# Characterising the soil fungal microbiome in metropolitan green spaces across a vegetation biodiversity gradient

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**Abstract**

Plant-soil feedbacks not only shape plant communities but also the abiotic and biotic nature of soils. These feedbacks are well-studied in natural and agricultural landscapes, but poorly studied in cities. Here, we investigated soil fungal communities, vegetation and soil abiotic properties in five urban green space types within urban Adelaide, South Australia. We surveyed eight, spatially-independent replicates of Sport Fields, Community Gardens, Parklands, Young Revegetation, and Old Revegetation sites. Vegetation strongly associated with soil fungal abundance and diversity. Revegetated urban green spaces had appreciably higher fungal diversity than other spaces, as well as greater richness in saprotrophic and pathotrophic fungi. We suggest that restoration of urban green space fungal microbiomes appears possible via replanting the native vegetation community. Such revegetation interventions will likely have positive outcomes for not only biodiversity conservation but also human health, via re-creating a biodiverse environmental microbiome.

**Key Words:** genomics; urban green spaces; fungal restoration; public health; functional ecology

## 1. Introduction

The soil microbiome is the richest component of terrestrial biodiversity and plays major roles in ecosystem processes, such as carbon and nitrogen cycling and soil formation. The important and multi-functional roles of fungi have led them to be put forward as a foundation of ecosystem stability (Nilsson et al., 2018; Yang et al., 2018). Indeed, biodiverse communities of fungi are indicators of healthy ecosystems and promote environmental resilience (Bardgett & Van Der Putten, 2014; Delgado-Baquerizo et al., 2017; Delavaux et al., 2019).

The soil fungal community is to a large degree shaped and modified by vegetation composition and structure, as well as by soil traits. For example, plant diversity, biomass and successional stage within a given site are associated with soil fungi abundance and diversity (Bardgett & Van Der Putten, 2014; Hiiesalu et al., 2014; Nguyen Williams et al., 2016; Peay et al., 2016; Hannula et al., 2017; Nilsson et al., 2018). Also, the vegetation community has important roles in shaping soil fungal diversity through the effects of root exudates (McGuire et al., 2012; Tedersoo et al., 2014; Lange et al., 2015; Zuo et al., 2016; Eisenhauer et al., 2017; Nilsson et al., 2018) and microclimate (Hiiesalu et al., 2014; Docherty & Gutknecht, 2019). In addition, soil abiotic properties shape fungal communities through the influence of soil pH, organic carbon, nitrogen, phosphorus, N:P ratio, cation exchange and water holding capacities and pore space (Güsewell & Gessner, 2009; Docherty et al., 2015; Tonn & Ibáñez, 2017; Liddicoat et al., 2018).

The soil fungal community is also subjected to time-dependant effects. Examples of shorter time-dependent drivers of fungal community change are resource pulses

(e.g. human watering, fertilization and tilling), during crop cultivation and gardening, and seasonal weather events (Bardgett & Van Der Putten, 2014). Concurrently, the structure and stability of soil microbiomes are impacted by fragmentation, fire, floods, land use changes, deforestation and contamination (Cho et al., 2017; Dove & Hart, 2017; Eldridge & Delgado-Baquerizo, 2018; House & Bever, 2018). Over longer timespans, the soil fungal microbiome is impacted by successional shifts of vegetation with time since ecosystem restoration or glacial retreat (Clemmensen et al., 2015; Hannula et al., 2017; Yan et al., 2018; Delgado-Baquerizo et al., 2019), and by climate change (Jumpponen & Jones, 2014; Classen et al., 2015; Maestre et al., 2015; Docherty & Gutknecht, 2019).

Although there is abundant information about the composition and structure of soil fungal communities across biogeographical, latitudinal and elevational gradients in agricultural and forest contexts (Köhl et al., 2014; Pellissier et al., 2014; Tedersoo et al., 2014; Bahram et al., 2015; Paungfoo-Lonhienne et al., 2015; Delavaux et al., 2019), there are few studies from urban green spaces. Of those studies that do exist, most are from parks in the northern hemisphere (Newbound et al., 2010; Ramirez et al., 2014; Tonn & Ibáñez, 2017; Guilland et al., 2018) or focused only on individual fungal groups (Martinová et al., 2016; Hosokawa et al., 2019). Since the often small urban green spaces are increasingly important for biodiversity conservation (Wintle et al., 2019) and for maintaining and promoting public health (Nieuwenhuijsen et al., 2017; Liddicoat et al., 2018; Mills et al., 2019; Robinson & Breed, 2019), there is a pressing need to better understand the composition and structure of fungal microbiomes in cities, especially since the world is becoming increasingly urbanised (Rydin et al., 2012; Guilland et al., 2018).

Emerging research suggests that the loss of contact with these diverse environmental microbiomes, and perhaps key microbial taxa, may compromise normal healthy immune system development and regulation in people (Von Hertzen et al., 2011; Rook, 2013). Exposure to environmental microbiomes can help shape commensal microbiota in the gut and elsewhere in the human body (Gilbert et al., 2018; Rothschild et al., 2018), which has implications for immune system development and predisposition to both infectious and non-infectious diseases (Ichinohe et al., 2011; Stein et al., 2016). Degraded, low biodiversity soils may favour more opportunistic and potential pathogenic microbes, which appear to be countered by ecosystem restoration (Liddicoat et al., 2019). It is, therefore, important to improve our understanding of the patterns in soil fungal diversity and composition in urban green spaces, given the potential for soil microbiomes to influence immune-related human health outcomes.

Urban green spaces display significant differences in use and management that have large effects on soil fungi (Newbound et al., 2012; Hui et al., 2017; Guilland et al., 2018). Human interventions can establish and maintain vegetation diversity and structure (e.g. via gardening activities), as well as influence soil via fertilisation, mulching and watering. However, it is largely unknown how these land use differences result in changes to soil fungal community composition and structure. In this study, we examined how different urban green space types associated with soil fungal microbiome composition and structure in Adelaide – a city of ca. 1.3 million inhabitants in southern Australia (June 2017 census data; www.abs.gov.au/ausstats/ [accessed 25 June 2019]). We sampled eight replicates of five urban green space types with contrasting vegetation coverage and plant biodiversity.

