Bacterial communities in peat swamps reflect changes associated with catchment urbanisation

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Abstract
Like many peat wetlands around the world, Temperate Highland Peat Swamps on Sandstone (THPSS), located in the Sydney Basin, Australia, have been impacted by urban development. In this paper, we used Illumina 16S rRNA DNA amplicon sequencing to characterise and compare the bacterial communities of surface (top 0–2 cm) and deep (50 cm) sediments in peat swamps that occur in both urbanised and non-urbanised catchments. Proteobacteria (32.2% of reads), Acidobacteria (23.6%) and Chloroflexi (10.7%) were the most common phyla of the dataset. There were significant differences in the bacterial community structure between catchment types and depths apparent at the phyla level. Proteobacteria, Bacteroidetes, Actinobacteria and Verrucomicrobia made up a greater proportion of the reads in the surface sediments than the deeper sediments, while Chloroflexi and Nitrospirae were relatively more common in the deeper than the surface sediment. By catchment type, Acidobacteria were more common in swamps occurring in non-urbanised catchments, while Nitrospirae, Bacteroidetes and Actinobacteria were more common in those in urbanised catchments. Microbial community structure was significantly correlated with sediment pH, as was the relative abundance of several phyla, including Acidobacteria (negative correlation) and Bacteroidetes (positive correlation). As an indicator of trophic shift from oligotrophic to copiotrophic conditions associated with urbanised catchment, we found significant differences ratios of β-Proteobacteria to Acidobacteria and Bacteriodetes to Acidobacteria between the catchment types. Based on SIMPER results we suggest the relative abundance of Nitrosomonadaceae family as a potential indicator of urban degradation. As the first study to analyse the bacterial community structure of THPSS using sequencing of 16S rDNA, we reveal the utility of such analyses and show that urbanisation in the Blue Mountains is impacting the microbial ecology of these important peatland ecosystems.

Keywords Peatland · Wetland · Swamp · Bacterial community · Temperate Highland Peat Swamps on Sandstone · Illumina sequencing · Urbanisation

Introduction
Wetlands have the potential to mitigate surface water pollution and carbon emissions, which are among the most pressing and widespread anthropogenic disturbances (Mitsch and Gosselink 2015). Yet, wetlands globally are threatened by a multitude of pressures (Asselen et al. 2013). Urbanisation is a major threat to the physical and biological structure and function of wetlands. It is well known that changes to the imperviousness of surfaces within a catchment negatively affects the ecology of wetlands (Arnold and Gibbons 1996) by altering the hydrology, augmenting nutrient and pollutant concentrations and changing physicochemical conditions leading to a loss of ecosystem services (Walsh et al. 2005; Tippler et al. 2014). For example, stormwater runoff can channelize wetlands, lower water tables, and impact water quality (Tippler et al. 2014; Cowley et al. 2016).

Wetland microbial communities are sensitive and responsive to changing environmental conditions and shifts in microbial assemblage have the potential to alter ecosystem function (Urakawa and Bernhard 2017). Given that the sediment microbial community is fundamental to carbon and nitrogen cycling in wetlands (Mitsch and Gosselink 2015),
understanding the implications of urbanisation to wetland microbial function is key, as is the ability to track and assess degradation associated with urbanisation, to ensure that ecosystem services and ecosystem integrity are maintained.

Since European colonisation and ensuing urbanisation, wetlands such as the endangered Temperate Highland Peat Swamps on Sandstone (THPSS) that occur in the Sydney Basin in south-eastern Australia, have undergone habitat loss and degradation (Kohlhagen et al. 2013). The THPSS, particularly those in the developed areas of the Blue Mountains, are increasingly under pressure from the effects of urbanisation (Belmer et al. 2015, 2018; Fryirs et al. 2016; Lane 2016). Impervious catchment area and the number of stormwater drains entering these swamps have significant negative impacts on the hydrological and geomorphic condition of these wetlands (Fryirs et al. 2016). THPSS in urbanised catchments have altered macroinvertebrate communities (Belmer et al. 2018; Hardwick 2019) and chemical properties (Belmer et al. 2015). Urbanisation is also associated with increased carbon exports and depletion of carbon stocks in these swamps at an ecologically and economically significant scale (Cowley et al. 2018a, b; Cowley and Fryirs 2016). As peat formation and carbon sequestration processes rely heavily on slow microbial decomposition of accumulated organic matter under saturated, anaerobic and naturally acidic conditions (Moore 1989), it is important to understand the microbial composition and processes of the ecosystem to improve management and protect these systems.

