9.65	0.91	0.54	7.93
U117	7.22	0.91	52.15	9.06	0.84	0.29	10.70
U118	6.65	0.35	22.49	5.24	0.41	0.35	9.55
U119	6.41	0.30	23.37	6.69	0.56	0.47	10.30
U120	6.45	0.29	21.50	4.98	0.40112	0.77	8.44

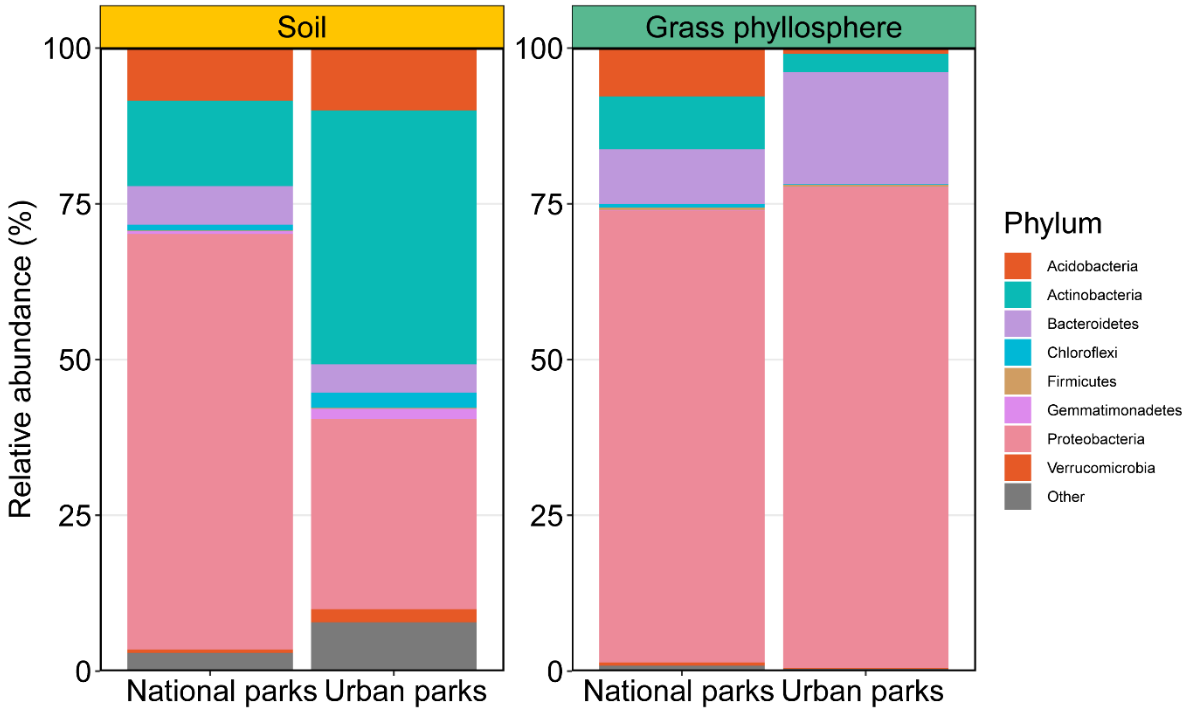

Fig. S1. Bacterial community compositions in soil and grass phyllosphere samples across sampling sites at phylum level.

