URBAN MICROBIOME RESTORATION

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Abstract

Humans are ecosystems where microbes – the human microbiome – provide functions and services. These services include production of vitamins and immunoregulatory short-chain fatty acids, pathogen resistance, and increased digestion and detoxification capacity. Our human ecosystem gets its integrity from the quality of the foods we eat and the health of the wider ecosystems that we live in and walk through, collecting and nourishing our microbes as we go. The state of this relationship governs much of human health. Many increasingly common noncommunicable diseases in urban populations are linked to degraded human and environmental microbiomes. Microorganisms are central to environmental and public health through their roles in biogeochemistry and immunoregulation. Urbanisation has led to disruptive changes to microbiota, both environmental and human; however, urban ecological restoration can help bring them back. Heywood Park in Unley Park, South Australia, has undergone small-scale ecological restoration through planting native understorey species in a patchwork beneath mature trees at different times over the past two decades, to improve amenity and tree health. This progressive, piecewise understorey planting has provided microbiome habitats at different stages of restoration from their previous state as irrigated turf. This study investigated whether soil microbiota differed between the different landscape treatments and ages, to inform whether companion plantings around remnant trees in Heywood Park have restored soil microbiota. Marker gene sequencing was used to compare soil bacteria and fungi in several areas of the park with long-established companion planting, younger companion plantings of staggered ages, wooded areas with no-companion planting, and turf grass areas. Results showed that mulched areas with companion plantings had healthier soil microbiomes than other areas. Soil pH, organic carbon, and effective cation exchange capacity were associated with differences in the microbiome. Time elapsed since mulching and companion planting also influenced soil microbiome assemblage.

Introduction

Urban development has progressively removed remnant vegetation that was managed by Indigenous peoples in pre-colonial times. Subsequently, indigenous plant and animal diversity has declined and biogeochemical, hydrological, and ecological processes have been interrupted (Theodorou 2022). Indigenous biodiversity, ecological processes and indigenous management practices supported soil microbial communities which play crucial roles in nutrient cycling and human, plant and environmental health.

Plant species exude compounds from their roots that nourish specific assemblages of microbiota – communities of microorganisms (Rosado *et al*. 2018). In return, these microbiota provide the roots with nutrients and disease protection. A dominant tree may support an understory assemblage that helps to create the soil conditions appropriate for beneficial microbiota to thrive and support the health of that whole plant community, including the dominant tree. As understory was removed from beneath trees to make way for turf grass for amenity and recreation, the soil health declined as did that of the remnant trees. The deterioration of soil microbial communities has not only been harmful to now isolated remnant trees, but to the health of human populations as well (Mills *et al*. 2019).

Within the Greater Adelaide area of South Australia, both rural and urban areas have shown recovery of soil microbial communities after ecological revegetation efforts (Gellie *et al*. 2017; Mills *et al*. 2020). Remnant tree health has also proved to benefit from such revegetation projects. Perhaps most interesting is that children exposed to restored urban green spaces, including in one study in Adelaide, have shown improvement in immune markers and recovery of skin and gut microbiota relative to unexposed children (Roslund *et al*. 2020;

Roslund *et al*. 2021; Mills *et al*. 2022). The important benefits of restoration of biodiversity and vegetation stratum for soil microbiota, remnant tree and public health are becoming ever clearer.

The original Grey box/South Australian blue gum (*Eucalyptus microcarpa*/*Eucalyptus leucoxylon*) woodland which covered the area to the south of Adelaide prior to European invasion and colonisation was so dense that they named it 'The Black Forest' (Kraehenbuehl 1996). Much of The Black Forest's biodiversity was subsequently lost through land clearing, farming, and development during the 19th century. A portion of The Black Forest in private ownership retained some tree and understorey species into the 20th century; this was purchased by the local authority in 1920 and dedicated as Heywood Park.

Heywood Park occupies 3.3 hectares of the traditional lands of the Kaurna people, or Kaurna Meyunna Yerta. Brown Hill Creek flows through the western edge of the park. The creek provided a reliable water source and sustained the woodland vegetation including River red gum (*Eucalyptus camaldulensis*), a widespread riparian species of substantial utility to the Aboriginal custodians. Irrigated turf planted for amenity and to support recreation competed with indigenous ground layer plant species, resulting in unheeded calls in the late 1970s to protect and rehabilitate areas of native understorey (Kraehenbuehl 1996).