Surprisingly, there have been few comparative studies investigating the impacts of urbanisation on wetland microbial assemblages. A number of studies undertaken in streams and rivers have recently reported impacts from urbanisation on microbial assemblage composition in the water column and/or sediments (Ibekwe et al. 2016; Wang et al. 2016, 2018; Hosen et al. 2017; Jani et al. 2018; Roberto et al. 2018; Simonin et al. 2019). In tidal wetlands, microbial assemblages responded to urbanisation stressors by shifting function toward increased nitrification and xenobiotic degradation (Gonzalez Mateu et al. 2019). We previously reported differences in the community fingerprints between THPSS in urbanised and non-urbanised catchments, and increased likelihood of finding the microbial functional genes for methanogen and methanotrophs (mcrA & pmoA) in the urbanised swamps (Christiansen et al. 2019), but an in-depth taxonomic analysis is lacking.

The aim of this study was to characterise bacterial assemblages in sediments in THPSS (hereafter called swamps) by high-throughput 16S rRNA amplicon sequencing, and to compare the assemblages in swamps within urbanised and non-urbanised catchments. Further, we sought to identify taxa indicative of urban-impacted conditions and microbially-mediated ecosystem community functions that may also be affected. These swamps are saturated peat-forming systems that are naturally nutrient poor and acidic (Pemberton 2005). Consequently, we expected that undisturbed swamps would harbor taxa with oligotrophic traits of slow carbon mineralisation. The ratio of copiotrophic to oligotrophic taxa has been suggested as a reliable indicator of trophic status and ecological condition in wetlands and soils (Smit et al. 2001; Sims et al. 2013), which we apply in this study using the ratio of Proteobacteria to Acidobacteria (Smit et al. 2001; Hartman et al. 2008). Bacteroidetes and β-Proteobacteria are more consistently copiotrophic (Fierer et al. 2007) compared with the large and diverse phyla of Proteobacteria, so we also considered the ratios of β-Proteobacteria to Acidobacteria and Bacteroidetes to Acidobacteria as indicators of disturbance to trophic status. Our hypothesis was that greater relative abundances of oligotrophic taxa would be found in the non-urbanised catchment swamps and conversely a greater relative abundance of copiotrophic taxa would be found in the urbanised catchment swamps. We anticipated taxa associated with an urbanised catchment would include those with nitrification or bioremediation capabilities, indicative of pollution associated with stormwater runoff. We consider potential indicator taxa and the applicability for microbial biomonitoring in these ecosystems and more broadly.

Materials and methods

Study sites

The study area is located in the Blue Mountains approximately 100 km west of Sydney (Fig. 1). The swamps are situated in shallow depressions atop the sandstone plateau of the Sydney Basin (Fryirs et al. 2019). The area experiences a temperate climate with mean maximum temperatures between 9.4 °C (July) to 23.3 °C (January) and mean minimum temperatures between 2.6 °C (July) to 12.9 °C (February), with mean annual rainfall of 1400 mm (BOM 2017). The swamps are generally covered by dense native vegetation that includes sedge, Melaleuca, Grevillea and Banksia species (Keith and Myerscough 1993) and have a characteristic stacking of sediment layers that comprise the valley fill (Freidman and Fryirs 2014; Fryirs et al. 2014a, b; Cowley et al. 2016). Typically these swamps lack standing water. The water table is (generally) about 20 cm below ground level, however the highly organic sediments are generally saturated (Hose et al. 2014; Cowley et al. 2018a, b).

Twelve swamps were sampled in this study. Six ‘urbanised catchment swamps’ were located within the towns of Katoomba, Wentworth Falls, Leura or Blackheath. The urbanised sites had impervious catchment area > 30% and between zero and eleven stormwater drains discharging into them (Fryirs et al. 2016) (Fig. 1, Table 1). Six
'non-urbanised catchment swamps' were located nearby in catchments that were dominated by natural vegetation, had <15% impervious catchment area and no stormwater drains discharging into them. Sediment physico-chemistry has been described in Christiansen et al. (2019) and summarised for each site in Table S1. Most notably, sediment pH and surface ammonium concentrations were higher in urbanised than in non-urbanised catchment swamps. The electrical conductivity, moisture and organic content of sediments were similar between the swamps in different catchment types (Table S1) (Christiansen et al. 2019).