We report on patterns of soil fungal diversity associated with vegetation and soils from these urban green space types. We integrate complete vegetation and soil surveys of well-replicated plots of five typical urban green space types. We assess the diversity and relative abundance of soil fungi with high-throughput amplicon sequencing. In our analysis, we controlled for spatial autocorrelation effects, which is an important ecological factor to consider, although it is seldom well-incorporated into urban ecology. We integrated these data to answer the following questions: (1) To what degree are vegetation, soil fungi and physico-chemistry associated with urban green space types? (2) Are there fungal taxa and functional groups that are representative of different urban green space types?

## 2. Methods

### *2.1. Field surveys and sampling*

Soils were sampled and vegetation was surveyed from eight spatially-independent 25 x 25 m replicates of five green space types from metropolitan Adelaide, South Australia (Figure 1). The urban green space types were Sport Fields (grass monocultures of cultivated lawns), Community Gardens (community spaces where vegetable crops are grown), Parklands (established trees over grass), Young Revegetation sites (< 10 years of native plant species revegetation), and Old Revegetation sites (>10 years of native plant species revegetation) (Table S1; Figure S1). Adelaide's climate is Mediterranean, with warm and dry summers and mild, wet winters. The mean annual air temperature is 12.3°C and rainfall is 551 mm (www.bom.gov.au, [accessed 26 June 2019]).

We generated pairwise linear distances between replicates. Maximum and minimum distances were 8.66 and 0.04 km, respectively (mean 3.70 ± 1.76 km) (Table S2). After removal of soil surface organic matter, core samples were collected to 10 cm at nine locations (five at plot corners and centre and four randomly dispersed) within each replicate to capture soil heterogeneity, which were then pooled, homogenised and stored at -20˚C. All field work was done in September to October 2017. Approximately 300 g per replicate of homogenised soil was put into polyethylene bags in the field and sent to a commercial soil laboratory for soil physico-chemical analysis (CSBP Laboratories, Perth). For each replicate, we assessed available nitrate, ammonium, phosphorus, potassium and sulphur, organic carbon, pH, total cations (calcium, magnesium, potassium and sodium), conductivity and soil texture as described in (Bissett et al., 2016). We also calculated cation exchange capacity (CEC) as the sum of all cations, and the ratio of available N and P (N:P).

We surveyed vegetation along five 25 m transects, separated by 5 m in each replicate. At every meter along each transect, the plant species present were recorded and sampled for later identification (= 130 intercept points/replicate). We were unable to identify some plants to species, in which case we used morphotype terms (e.g. graminoid, herbaceous weeds). For analysis and interpretation, the cover corresponding to bare soil (no vegetation cover; intercept points = 0) was scored due to its importance, for example, on microclimate. Plants were also scored by their growth form, and characterised as trees, shrubs, herbs or graminoids as a proxy of functional groups. Relative number of intercept points and relative number of species by growth form were integrated into a percentage importance value (IVI = (Intercept point / ∑ intercept points) * 100) + (number of species / ∑ number of species) * 100);

maximum IVI = 200 %). The proportion of each growth form provides a good characterisation of urban green space vegetation architecture and structure. We also took a panoramic photo from the centre of each replicate (example for each urban green space type shown in Figure S1). Due to logistical limitations (e.g. no permission to dig surface soils), we were only able to survey the vegetation of seven Community Garden replicates and three Sport Fields.

*2.2. Soil fungi metabarcode analysis*

To characterise the soil fungal microbiome, we subsampled 50 g soil per replicate, which was stored in a sterilized 50 ml falcon tube and preserved for two weeks at -20˚C. In the field, all soil sampling equipment was either sterile (e.g. collection bags, falcon tubes, gloves) or sterilized with ethanol and 5% Decon 90 (Decon Laboratories Ltd) prior to use (e.g. trowel).

DNA was extracted in duplicate from well-mixed 0.25 g subsamples of soil using the DNeasy® PowerLyzer® PowerSoil® Kit (QIAGEN, Hilden, Germany) automated on the QIAGEN QIAcube® (QIAGEN, Hilden, Germany). Each QIAcube run consisted of 10 soil samples plus an extraction blank control (EBC) to monitor background DNA levels. The internal transcribed spacer 1 (ITS1) was used to characterise fungal diversity using the barcoded Illumina fusion primers as described in the Earth Microbiome Project (http://press.igsb.anl.gov/earthmicrobiome/) (Thompson et al., 2017).

PCR amplifications were run in 25 µL reaction mixes, each containing 2 mM MgSO4, 0.6 mM dNTPs, 0.4 µM of each primer, 0.3 U Platinum™ Taq DNA Polymerase High

Fidelity in 10× reaction buffer (Invitrogen™, Carlsbad, CA, USA), and 2 µL DNA extract. The standard Earth Microbiome Project (Thompson et al. 2017) primers ITS1f (AATGATACGGCGACCACCGAGATCTACAC GGCTTGGTCATTTAGAGGAAGTAA) and ITS2 (CAAGCAGAAGACGGCATACGAGAT NNNNNNNNNN CGGCTGCGTTCTTCATCGATGC) were used, which include Illumina adapter sequences and a Golay Barcode (in bold) on the reverse primer. The PCR conditions were 1 min at 94°C, followed by 35 cycles of 94°C for 30 s, 52°C for 30 s, and 68°C for 30 s, and a final extension at 68°C for 7 min. A no-template control (NTC) was included for each PCR run to monitor background DNA levels in PCR reagents. For each DNA extract, triplicate PCR amplifications were included and pooled to minimise PCR bias. PCR products from each DNA extract were quantified using the LabChip GX (Waltham, MA, USA), purified using Agencourt AMPure XP PCR Purification (Beckman Coulter Genomics, NSW) and pooled to equimolar concentration. The final library was quantified using the Agilent 2200 TapeStation (Agilent Technologies, Santa Clara, CA, USA) and sequenced using a 300 cycle Illumina MiSeq kit at the Australian Genome Research Facility (AGRF).

Raw sequences were demultiplexed into samples using the unique Golay barcode sequence assigned to each sample at the PCR step and the resulting read1 (forward) only FASTQ data files were processed with QIIME2 (version 2018.6; http://qiime2.org/). DADA2 (Callahan et al., 2016) was used for error-correction, quality filtering (minimum 120 bp, minimum quality score 30), chimera removal and sequence variant calling. The UNITE v7.2 (2017-12-01) ‘dynamic species hypotheses’ fungal was used to assign taxonomy. Amplicon sequence variants

(ASV) abundance and taxonomy tables produced from the QIIME2 pipeline were then analysed using R (R Core Team 2018) and the microbiome data analysis framework of the R phyloseq package (McMurdie & Holmes, 2013). During DNA extractions, we included one extraction blank control (EBC) for every batch of 10 subsamples. Subsamples and negative controls were labelled 1, 2, 3,…, 44, and R1, R2, R3,…, R44 for corresponding duplicates. Control sample 11 was the EBC for subsamples 1-10, while control samples 22, 33, 44, R11, R22, R33, and R44 were the corresponding EBCs for remaining extraction batches. Samples 5, 17, 18, 24, 25, 26, *33*, *R22*, *R33*, and R34 (note EBCs in italics) failed sequencing and could not be considered in the analysis. A no-template control was also included to flag potential contaminating sequences relevant to all samples.