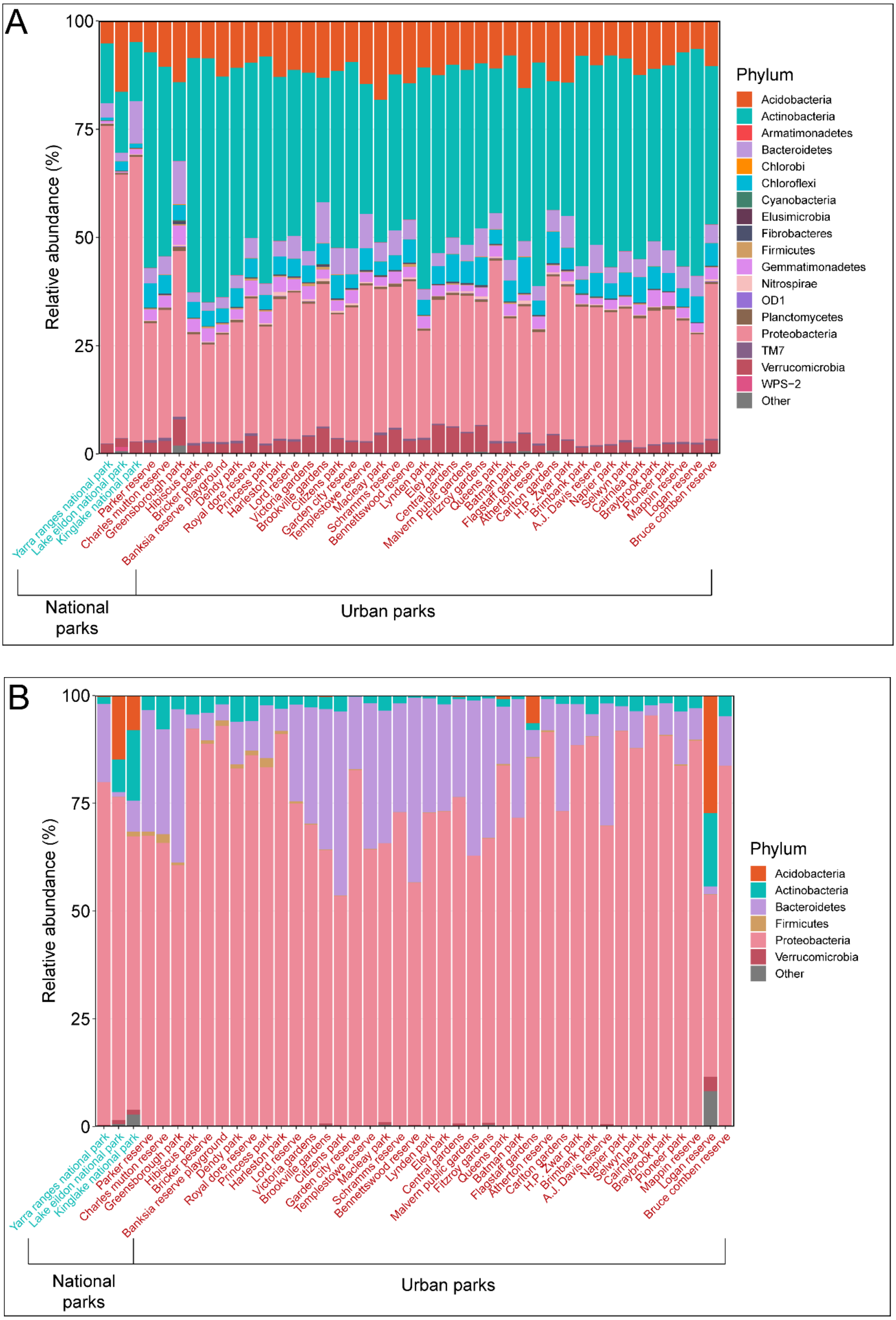

Fig. S2. Distribution bar-plot of bacterial community compositions in soil (A) and grass phyllosphere (B) samples across different urban green spaces and national parks at the phylum level.