As is common with mature trees when their environment changes, aged Grey box and South Australian blue gums in Heywood Park's turf areas slowly deteriorated. Deteriorating tree health and potential for branch failure in some of the large trees fueled increasing pressure for tree protection and understorey restoration from conservationists (CCSA 2004) and led to an area surrounding some of the park's remnant Grey box trees being fenced, mulched, and planted with native shrub and ground layer species in 2005 to help to restore tree health and to manage risk. Following community acceptance of understorey restoration, over time further areas were subsequently mulched and planted.

The City of Unley's progressive roll-out of mulching and understorey planting across different parts of Heywood Park, over nearly two decades, established the environment which enabled this study. The study assessed fungal and bacterial diversity and abundance in soil samples, to determine whether the soil microbiota differed in the park's different landscape treatments.

Methods

The study was conducted in Heywood Park, in the suburb of Unley Park, in South Australia (34°57'40.4"S 138°36'02.5"E). This study assessed soil bacterial and fungal communities associated with native companion plantings in woodchip mulch around old Grey box (*Eucalyptus microcarpa*) and South Australian blue gum (*Eucalyptus leucoxylon* ssp. *leucoxylon*) trees. Microbial diversity and abundance in areas with younger companion plantings were also assessed and compared with areas of turf, River red gums (*Eucalyptus camaldulensis*) without companion plantings, and with the older companion planting.

Soils were sampled from four different landscape treatment types within Heywood Park on the 15th of March 2022 (Figure 1). A total of 24 soil samples were taken, each from 0-10 cm deep. The sample types were: companion plantings (CP, 3 independent areas with 3 replicates each, 9 total samples), old fenced companion planting (FC, 1 area with 3 replicates, 3 total samples), trees with no companion plantings (NC, 2 areas with 3 replicates each, 6 total samples), and turf (T, 2 areas with 3 replicates each, 6 total samples).

Each sample site had one sample taken for microbiome analysis and one for physicochemical analysis. Samples for microbiome analysis were collected with sterilised equipment in sterile 50 mL centrifuge tubes and sent to the Australian Genome Research Facility (AGRF) for analysis. Samples for physicochemical analysis were collected in zip-lock bags supplied by the Australian Precision Ag Laboratory (APAL) which also conducted the analysis.

At the AGRF's laboratory DNA was extracted from the soil samples using the DNeasy PowerSoil Pro Kit (QIAGEN). One extraction blank control was used. Polymerase chain reaction (PCR) amplification and sequencing of all samples were done by the AGRF. Further details of the methods used will shortly be published elsewhere.

Prior to statistical analysis of both bacterial and fungal datasets, amplicon sequence variants (ASVs) were removed if they were unassigned at Kingdom, assigned as Archaea, Mitochondria or Chloroplast, were not prevalent in at least two samples, or had less than 10 sequence reads across all samples. Microbial taxonomic units in this study were ASVs.

Alpha diversity was measured as observed ASV diversity, Shannon's ASV diversity and Simpson's ASV diversity. Shannon and Simpson diversity indices were converted to effective species number by taking the exponent of Shannon's values and the inverse of Simpson's values. Generalised Linear Mixed Models with negative binomial distributions were used to model differences in alpha diversity between 'sample types' and 'sites', with sites as a random variable. Beta diversity of bacterial and fungal ASV communities was analysed using Bray-Curtis (abundance weighted) and Jaccard (abundance unweighted) distance matrices in Constrained Analysis of Principal Coordinates (CAP). The physicochemical variables were used to constrain the microbial samples. Differences between microbial communities of the sample types and sites were tested with permanova. Differential abundance testing of ASVs significantly varying in abundance by sample type was done with DESEQ2. The top 40 differentially abundant taxa by sample type were visualised with heatmaps.

Figure 1. Images of vegetation associations from which soil samples were collected:

Left, top: FC1, fenced, 17 yr old mulch & planting around remnant Grey box
Left, 2nd from top: CP1, 17 yr old mulch & planting around remnant Grey box
Left, 2nd from bottom: CP2, 7 yr old mulch & planting near River red gum & SA blue gum
Left, bottom:CP3, mulched & young planting area near River red gum and Spotted gum (*Corymbia maculata*)
Right, top: T1, turf area, no mulch, no companion planting or trees
Right, 2nd from top: T2, turf area, no mulch, no companion planting or trees
Right, 2nd from bottom: NC1, young mulched area near River red gum, no planting
Right, bottom: NC2, young mulched area around mature River red gums, no planting

Results

Soil Physicochemical Properties

Soil physiochemical properties differed between landscape treatments. FC and CP1 samples had high sand content, high organic carbon content, and high Ca:Mg ratio. NC1 samples had higher silt content and higher salinity. Turf samples had higher pH, higher clay content, and higher exchangeable cation ratio.