We identified and removed contaminating sequences using the R decontam package (Davis et al., 2018). Specifically, we used the isContaminant() function where contaminants were identified by increased prevalence in negative controls (i.e. extraction blank controls, PCR negative samples), with a threshold value of 0.5. Subsequently, all taxa identified as contaminants, and the control samples, were removed from further analysis. We also excluded taxa that were not identified as belonging to Fungi or that were unclassified at the phylum level. Unclassified phyla comprised 68% of all ASVs, with a mean ASV relative abundance across samples of 39.2% (SD 23.4%). This meant that ~61% (100-39%) of the sequence reads were classified at the Phylum level and considered in the analysis. We then joined the duplicate subsamples by summing sequence reads, i.e. 1 + R1, 2 + R2, etc, to obtain 40 study samples in total. Rare taxa with less than 50 sequence reads across all samples were also removed. To normalise for sampling effort, we rarefied the

microbiome data to the minimum sequence read depth (n = 22,902) of all samples. For ASVs identified at least to Class level, we analysed fungal trophic mode with the online version of FUNGuild (Nguyen et al., 2016) (http://www.stbates.org/guilds/app.php). In our analysis, we included the 'possible' and 'probable' identifications from FUNGuild, and considered only the main trophic modes (i.e. pathotrophs, saprotrophs and symbiotrophs). The pathotroph:saprotroph abundance ratio was also calculated.

*2.3. Statistical analysis*

Vegetation cover, soil physico-chemical traits, soil fungal ASV reads and inter-plot linear distance data were converted into matrices. We calculated plant species and ASV richness, Shannon H', Simpson's D, and equity (Shannon's E). The heterogeneity of vegetation and fungal ASVs profile within each green space was assessed by their β-diversity. Fungal ASV richness was corrected with the Chao2 estimator (Chao, 1987). We used ANOVAs to determine differences in variable means between urban green space types, and Pearson correlations to explore univariate relationships between vegetation, soil physico-chemistry, and fungal ASVs. We applied sequential Bonferroni adjustments of P values to correct for multiple testing, and visually assessed normality of residuals of fitted models.

Multivariate relationships among urban green space types for vegetation, soil, fungal ASVs abundance, richness and trophic mode were tested statistically with multifactor PERMANOVA (Anderson, 2001). Ordination of plots within soil physico-chemical, vegetation and soil fungal ASVs multidimensional spaces was done with PCA or PCoA to visualize the relationships among them. Due to the difficulty in identifying

some plant species, vegetation cover was transformed to presence/absence before ordination.

The most important ASVs from each urban green space type were determined by indicator species analysis, based on the abundance and constancy of a given ASV in and to a group (Dufrêne & Legendre, 1997). The indicator value (IV) is interpreted as quantifying the degree to which an ASV designates that group. The IV results were followed by a Monte Carlo randomization test (5,000 permutations) to generate a P value that represents the statistical significance of that particular IV. Only the ASVs identified to class were analysed, and if $P < 0.05$, they were considered as indicator ASVs.

The multivariate associations among fungi, soil, vegetation and geographical distance matrices were assessed with Mantel tests (Mantel, 1967), with partial Mantel tests used where either soil, vegetation or distance were used as control variables (Smouse et al., 1986). All multivariate analyses were run in PC-Ord V7.0 (McCune & Mefford, 2016).

## 3. Results

### *3.1. Soil abiotic characteristics*

Soils from the urban green space types differed across all abiotic variables (Table S3), and differed significantly when considering all soil traits together (multifactor PERMANOVA $F_{(4,35)} = 2.64$; $P = 0.0098$). Overall, Community Gardens differed the most from the others (Table S4). The Community Garden soils were high in phosphorus, sulphur, electrical conductivity and calcium content, and had low N:P

ratios. Jointly with the Sports Field soils, Community Garden soils differed in texture and available nitrogen (Table S4). Accordingly, the replicates from these two green space types generated the largest and most diverging and distorted polygons within the soil-based ordination (Fig. 2A).

### ***3.2.*** *Vegetation*

We recorded a total of 372 plant species (or plant morphotypes) across all urban green spaces. After excluding uncertain vouchers, we used 340 specimens for further analysis (Table S5). Due to the unbalanced number of replicates across green space types (Young and Old Revegetation n = 8 plots; Community Gardens and Parklands n = 7 plots; Sport Fields n = 3 plots), overall differences between vegetation composition were established with the Sum F test (Warton & Hudson, 2004) and Jaccard distances. We observed significant differences in vegetation richness and cover between all urban green space types, except between Young and Old Revegetation replicates (Mean F value = 1.68; $P < 0.001$).

The percentage of intercept points occupied by vegetation was significantly higher in Old Revegetation and Parklands than the other green space types, with the exception of Sport Fields, where cover was 100% (Table 1). Correspondingly, the proportion of bare soil showed the opposite trends. Plant species richness, species equity, and Shannon H' and Simpson diversity indices were highest in the Community Gardens and lowest in Parklands (Table 1).

The multivariate representation of urban green spaces in vegetational space (except those from Sports Fields) clearly separated Community Gardens and Parklands from

Old and Young Revegetation sites along both ordination axes (Fig. 2B). Despite plotting only species presence or absence, this ordination corresponded well with the results of multivariate group analysis and urban green space plant species richness and diversity, as described above. As expected, graminoids and herbs defined Sport Fields and Community Gardens, respectively, and shrubs and trees distinguished Young and Old Revegetation and Parkland sites (Table 2).