Sample collection

Sampling was conducted in September 2015. Sediment was collected at three representative random locations along the central axis of each swamp using a Russian D-corer. From each core, sediment was sampled from the top 1–2 cm and at 50 cm depth. These depths target contrasting sediment composition and conditions (Cowley et al. 2016). The surface is comprised of surface organic fines of living and decomposing organic matter and fine silt and sand and is likely to favour aerobic microbial metabolic processes. Sediments at 50 cm depth were generally alternating organic sands, which include layers of peat and mineral sand. These deeper sediments had lower electrical conductivity, ammonium, moisture and organic content than the surface sediments (Table S1). Sediment pH was similar between the two depths (Table S1) (Christiansen et al. 2019). Samples for molecular analysis were placed directly into separate sterile tubes (2 mL Eppendorf tubes) with a stainless steel spatula sterilised with ethanol in between samples. All sediment samples were snap-frozen immediately in the field using dry ice and stored upon return to the lab at –80 °C.

Table 1 Location and attributes of upland swamp sampling sites in the Blue Mountains NSW, Australia

| Catchment type | Swamp               | Channel |
|---------------|---------------------|---------|
| Non-urbanised | Fortress Creek (FC) | N       |
|               | Grand Canyon (GC)   | 4.9     |
|               | Michael Eade’s Reserve (ME) | 13.1 |
|               | Mt Hay Creek (MtH)  | 0       |
|               | Timmy’s Swamp (TS)  | 0       |
|               | Vista (VS)          | 0       |
| Urbanised     | Fifth Avenue (5A)   | 44.3    |
|               | Katoomba Falls Rd (KF) | 51.9 |
|               | Marmion Rd (MA)     | 51.6    |
|               | Pitt Park (PP)      | 31.9    |
|               | Popes Glen (PG)     | 38.5    |
|               | Wentworth Falls Lake (WFL) | 62.7 |

Impervious catchment and stormwater drain data are from (Fryirs et al. 2016). Swamp names as follows: FCFortress Creek, GCGrand Canyon, MEMichael Eade’s Reserve, MtHMt Hay Creek, TSTimmy’s Swamp, VSVista Swamp, 5AFifth Avenue, KFKatoomba Falls Road, MA Marmion Road, PP Pitt Park, PGPopes Glen, WFLWentworth Falls Lake.
Molecular analysis

Extraction

Environmental DNA was extracted from sediment samples using the PowerSoil Total RNA Isolation Kit and DNA Elution Accessory Kit (MoBio) according to the manufacturer’s protocol. In this study only the DNA extraction was utilised. The purity and yield of the DNA was quantified using a NanoDrop 2000 spectrophotometer (Thermo Scientific Inc.). Eluted DNA was diluted with sterile MilliQ water (1:150) to avoid PCR inhibitors based on qPCR efficiency curves of serial dilutions.

Sequencing and pipeline processing

Amplicon sequencing was performed on the V1-V3 region of the 16S rRNA bacteria gene via the Illumina MiSeq platform using the primers 27f and 519r (Lane et al. 1985; Lane 1991). PCR amplification and sequencing were completed by Ramaciotti Centre for Genomics, University of New South Wales, Sydney (ISO/IEC 17,025 accredited laboratories).

Sequencing reads were processed using the EBI genomics project processing pipeline Version 4.0 (Mitchell et al. 2017). In summary, paired overlapping raw reads were merged to form longer reads using SeqPrep (v 1.1). For quality control, low quality ends and sequences (> 10% undetermined nucleotides) were removed using Trimomatic (v 0.35), and sequences less than 100 nucleotides were removed using Biopython (v 1.65). Infernal (v 1.1.2) was used to identify ncRNA and lower quality overlaps removed by Cmsearch deoverlap script (v 1.0). Taxonomy and OTU classifications were assigned using MAPseq (v 1.2) with SILVAs SSU/LSU (v 128) database. Data are publicly available and stored in the European Nucleotide Archive (ENA) https://www.ebi.ac.uk/ena/browser/view/PRJEB23135.