Bacteria

Bacterial alpha diversity was not different between sample sites or between any landscape treatments. Differences were observed in bacterial beta diversity between turf areas and the other treatments, most likely due to unnatural management regimes including chemical fertilisers and past irrigation with potable mains water prior to the more recent use of reclaimed water. In both the Bray-Curtis (abundance weighted) and Jaccard (binary presence) constrained analyses the turf grass communities were different from the rest of the samples. The non-turf samples also differed; they formed two distinct groups. One group was the FC1 and CP1 areas that represent the oldest companion plantings; these were most strongly associated with soil organic carbon, sand, and nitrogen species. The other group included both no-companion areas (NCx) and the CP3 area that represented the youngest companion planting. The CP2 samples straddled both groups, suggesting that restoration of bacterial diversity and abundance is related to the age of the companion planting and that the CP2 area (with an intermediate community between CP1 and CP3) is transitioning from a soil bacterial community associated with absence of companion planting to a community more typically associated with mature diverse plantings.

Soil physicochemical variables most strongly associated with variation in the bacterial communities between samples were pH and organic carbon. Higher pH was more characteristic of turf and young companion planting (CP3) samples. Organic carbon, which was correlated with the microbially important variable effective cation exchange capacity (ECEC), was more characteristic of the more mature companion plantings (FC1 and CP1). Nitrogen species (nitrate and ammonium) were also significant to the variation in bacterial communities, and these were characteristic of the more mature companion plantings.

Dominance of bacterial phyla varied between the sample types. The more mature plantings (FC1 and CP1) had distinctly more Firmicutes in relative abundance (4.2-11.7 %) than less mature companion plantings (CP2 and CP3: 0.8-4.3 %), areas with no companion plantings (0.5-3.5 %), and turf (0.9-1.8 %). Proteobacteria were generally higher in relative abundance in sites with companion plantings (15.2-35.2 %) than without (14.0-25.8 %). The turf grass samples had higher relative abundance of Verrucomicrobia and Nitrospirae (1.7-3.3 % & 0.5-4.0 %, respectively) than all other sites (0.2-1.3 % & 0.0-0.6 %, respectively). Acidobacteria were higher in relative abundance in CP3 and turf samples, which were also characterised by higher pH.

Fungi

Fungal alpha diversity did not differ between treatments for all three measures (observed ASV richness, Shannon's effective number of ASVs, Simpson's effective number of ASVs). Fungal beta diversity in turf communities was different from other sample types for both Bray-Curtis and Jaccard analyses. In the abundance weighted Bray-Curtis analysis, FC, CP, and NC samples formed a large cluster with mature *Eucalyptus* trees being the main commonality between them. pH, organic carbon, and nitrate were significant constraining variables to both Bray-Curtis and Jaccard beta-diversity analyses. Further, ammonium, magnesium, and the Ca:Mg ratio were significant to the Jaccard model.

Three of these samples with mature *Eucalyptus* trees were outliers (FC1_2, CP1_2, NC1_1) with nearly 90 % relative abundance of Ascomycota, far more dominant than in other samples. Ascomycota generally dominated most samples. However, Basidiomycota were dominant in several samples, including all from CP3. Most differentially abundant fungi were highly abundant in just one sample, showing that fungal species were not characteristic of any particular areas and that their sphere of individual influence was smaller than the space between samples. It was noteworthy that *Armillaria luteobubalina*, responsible for root-rot in some parts of Adelaide, was not identified in these samples.

Discussion

The age of the understorey companion plantings appeared to be an important factor for community composition of bacteria and fungi in the Heywood Park samples. The communities in the older planting, CP1, were quite separated from the youngest planting in CP3, with those of CP2 spanning the space between them in the constrained ordinations. Previous studies in the Adelaide area have found there to be a multi-year time lag in soil community development between time of planting and reaching a reference community state (Gellie *et al*. 2017; Baruch *et al*. 2020). That is likely the successional process at play here as there was no intervention by inoculation. While there is no remnant reference for these samples, there is a stronger similarity with FC1, the oldest companion planting, to the older CP1 than the younger CP2 and CP3 plantings.