### ***3.3.*** *Fungi*

We generated 7,399,078 fungal reads that were assigned to 18,541 ASVs, of which 1,898 ASVs (916,080 reads) were classified at least to phylum and had at least 50 reads in our dataset. We rarefied the 1,898 ASV dataset to the minimum sequence depth of 22,902 reads for further analysis (Raw sequence data accessible at DOI:10.25909/5cd0e267156b1). There were 445 unique ASVs in the ASV replicate matrix, and the Chao2 species estimated number was 2,247 ASVs. Sport Field replicates displayed the lowest number of ASVs (Table 3) and Ascomycota was the dominant phylum with >89% of all ASVs in all green spaces and 43% ASVs were classified into 12 classes (Table 3). Although fungal abundance and diversity parameters did not differ significantly between urban green space types, those with woody elements (i.e. Young Revegetation, Old Revegetation and Parkland sites) had appreciably higher ASV mean richness (Table 4). Conversely, inter-site β-diversity was highest in Community Gardens and Sports Field sites (Table 4).

Pairwise PERMANOVA of fungal ASV composition and abundance indicated that urban green space types were statistically different ($F_{(4,35)}$ = 1.86; P = 0.0002), of which Community Gardens and Sport Fields diverged the most in soil physico-

chemical traits and ASV abundance (Table S6). The ordination of urban green space replicates in fungal ASV multidimensional space showed a clear split of Sport Field and Community Garden replicates from the rest (Fig. 3). By the size of their large plotted polygons, the Sport Fields and Community Gardens urban spaces were also the most variable, confirming their large β-diversity.

Within the group of 810 ASVs classified at least to class level, only 53 met the indicator criterion we employed (= P <0.05 with the Monte Carlo's randomization test) and were subsequently used as indicator ASVs. Eight ASVs designated Community Gardens and Young Restoration replicates each, 19 signalled Old Restoration replicates, 13 for Parklands and five for Sport Fields (Table S7). Five genera were specific only in one urban green space: *Coprinellus* sp. in the Community Gardens; *Eutypa* sp*.* in Young Revegetation replicates; *Pseudolachnea* sp., *Reddellomyces* sp. and *Russula* sp*.* in Old Revegetation replicates and *Vishniacozyma* sp. in Sport Field replicates.

Pathotroph and saprotroph ASV richness was lowest in the Community Garden and Sport Fields replicates but symbiotrophs ASV richness was highest in the Old Revegetation replicates. The pathotroph:saprotroph ratios did not differ among green spaces (Table 5 and Fig. 4).

### ***3.4.*** *Mantel tests*

Fungal and vegetation distance matrices were significantly associated (r = 0.123; P = 0.036), even when controlling for soil traits (r = 0.133; P = 0.023) and for geographic inter-replicate distances ( r = 0.121; P = 0.034). Fungal and abiotic traits of soils

were uncorrelated. Vegetation and abiotic characteristics of soils also showed a significant association (r = 0.145; P = 0.033), whereas inter-replicate distance among plots was not related to any other variable.

### ***3.5.*** *Univariate Correlations*

Overall, soil traits were uncorrelated to soil fungal parameters (Table S8). For the Young and Old Revegetation sites, the ASV diversity indices were associated negatively with most soil traits (Table S8). Vegetation traits did not have significant effects on soil fungal abundance nor its diversity. Vegetation cover was not correlated with soil fungal ASVs (Table S9). For Young and Old Revegetation green spaces, vegetation cover was weakly correlated with soil fungal abundance (Table S9).

## 4. Discussion

We show strong associations between plant species richness, vegetation cover and soil fungal communities in urban green spaces in Adelaide, South Australia. We showed this with a sampling design that allowed us to control for geographic proximity of different urban green space types. In general, urban sites that had multi-layered woody vegetation (e.g. revegetated areas) also had greatest fungal diversity. These sites also had large numbers of indicator species and high richness in saprotrophic and pathotrophic fungi. We discuss the implications of these findings on urban landscape management, biodiversity conservation and public health.

Finding such clear differences in soil fungal diversity and abundance (even in replicated urban plots) challenges the belief that fungal spores are numerous and

everywhere (Newbound et al., 2010). Although our urban green spaces would include a range of soil types (e.g. brown sandy to clay soils, red-brown clay soils (Ultisols) and alluvial silts and sands (Sheard & Bowman, 1996)), we found that soil traits alone were not correlated with soil fungal parameters. We expect that differences in use (e.g. vegetation) and management, and therefore microclimate, represented key influences on the respective soil microbiomes. It is expected that fungi are negatively affected by urbanisation due to heat island and edge effects, increased nitrogen deposition and pollution (e.g. heavy metals), and habitat fragmentation that limits spore dispersal and lowers population densities (Tonn & Ibáñez, 2017). However, some fungal taxa may be introduced into urban areas, particularly those that are mycorrhizal partners of exotic invaders or cultivated plants or saprotrophs to their specific necromass (Newbound et al., 2010). The complex direct and indirect associations between soil, vegetation and management, plus the relative uncertainty of the identification of fungal ASVs by high-throughput sequencing (Nilsson et al., 2018), make it difficult to associate a particular biophysical trait and/or management practice with diversity and abundance of soil fungi.

*Fungal community patterns in metropolitan green spaces*

Our herbaceous urban green spaces (i.e. Sports Fields, Community Gardens) were vastly different from the other green space types. The Community Gardens had higher ASV richness and Shannon's diversity, and higher aboveground vegetation diversity than the Sport Fields. The intensive use and management of the Community Gardens is likely to comprise crop rotation, continuous tilling, watering, fertilization and weeding. Most of these procedures are likely to decrease temporal

stability, affecting soil fungal communities due to high turnover, triggering rapid fungal responses (Bardgett & Van Der Putten, 2014; Hernandez & Menendez, 2019). Continuous soil tillage can destroy hyphal networks, which decreases the presence of mycorrhizas (Köhl et al., 2014), while weeding should decrease fungal diversity by depriving some species of their required symbionts.

The likely fertilisation of the Community Gardens upheld high soil phosphorus and calcium content, and increased salinity, which resulted in a low N:P ratio but relatively high CEC. Low N:P ratio tends to favour more soil bacteria than fungi (Güsewell & Gessner, 2009), whereas high N fertilization and low P promote fungal abundance of genera with pathogenic traits (Paungfoo-Lonhienne et al., 2015). Relatively high CEC and higher soil fertility are positively correlated with fungal diversity (Liddicoat et al., 2018), likely due to effects related to vegetation growth such as higher necromass for saprotrophic fungi and larger root area for mycorrhizal attachment. The diversity of crops in Community Gardens is also likely to contribute to soil microbiome diversity, offering more opportunities for fungal symbioses and leading to stronger niche differentiation in soil organisms (Bardgett & Van Der Putten, 2014). However, the incorporation of legumes into the crop cycle schedule might decrease mycorrhizal infection as described above. These rather contradictory associations between cause and effect and low temporal stability help to explain why the Community Garden was the most dynamic of urban soil fungal microbiomes.