Statistical analysis

Diversity, measured with Shannon diversity index, richness, estimated by Chao1 index, the relative abundances of phyla, and ratios of taxa were compared using a 3-factor repeated measures analysis of variance (ANOVA). In these analyses, catchment type (between-subject factor) and depth (within-subject factor) were considered fixed factors and site was considered a random factor and nested within catchment type. Sphericity was tested using Mauchley’s Test and the Geisser-Greenhouse Adjustments used where the assumption was not met. Tukey’s post hoc pair-wise comparisons were used to test for differences between levels where there was a significant interaction. Relationships between phyla relative proportions, diversity and richness were explored using Pearson’s correlation.

Bacterial assemblages were analysed at the operational taxonomic unit (OUT) level. Assemblages were compared between catchment types and depth using a 3-factor PERMANOVA, replicating the ANOVA analysis described above. The ‘catchment type’ and ‘depth’ were considered fixed factors with ‘site’ nested within catchment type as a random factor. Profiles at the OTU level were visualised using non-metric multidimensional scaling (NMDS). The OTU reads were standardised by total number of reads and square root transformed (Hellinger transformation) and made into a resemblance matrix using the Bray–Curtis similarity index (Clarke et al. 2006). Where differences were found, we followed up with SIMPER analysis to determine the OTU(s) that contributed most to the dissimilarities between groups.

Relationships between bacterial community assemblages and sediment conditions at the time of sampling (from Christiansen et al. 2019, Table S1) were analysed at each depth using distance based linear models (DistLM) with stepwise selection based on Bray–Curtis similarity. Sediment variables that were significant in explaining the community variation were further correlated (Pearson’s correlation) with the relative abundance of key phyla and Proteobacteria classes.

Analysis of taxonomic data was based on relative abundance (number of reads per OTU over the total number of reads for that sample). The phyla level dataset was limited to those contributing at least 1% of the total, and the OTU level dataset was limited to those contributing at least 0.1% of the total dataset. The diversity and richness index calculations were based on the complete dataset. The significance level (α) for all analyses was 0.05. The 3-factor ANOVA and Pearson’s correlation analyses were performed using Minitab (Minitab Inc, PA, USA). The PERMANOVA, SIMPER and DistLM analyses were performed with PRIMER 6 & PERMANOVA + add on version 1.0.8 (PRIMER-E Ltd., Plymouth, UK).

Results

Sample reads

The mean number of sequence reads per sample was 97,430, ranging from 25,877 to 175,294. A total of 2754 OTU were found. The mean number of OTUs per sample was 544 OTU and ranged from 128 to 1098. There were 179 OTUs that individually made up at least 0.1% of the dataset. The greatest number of those were found in the surface of the urbanised catchments (154 OTU), while 79 OTU were found at both depths and catchment types.
Shannon Diversity index values differed significantly between catchment types ($p = 0.022$), depths ($p < 0.001$) and sites (nested within catchment type) ($p = 0.041$). Overall, mean (± SD) diversity was greater in the urban catchment surface sediments (4.53 ± 0.33) than non-urbanised catchment surface sediments (4.17 ± 0.21), which were greater than deep urban (3.84 ± 0.39) and non-urbanised catchment sediments (3.57 ± 0.36) (Fig. 2). Mean OTU richness differed significantly between catchment types ($p = 0.032$) and depths ($p < 0.001$), but not sites nested within catchment type ($p > 0.05$). Mean OTU richness (± SD) was greatest in the urban catchment surface sediments ($1058 ± 233$) followed by non-urbanised catchment surface sediments ($782 ± 112$), deep urban catchment sediments ($574 ± 180$) and deep non-urbanised catchment sediments ($500 ± 143$) (Fig. 2).

Phyla analysis

Proteobacteria was the most common phylum, comprising 32.2% of reads of the dataset, followed by Acidobacteria with 23.6% and Chloroflexi with 10.7%. Other phyla that comprised more than 1% of the total reads were Planctomycetes (9.0%), Nitrospirae (3.3%), Bacteroidetes (3.0%), Actinobacteria (2.5%), Verrucomicrobia (2.3%), Firmicutes (1.4%) and Spirochaetes (1.1%).