The bacterial and fungal communities of older FC1 and CP1 plantings were both strongly linked to higher organic carbon soil loads than other sites, a variable that was a significant predictor in the models. Organic carbon is important as a food source for microbiota and is generally associated with greater water holding capacity. Furthermore, organic carbon in these samples was collinear (i.e., strong positive correlation) with effective cation exchange capacity, a soil variable identified in an Australia wide study as being inversely associated with infectious and parasitic disease risk in humans (Liddicoat *et al*. 2018).

The relative abundance of Firmicutes was higher in the older FC1 and CP1 plantings relative to other areas. This finding is at odds with a previous study from the Mt Bold Reservoir near Adelaide, where older *Eucalyptus leucoxylon* dominated restoration sites had relatively fewer Firmicutes than younger sites (Gellie *et al*. 2017). However, in agricultural contexts, higher relative abundance of Firmicutes has been associated with disease suppressive soil (Lee *et al*. 2021). Firmicutes, as a broad group of taxa representing a broad range of functions, likely need to exist in a ‘goldilocks’ zone of dominance, where over or under representation could lead to poorer soil function.

Proteobacteria were slightly lower in relative abundance in unplanted mulch (NC) and in turf soil samples than in samples from sites with companion planting. Proteobacteria are heavily involved in carbon, nitrogen, and sulphur cycling (Kersters *et al*. 2006), and their relative abundances in this study coincided with higher levels of organic carbon and nitrogen species. Proteobacteria are also important in the nexus between environmental microbiomes and improved human immune function (Hanski *et al*. 2012; Roslund *et al*. 2020).

The Ascomycota dominated the fungal community in most samples. Ascomycota dominance is common in soils the world over (Egidi *et al*. 2019); however, there were several instances when the Basidiomycota were most dominant in this study. One study from China found that increased Basidiomycota relative to Ascomycota was indicative of degraded soils (Qi *et al*. 2021). The Basidiomycota-dominated samples were spread across all sample types, including all CP3 samples, perhaps indicating that urban soils maintain pockets of degradation even after restoration.

Ecological benefits additional to recovering soil microbiomes and human health are also significant and can add further value to soil health restoration, including contributing to the conservation of locally endangered species (plant and animal) and educating the public about these. Indigenous plant species support indigenous pollinators, and can reduce chemical fertiliser requirements, pesticides, and emissions associated with turf grass maintenance (Townsend-Small & Czimczik 2010). That is not to say that turf grass should be excluded from reserves in lieu of biodiverse plantings, as open areas for recreation are socially beneficial and healthy. However, a balance of turf with biodiversity such as is being progressively developed at Heywood Park is a healthier option. Local government and other land managers should be broadly encouraged to use indigenous understorey plantings in association with tree cover in parks and other urban sites, and to progressively increase plant species diversity over time, to benefit the environment and human population.

Conclusion and Recommendations

Mulching and planting with understorey species appear to have generally improved the bacterial and fungal soil communities and soil health at Heywood Park. As microbe community maturation can take years after planting, with time the microbiome communities in the most recently planted areas may reflect the communities present at the older fenced and planted site more closely. Benefits of planting and mulching identified at Heywood Park, specifically the increased relative abundance of Firmicutes, higher effective cation exchange capacity, and higher levels of Proteobacteria, are all associated with improved human health and wellbeing. The City of Unley is to be congratulated on the ecological restoration work at the park, for its potential benefit to human health and wellbeing and to environmental health.

Expanding the planting of indigenous understorey species and incorporating well-planned experiments is encouraged to further inform this developing field of investigation. Companion planting studies with networked and embedded experiments built into working demonstrations could be used to track the ecological outcomes. Replicating and staggering plantings could allow for rigorous ecological monitoring of microbiota, to support threatened species recovery and pollinator services, and to encourage more projects for urban ecological recovery. Such experimentation would provide an evidence base to inform increasingly effective restoration, conservation, and preventative health measures. Such experimentation could investigate whether biogeochemical cycling can recover in urban areas and whether widespread biodiverse planting can influence the human microbiome and immune system at the suburb, local government, and whole-of-city scale.

The City of Unley and councils nationally can drive urban ecological restoration further for environmental and human benefit. The growing evidence of public health benefits is encouraging. Areas with under-used green space could not only increase tree canopy cover but also restore the urban microbiome. Local, state and territory governments can progress this preventative health approach on parks, greenways, streets and road corridors, at schools, and in gardens at major facilities that are accessed by large groups of people. Such a program could have health benefits coupled with important ecological and nature education opportunities. Governments that combine urban ecological restoration with embedded experimentation will be seen as innovative global leaders in public and environmental health.

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