The Sports Fields had minimal vegetation diversity with shallow but densely packed graminoid roots and 100% coverage of soil surfaces, preventing exposure to direct sun. Sports Fields are frequently mowed, fertilized and trampled (causing soil

compaction), which together with minimal vegetation diversity is likely to contribute to these sites having the lowest fungal ASV richness. The negative effects of fertilization on soil fungi were described above, and together with the slightly acidic soil pH, are likely to contribute to the lower fungal diversity present in these sites (Maestre et al., 2015). The absence of a shading overstory increases soil temperature which has also been shown to decrease fungal diversity (McLean et al., 2005; Pellissier et al., 2014; Docherty & Gutknecht, 2019). Here again, these contradictory influences may explain the complexity of soil fungal community responses.

The three urban green spaces with woody vegetation – Parklands, Young and Old Revegetation sites – are presumably less managed and displayed similar ASV richness and Shannon's H' diversity. Also, these sites share a multi-layered canopy that would help moderate soil temperature and preserve soil moisture. These three green spaces also represent landscapes with permanent deep-rooted plants, and probably significant root exudation, which are major drivers of soil fungal community dynamics (Bardgett & Van Der Putten, 2014). However, these wooded urban green spaces differ in several important aspects. Old Revegetation replicates had the highest ASV richness, which is an expected consequence of their longer establishment and temporal stability. Also, Old Revegetation sites displayed higher numbers of indicator species than Young Revegetation plots (19 vs. 8 ASVs). The Young Revegetation replicates had more bare soil, likely resulting in higher soil temperatures with the negative flow-on effects to fungi as discussed above. The Parklands had the lowest aboveground plant species richness and Shannon's diversity, despite the mix of native and introduced species, which could potentially

increase fungal abundance and diversity. The regular removal of dead leaves and branches typical of parklands might also help explain their lowest fungal diversity, which would largely affect the saprophytic community (Newbound et al., 2010) – consistent with our data. Revegetation sites probably have larger stored necromass, which is a promoter of soil microbial diversity (Bahram et al., 2015). Larger stored necromass should, therefore, have positive effects on saprotrophs, such as Agaricomycetes and other Basidiomycota involved in wood decay and ectomycorrhizal symbioses, which our results support and are also reported by Yan et al. (2018) from a nearby ecological restoration chronosequence.

Pathotroph and saprotroph ASV richness was lowest in the Community Gardens and Sport Fields, and highest in the three wooded urban green spaces. This result was perhaps unexpected, as the Community Gardens and Sport Fields are the most altered green spaces and may, therefore, be expected to contain the highest richness of pathotrophs (Paungfoo-Lonhienne et al., 2015; Hannula et al., 2017; Brinkmann et al., 2019). On the other hand, the greater richness in saprotrophs in the wooded urban spaces is an expected consequence of the larger amount of leaf litter and dead branches in the revegetation replicates and mulching in parks, as already discussed.

Our Old Revegetation sites differed substantially from those restored over similar timeframes in nearby eucalypt grassy woodlands (Yan et al., 2018). Although the ASV richness in our Old Revegetation sites was similar to those reported in Yan et al. (2018), they reported a lower percentage of fungi unclassified to class level (Yan et al. 2018 = 6.1% *vs.* 28.6% in our study). Basidiomycota was much more important

in the eucalypt grassy woodlands in Yan et al. (2018) than in our urban woodland plots. This might be a result of denser vegetation in the eucalypt grassy woodlands in Yan et al. (2018), which would provide more plant litter rich in lignin and cellulose that Basidiomycota degrade (Hui et al., 2017). The percentage of predicted symbiotrophs was substantially higher than those reported in Yan et al. (2018), which might be associated with the relatively high soil N:P ratio in this previous study. However, these comparisons are only indicative as the samples were not analysed jointly.

*Public health implications*

Reduced soil fungal diversity may have the potential to impact human health through adverse and deficient immunomodulatory stimuli (Von Hertzen et al., 2011; Rook, 2013). Liddicoat et al. (2018) found rates of infectious and parasitic disease increased in areas with low soil cation exchange capacity, a proxy indicator for soil microbial diversity. They suggested this adverse health outcome may be partly explained by reduced immune training in populations exposed to poorer, low microbial diversity soils; an inference supported by a followup randomised controlled mouse study (Liddicoat et al., 2020). Also, depauperate fungal communities may not provide adequate ecological controls on particular taxa, with potential for overgrowth and elevated allergen exposures (e.g. fungal spores), which could contribute to adverse respiratory health outcomes. Conversely, greater microbial diversity in soils can also resist the invasion and establishment of potentially pathogenic species (van Elsas et al., 2012). Consequently, our results suggest that revegetated urban landscapes and parklands provide more healthy environments for humans as a consequence of their higher soil microbiome diversity.

## Conclusions

We observed clear differences in the diversity and structure of soil fungal communities among metropolitan urban green spaces, most likely as a consequence of differences in soil traits, aboveground vegetation and management practices. Aboveground vegetation composition and structure had the strongest influence on soil fungal abundance and diversity. However, even the most complex vegetated urban green spaces (i.e. old revegetation sites) displayed lower levels of soil fungal diversity and differed substantially in composition from those of similarly restored woodlands in the region outside the metropolitan area, emphasizing the generalised impact that urbanisation has on soil fungi. Such impacts on soil fungi have potential health implications for people accessing these green spaces, with some degree of optimism found by the increased functional diversity of soil fungi observed in more complex vegetated urban areas – results that are consistent with studies on airborne bacterial communities in urban areas (Mhuireach et al., 2016; Mhuireach et al., 2019). As such, with further development of urban green space management practices and design principles (Robinson et al., 2018), it should be possible to optimise urban green space management to not only return native and functional soil fungal communities (Breed et al., 2019) but also provide opportunities for naturally-diverse microbiome exposures with potential for human health gains.

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**Data Availability**

Data that support the findings of this study are available in figshare at DOI: 10.25909/5cd0e267156b1.

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## References


Anderson, M. J. (2001). A new method for non‐parametric multivariate analysis of variance. *Austral Ecology, 26*(1), 32-46.

Bahram, M., Peay, K. G., & Tedersoo, L. (2015). Local‐scale biogeography and spatiotemporal variability in communities of mycorrhizal fungi. *New Phytologist, 205*(4), 1454-1463.