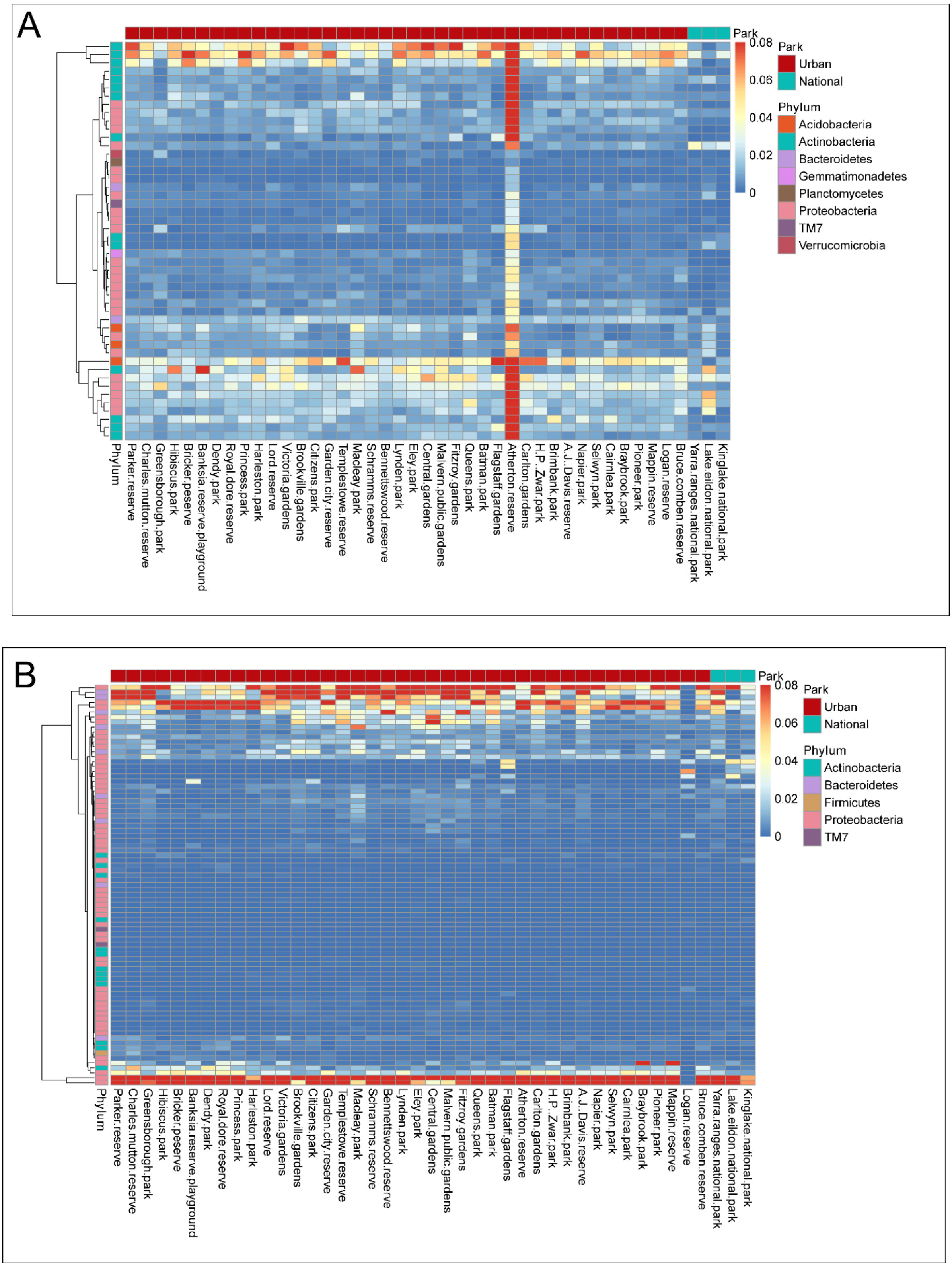

Fig. S3. Heatmap of bacterial community compositions in soil (A) and grass phyllosphere (B) samples across the investigated urban green spaces and national parks at genera level.