Several phyla were more abundant in either the deeper or surface sediments. Proteobacteria, Bacteroidetes, Actinobacteria and Verrucomicrobia made up a greater proportion of the reads in the surface sediments than the deeper sediments, while Chloroflexi and Nitrospirae were relatively more common in the deeper than the surface sediment (all $p < 0.001$). Acidobacteria had greater relative abundance in swamps in non-urbanised catchments ($p = 0.046$), while Bacteroidetes ($p = 0.008$), Nitrospirae ($p = 0.008$), and Actinobacteria ($p = 0.007$) were more abundant in swamps in urbanised catchments. The relative abundance of Proteobacteria as a whole did not differ significantly by catchment type, but there were differences of some Proteobacteria classes between catchment types. The proportion of α-Proteobacteria was greater in the swamps in non-urbanised catchments ($p = 0.002$) than the swamps in urbanised catchments, while the proportion of β-Proteobacteria ($p = 0.004$) and δ-Proteobacteria ($p = 0.005$) were greater in the swamps in urbanised than non-urbanised catchments. The proportion of each Proteobacteria class varied with depth. There was a significant interaction between catchment type and depth for Planctomycetes ($p = 0.025$), Nitrospirae ($p = 0.012$), Actinobacteria ($p = 0.003$) and Firmicutes ($p = 0.002$). In the swamps in non-urbanised catchments Planctomycetes was relatively more common in the deeper than surface sediments and Firmicutes made up a greater proportion of the surface sediment compared to the deep sediment assemblage but neither taxon differed markedly with depth in urbanised catchments (Fig. 3). In the urbanised catchments, Nitrospirae made up a greater proportion of the deep than the surface sediment assemblages and Actinobacteria made up a greater proportion of the surface compared to the deep sediment assemblages but neither taxon differed markedly with depth in non-urbanised catchments (Fig. 3).

Bacterial community analysis

The ordination of the bacterial community shows a division among samples along the X axis that reflects catchment type (Fig. 4). There was further division along the Y axis, with surface sediments clustering toward the top of the plot and deep samples toward the bottom (Fig. 4). In general, the surface samples were clustered together more tightly, suggesting greater within group similarity, than the
deep sediments (Fig. 4). This visualisation was supported by the PERMANOVA results. The bacterial community composition varied significantly by catchment type (p = 0.001) and depth (p = 0.001), site within catchment (p = 0.001) and there was an interaction between catchment type and depth (p = 0.037) and site and depth (p = 0.004) (Fig. 4). Pairwise tests of the sites revealed that Pitt Park (PP) and Popes Glen (PG) were significantly different from the other swamps in urbanised catchments, but not each other. Vista Swamp (VS) was different from all but Fortress Creek (FC), and Grand Canyon (GC) and Timmy’s Swamp (TS) were different.

Having found a significant difference between surface and deep sediments and a significant interaction between catchment type and depth, we ran a second PERMANOVA and SIMPER analysis on the surface and deep samples separately. For the surface sediments, the catchment type (p = 0.004) and

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**Fig. 3** Mean phylum composition (including Proteobacteria classes) for each swamp and overall mean by depth and arranged by catchment type (urbanised or non-urbanised). Asterisk (*)/(**) adjacent to phyla/class indicates significant differences (p < 0.05) between catchment types relative abundance in non-urbanised catchments. One (*) indicates greater relative abundance in non-urbanised catchment swamps and two (**) indicates greater relative abundance in urbanised catchment swamps. Swamp names as follows: FC Fortress Creek, GC Grand Canyon, ME Michael Eade’s Reserve, MHH Mt Hay Creek, TS Timmy’s Swamp, VS Vista Swamp, 5A Fifth Avenue, KF Katoomba Falls Road, Ma Marmion Road, PP Pitt Park, PG Popes Glen, WFL Wentworth Falls Lake

**Fig. 4** NMDS of bacterial community based on OTU with greater than 0.1% relative abundance for the dataset. Black triangles are for swamps in non-urbanised catchments and grey circles are for swamps in urbanised catchments. Solid symbols are from surface sediment samples and open are from deep sediment (50 cm). Swamp names as follows: FC Fortress Creek, GC Grand Canyon, ME Michael Eade’s Reserve, MHH Mt Hay Creek, TS Timmy’s Swamp, VS Vista Swamp, 5A Fifth Avenue, KF Katoomba Falls Road, Ma Marmion Road, PP Pitt Park, PG Popes Glen, WFL Wentworth Falls Lake