Bardgett, R. D., & Van Der Putten, W. H. (2014). Belowground biodiversity and ecosystem functioning. *Nature, 515*(7528), 505.

Bissett, A., Fitzgerald, A., Meintjes, T., Mele, P., Reith, F., Dennis, P., . . . Brugger, J. (2016). Introducing BASE: the Biomes of Australian Soil Environments soil microbial diversity database, Gigascience, 5, 21.

Brinkmann, N., Schneider, D., Sahner, J., Ballauff, J., Edy, N., Barus, H., . . . Polle, A. (2019). Intensive tropical land use massively shifts soil fungal communities. *Scientific Reports, 9*. doi: 10.1038/s41598-019-39829-4

Breed, M., Harrison, P., Blyth, C., Byrne, M., Gaget, V., Gellie, N., . . . Mohr, J. (2019). The potential of genomics for restoring ecosystems and biodiversity. *Nature Reviews Genetics, 20*. doi: 10.1038/s41576-019-0152-0

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., & Holmes, S. P. (2016). DADA2: high-resolution sample inference from Illumina amplicon data. *Nature methods, 13*(7), 581.

Chao, A. (1987). Estimating the population size for capture-recapture data with unequal catchability. *Biometrics*, 783-791.

Cho, H., Kim, M., Tripathi, B., & Adams, J. (2017). Changes in soil fungal community structure with increasing disturbance frequency. *Microbial Ecology, 74*(1), 62-77. doi: 10.1007/s00248-016-0919-1

Classen, A. T., Sundqvist, M. K., Henning, J. A., Newman, G. S., Moore, J. A., Cregger, M. A., . . . Patterson, C. M. (2015). Direct and indirect effects of climate change on soil microbial and soil microbial‐plant interactions: What lies ahead? *Ecosphere, 6*(8), 1-21.

Clemmensen, K. E., Finlay, R. D., Dahlberg, A., Stenlid, J., Wardle, D. A., & Lindahl, B. D. (2015). Carbon sequestration is related to mycorrhizal fungal community shifts during long‐term succession in boreal forests. *New Phytologist, 205*(4), 1525-1536.

Davis, N. M., Proctor, D. M., Holmes, S. P., Relman, D. A., & Callahan, B. J. (2018). Simple statistical identification and removal of contaminant sequences in marker-gene and metagenomics data. *Microbiome, 6*(1), 226.

Delavaux, C. S., Weigelt, P., Dawson, W., Duchicela, J., Essl, F., van Kleunen, M., . . . Bever, J. D. (2019). Mycorrhizal fungi influence global plant biogeography. *Nature Ecology & Evolution, 3*(3), 424-+. doi: 10.1038/s41559-019-0823-4

Delgado-Baquerizo, M., Bardgett, R. D., Vitousek, P. M., Maestre, F. T., Williams, M. A., Eldridge, D. J., . . . García-Velázquez, L. (2019). Changes in belowground biodiversity during ecosystem development. *Proceedings of the National Academy of Sciences*, 201818400.

Delgado‐Baquerizo, M., Eldridge, D. J., Ochoa, V., Gozalo, B., Singh, B. K., & Maestre, F. T. (2017). Soil microbial communities drive the resistance of ecosystem multifunctionality to global change in drylands across the globe. *Ecology Letters, 20*(10), 1295-1305.

Docherty, K. M., Borton, H. M., Espinosa, N., Gebhardt, M., Gil-Loaiza, J., Gutknecht, J. L., . . . Purdy, G. (2015). Key edaphic properties largely explain temporal and geographic variation in soil microbial communities across four biomes. *Plos One, 10*(11), e0135352.

Docherty, K. M., & Gutknecht, J. L. (2019). Soil microbial restoration strategies for promoting climate‐ready prairie ecosystems. *Ecological Applications*, e01858.

Dove, N. C., & Hart, S. C. (2017). Fire reduces fungal species richness and in situ mycorrhyzal colonization: A meta analysis. *Fire Ecology, 13*(2), 37-65. doi: 10.4996/fireecology.130237746

Dufrêne, M., & Legendre, P. (1997). Species assemblages and indicator species: the need for a flexible asymmetrical approach. *Ecological Monographs, 67*(3), 345-366.
Eisenhauer, N., Lanoue, A., Strecker, T., Scheu, S., Steinauer, K., Thakur, M. P., & Mommer, L. (2017). Root biomass and exudates link plant diversity with soil bacterial and fungal biomass. *Scientific Reports, 7*, 44641. doi: 10.1038/srep44641
Eldridge, D. J., & Delgado-Baquerizo, M. (2018). Functional groups of soil fungi decline under grazing. *Plant & Soil, 426*(1-2), 51-60. doi: 10.1007/s11104-018-3617-6
Gilbert, J. A., Blaser, M. J., Caporaso, J. G., Jansson, J. K., Lynch, S. V., & Knight, R. (2018). Current understanding of the human microbiome. *Nature medicine, 24*(4), 392.
Guilland, C., Maron, P., Damas, O., & Ranjard, L. (2018). Biodiversity of urban soils for sustainable cities. *Environmental chemistry letters, 16*(4), 1267-1282.
Güsewell, S., & Gessner, M. O. (2009). N: P ratios influence litter decomposition and colonization by fungi and bacteria in microcosms. *Functional Ecology, 23*(1), 211-219.
Hannula, S. E., Morriën, E., de Hollander, M., Van Der Putten, W. H., van Veen, J. A., & De Boer, W. (2017). Shifts in rhizosphere fungal community during secondary succession following abandonment from agriculture. *The ISME journal, 11*(10), 2294.
Hernandez, M. M., & Menendez, C. M. (2019). Influence of seasonality and management practices on diversity and composition of fungal communities in vineyard soils. *Applied Soil Ecology, 135*, 113-119. doi: 10.1016/j.apsoil.2018.11.008
Hiiesalu, I., Pärtel, M., Davison, J., Gerhold, P., Metsis, M., Moora, M., . . . Wilson, S. D. (2014). Species richness of arbuscular mycorrhizal fungi: associations with grassland plant richness and biomass. *New Phytologist, 203*(1), 233-244.
Hosokawa, A., Reid, C. R., & Latty, T. (2019). Slimes in the city: The diversity of myxomycetes from inner-city and semi-urban parks in Sydney, Australia. *Fungal Ecology, 39*, 37-44. doi: 10.1016/j.funeco.2018.11.004
House, G. L., & Bever, J. D. (2018). Disturbance reduces the differentiation of mycorrhizal fungal communities in grasslands along a precipitation gradient. *Ecological Applications, 28*(3), 736-748.
Hui, N., Jumpponen, A., Francini, G., Kotze, D. J., Liu, X., Romantschuk, M., . . . Setälä, H. (2017). Soil microbial communities are shaped by vegetation type and park age in cities under cold climate. *Environmental microbiology, 19*(3), 1281-1295.
Ichinohe, T., Pang, I. K., Kumamoto, Y., Peaper, D. R., Ho, J. H., Murray, T. S., & Iwasaki, A. (2011). Microbiota regulates immune defense against respiratory tract influenza A virus infection. *Proceedings of the National Academy of Sciences, 108*(13), 5354-5359.
Jumpponen, A., & Jones, K. L. (2014). Tallgrass prairie soil fungal communities are resilient to climate change. *Fungal Ecology, 10*, 44-57.
Köhl, L., Oehl, F., & van der Heijden, M. G. A. (2014). Agricultural practices indirectly influence plant productivity and ecosystem services through effects on soil biota. *Ecological Applications, 24*(7), 1842-1853. doi: doi:10.1890/13-1821.1
Lange, M., Eisenhauer, N., Sierra, C. A., Bessler, H., Engels, C., Griffiths, R. I., . . . Scheu, S. (2015). Plant diversity increases soil microbial activity and soil carbon storage. *Nature Communications, 6*, 6707.
Liddicoat, C., Bi, P., Waycott, M., Glover, J., Breed, M., & Weinstein, P. (2018). Ambient soil cation exchange capacity inversely associates with infectious and parasitic disease risk in regional Australia. *Science of the Total Environment, 626*, 117-125. doi: doi.org/10.1016/j.scitotenv.2018.01077
Liddicoat, C., Sydnor, H., Cando-Dumancela, C., Dresken, R., Liu, J., Gellie, N. J., . . . Hutchinson, M. R. (2020). Naturally-diverse airborne environmental microbial exposures modulate the gut microbiome and may provide anxiolytic benefits in mice. *Science of the Total Environment, 701*, 134684.