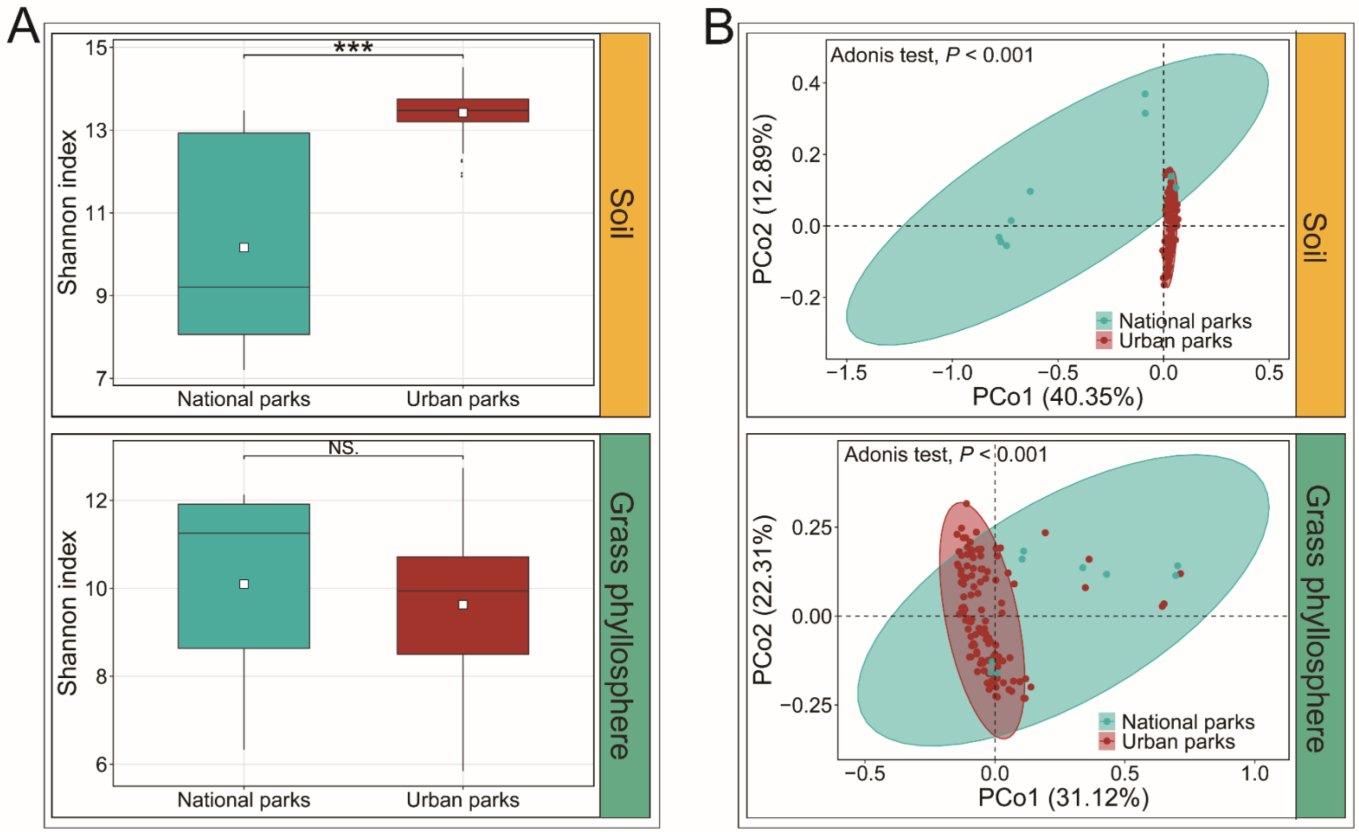

Fig. S4. Boxplots showing the Shannon index (α diversity) in soil and grass phyllosphere samples in the 40 urban green spaces and three national parks. *** indicates $P < 0.001$ determined by Mann-Whitney-Wilcoxon test. The white squares indicate the mean values (A). Principal coordinate analysis (PCoA) based on the Bray-Curtis distance showing the distribution patterns of microbiome in soil and grass phyllosphere samples (β diversity) (B).