Liddicoat, C., Weinstein, P., Bissett, A., Gellie, N. J., Mills, J. G., Waycott, M., & Breed, M. F. (2019). Can bacterial indicators of a grassy woodland restoration inform ecosystem assessment and microbiota-mediated human health? *Environment International, 129*, 105-117.

Maestre, F. T., Delgado-Baquerizo, M., Jeffries, T. C., Eldridge, D. J., Ochoa, V., Gozalo, B., . . . Ulrich, W. (2015). Increasing aridity reduces soil microbial diversity and abundance in global drylands. *Proceedings of the National Academy of Sciences, 112*(51), 15684-15689.

Mantel, N. (1967). The detection of disease clustering and a generalized regression approach. *Cancer research, 27*(2 Part 1), 209-220.

Martinová, V., van Geel, M., Lievens, B., & Honnay, O. (2016). Strong differences in Quercus robur-associated ectomycorrhizal fungal communities along a forest-city soil sealing gradient. *Fungal Ecology, 20*, 88-96. doi: 10.1016/j.funeco.2015.12.002

McCune, B., & Mefford, M. J. (2016). *PC-ORD: Multivariate analysis of ecological data. Version 7.* Gleneden Beach, Oregon, USA: MjM Software Design.

McGuire, K. L., Fierer, N., Bateman, C., Treseder, K. K., & Turner, B. L. (2012). Fungal community composition in neotropical rain forests: the influence of tree diversity and precipitation. *Microbial Ecology, 63*(4), 804-812.

McLean, M. A., Angilletta, M. J., & Williams, K. S. (2005). If you can't stand the heat, stay out of the city: Thermal reaction norms of chitinolytic fungi in an urban heat island. *Journal of Thermal Biology, 30*(5), 384-391. doi: 10.1016/jtherbio.2005.03.002

McMurdie, P. J., & Holmes, S. (2013). phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. *Plos One, 8*(4), e61217.

Mhuireach, G., Betancourt-Román, C. M., Green, J. L., & Johnson, B. R. (2019). Spatiotemporal controls on the urban aerobiome. *Frontiers in Ecology and Evolution, 7*, 43.

Mhuireach, G., Johnson, B. R., Altrichter, A. E., Ladau, J., Meadow, J. F., Pollard, K. S., & Green, J. L. (2016). Urban greenness influences airborne bacterial community composition. *Science of the Total Environment, 571*, 680-687.

Mills, J. G., Brookes, J. D., Gellie, N. J., Liddicoat, C., Lowe, A. J., Sydnor, H. R., . . . Breed, M. F. (2019). Relating urban biodiversity to human health with the 'holobiont'concept. *Frontiers in Microbiology, 10*.

Newbound, M., Bennett, L. T., Tibbits, J., & Kasel, S. (2012). Soil chemical properties, rather than landscape context, influence woodland fungal communities along an urban ‐ rural gradient. *Austral Ecology, 37*(2), 236-247.

Newbound, M., Mccarthy, M. A., & Lebel, T. (2010). Fungi and the urban environment: A review. *Landscape and urban planning, 96*(3), 138-145.

Nguyen, N. H., Song, Z. W., Bates, S. T., Branco, S., Tedersoo, L., Menke, J., . . . Kennedy, P. G. (2016). FUNGuild: An open annotation tool for parsing fungal community datasets by ecological guild. *Fungal Ecology, 20*, 241-248. doi: 10.1016/j.funeco.2015.06.006

Nguyen, N. H., Williams, L. J., Vincent, J. B., Stefanski, A., Cavender-Bares, J., Messier, C., . . . Kennedy, P. G. (2016). Ectomycorrhizal fungal diversity and saprotrophic fungal diversity are linked to different tree community attributes in a field-based tree experiment. *Mol Ecol, 25*(16), 4032-4046. doi: 10.1111/mec.13719

Nieuwenhuijsen, M. J., Khreis, H., Triguero-Mas, M., Gascon, M., & Dadvand, P. (2017). Fifty shades of green. *Epidemiology, 28*(1), 63-71.

Nilsson, R. H., Anslan, S., Bahram, M., Wurzbacher, C., Baldrian, P., & Tedersoo, L. (2018). Mycobiome diversity: high-throughput sequencing and identification of fungi. *Nature Reviews Microbiology*. doi: 10.1038/s41579-018-0116-y

Paungfoo-Lonhienne, C., Yeoh, Y. K., Kasinadhuni, N. R. P., Lonhienne, T. G., Robinson, N., Hugenholtz, P., . . . Schmidt, S. (2015). Nitrogen fertilizer dose alters fungal communities in sugarcane soil and rhizosphere. *Scientific Reports, 5*, 8678.

Peay, K. G., Kennedy, P. G., & Talbot, J. M. (2016). Dimensions of biodiversity in the Earth mycobiome. *Nature Reviews Microbiology, 14*(7), 434.

Pellissier, L., Niculita‐Hirzel, H., Dubuis, A., Pagni, M., Guex, N., Ndiribe, C., . . . Sanders, I. (2014). Soil fungal communities of grasslands are environmentally structured at a regional scale in the A lps. *Molecular Ecology, 23*(17), 4274-4290.

Ramirez, K. S., Leff, J. W., Barberan, A., Bates, S. T., Betley, J., Crowther, T. W., . . . Fierer, N. (2014). Biogeographic patterns in below-ground diversity in New York City's Central Park are similar to those observed globally. *Proceedings of the Royal Society B-Biological Sciences, 281*(1795). doi: doi.org/10.1098/rspb.2014.1988

Robinson, J., Mills, J., & Breed, M. (2018). Walking ecosystems in microbiome-inspired green infrastructure: an ecological perspective on enhancing personal and planetary health. *Challenges, 9*(2), 40.

Robinson, J. M., & Breed, M. F. (2019). Green prescriptions and their co-benefits: Integrative strategies for public and environmental health. *Challenges, 10*(1), 9.

Rook, G. A. (2013). Regulation of the immune system by biodiversity from the natural environment: an ecosystem service essential to health. *Proceedings of the National Academy of Sciences, 110*(46), 18360-18367.

Rothschild, D., Weissbrod, O., Barkan, E., Kurilshikov, A., Korem, T., Zeevi, D., . . . Bar, N. (2018). Environment dominates over host genetics in shaping human gut microbiota. *Nature, 555*(7695), 210.

Rydin, Y., Bleahu, A., Davies, M., Dávila, J. D., Friel, S., De Grandis, G., . . . Howden-Chapman, P. (2012). Shaping cities for health: complexity and the planning of urban environments in the 21st century. *The lancet, 379*(9831), 2079-2108.

Sheard, M., & Bowman, G. (1996). *Soils, stratigraphy and engineering geology of near surface materials of the Adelaide Plains*: Department of Mines and Energy South Australia.

Smouse, P. E., Long, J. C., & Sokal, R. R. (1986). Multiple regression and correlation extensions of the Mantel test of matrix correspondence. *Systematic zoology, 35*(4), 627-632.

Stein, M. M., Hrusch, C. L., Gozdz, J., Igartua, C., Pivniouk, V., Murray, S. E., . . . Metwali, N. (2016). Innate immunity and asthma risk in Amish and Hutterite farm children. *New England journal of medicine, 375*(5), 411-421.

Tedersoo, L., Bahram, M., Põlme, S., Kõljalg, U., Yorou, N. S., Wijesundera, R., . . . Abarenkov, K. (2014). Global diversity and geography of soil fungi. *Science, 346*(6213), 1256688. doi: 10.1126/science.1256688

Thompson, L. R., Sanders, J. G., McDonald, D., Amir, A., Ladau, J., Locey, K. J., . . . Ackermann, G. (2017). A communal catalogue reveals Earth's multiscale microbial diversity. *Nature, 551*(7681), 457. doi: 10.1038/nature24621

Tonn, N., & Ibáñez, I. (2017). Plant-mycorrhizal fungi associations along an urbanization gradient: implications for tree seedling survival. *Urban Ecosystems, 20*(4), 823-837. doi: 10.1007/s11252-016-0630-5

van Elsas, J. D., Chiurazzi, M., Mallon, C. A., Elhottovā, D., Krištůfek, V., & Salles, J. F. (2012). Microbial diversity determines the invasion of soil by a bacterial pathogen. *Proceedings of the National Academy of Sciences, 109*(4), 1159-1164. doi: 10.1073/pnas.1109326109

Von Hertzen, L., Hanski, I., & Haahtela, T. (2011). Natural immunity: biodiversity loss and inflammatory diseases are two global megatrends that might be related. *EMBO reports, 12*(11), 1089-1093.

Warton, D. I., & Hudson, H. M. (2004). A MANOVA statistic is just as powerful as distance‐based statistics, for multivariate abundances. *Ecology, 85*(3), 858-874.

Wintle, B. A., Kujala, H., Whitehead, A., Cameron, A., Veloz, S., Kukkala, A., . . . Cadenhead, N. C. (2019). Global synthesis of conservation studies reveals the importance of small habitat patches for biodiversity. *Proceedings of the National Academy of Sciences, 116*(3), 909-914.

Yan, D., Mills, J. G., Gellie, N. J., Bissett, A., Lowe, A. J., & Breed, M. F. (2018). High-throughput eDNA monitoring of fungi to track functional recovery in ecological restoration. *Biological Conservation, 217*, 113-120.

Yang, G. W., Wagg, C., Veresoglou, S. D., Hempel, S., & Rillig, M. C. (2018). How Soil Biota Drive Ecosystem Stability. *Trends in Plant Science, 23*(12), 1057-1067. doi: 10.1016/j.tplants.2018.09.007

Zuo, X., Wang, S., Lv, P., Zhou, X., Zhao, X., Zhang, T., & Zhang, J. (2016). Plant functional diversity enhances associations of soil fungal diversity with vegetation and soil in the restoration of semiarid sandy grassland. *Ecology and Evolution, 6*(1), 318-328.

**Figure 1.** Map of the city of Adelaide, South Australia, displaying the sampling replicates from the five urban green space types surveyed in this study.

**Figure 2**. Ordination of replicates within multivariate spaces. (A) PCA of soil traits space; Variance explained Axis 1 = 36.75%; Axis 2 = 17.59%. (B) PCoA of vegetation cover; Jaccard distance matrix. Variance explained Axis 1 = 12.34%; Axis 2 = 9.01%. Sport Fields replicates were deleted from analysis.

**Figure 3**. PCoA Ordination of soil fungi ASV reads; Sörensen distance matrix. Variance explained Axis 1 = 10.84%; Axis 2 = 6.58%.

**Figure 4**. Boxplots of ASV richness for each of the five urban green space types of the three fungal trophic modes, determined by FUNGuild (Nguyen et al. 2016), showing (A) saprotrophs, (B) pathotrophs, (C) symbiotrophs, and (D) pathotroph:saprotroph ratios.