2D Stress: 0.11
sites (within catchment) \( (p = 0.036) \) were significantly different. The taxa that contributed most to the differences between catchment types were *Candidatus_Koribacter* (genus) and Acidobacteriales (order) from Acidobacteria, and *Rosearcus* (genus) from α-Proteobacteria which were all more abundant in the swamps in non-urbanised catchments than the swamps in urbanised catchments. Nitrosomonadaceae (family) from β-Proteobacteria and *Geobacter* (genus) from δ-Proteobacteria were both more abundant in the swamps in urbanised than non-urbanised catchments. The assemblage composition at depth differed significantly with catchment type \( (p = 0.002) \) and site (within catchment type) \( (p = 0.002) \). The taxa that contributed most to the differences between the catchment types were Ktedonobacterales (order) from phylum Chloroflexi, *Candidatus_Koribacter* (genus) from Acidobacteria, *Isosphaera* (genus) and Isosphaeraceae (family) from Planctomycetes, and Acidobacteriales (order) from Acidobacteria, all of which had greater relative abundance in the swamps in non-urbanised than urbanised catchments (Fig. 5).

**Indicator of trophic status**

The ratios of Proteobacteria to Acidobacteria were not significantly higher in swamps in urbanised catchments \( (p = 0.063) \) than swamps in non-urbanised catchments (Fig. 6a). However, the ratios of β-Proteobacteria to Acidobacteria \( (p = 0.004) \) and the ratios of Bacteriodetes to Acidobacteria \( (p = 0.006) \) were (Fig. 6b, c, respectively). For each of these three ratios, the values were higher in

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**Fig. 5** NMDS of bacterial assemblages in A surface (0–2 cm) and B deep (50 cm) sediments from swamps in unurbanised and non-urbanised catchments. Black triangles are for swamps in non-urbanised catchments and grey circles are for swamps in urbanised catchments. The vectors show the relationship of the relative abundance of the five OTUs that contributed the most variation between the catchment types based on SIMPER results. The OTUs are reported to the lowest taxonomic level possible and the taxonomic level of identification is indicated by the letter preceding the taxonomic name as follows: o order, f family, g genus. Swamp names as follows: FC Fortress Creek, GC Grand Canyon, ME Michael Eade’s Reserve, MTH Mt Hay Creek, TS Timmy’s Swamp, VS Vista Swamp, 5A Fifth Avenue, KF Katoomba Falls Road, Ma Marmion Road, PP Pitt Park, PG Popes Glen, WFL Wentworth Falls Lake.
the surface sediments than the deep sediments (p < 0.001 for all) and there were significant differences between sites within catchment types (p ≤ 0.001 for all). The Pitt Park and Popes Glen sites had higher values than other sites (Fig. 6).

Ammonia oxidizing bacteria (AOB) has been suggested as an indicator of wetland ecosystem condition (Sims et al. 2013). The Nitrosomonadaceae family, which was highlighted with the SIMPER results as a key taxon contributing to differences between the catchment types, includes AOB. The relative abundance of Nitrosomonadaceae was significantly higher in swamps in urbanised catchments (p = 0.002) than in swamps in non-urbanised catchments. Nitrosomonadaceae reads were also more common in the surface than in deep sediments (p < 0.001) and there were differences between the sites within the catchment types (p = 0.001) (Fig. 6).

### Sediment environment and bacterial community

Sediment pH explained the greatest portion of the variation in the microbial community composition in the surface sediments (r² = 0.51, p = 0.001). Ammonium (r² = 0.03, p = 0.031) and moisture content (r² = 0.03, p = 0.023) explained a small but statistically significant portion variation in the surface sediments (Fig. 7). By phyla, Acidobacteria (r = −0.820, p < 0.001) and Firmicutes (r = −0.625, p < 0.001) were negatively correlated with pH, as was class β-Proteobacteria (r = −0.771, p < 0.001). Bacteroidetes (r = 0.702, p < 0.001) and Nitrospirae (r = 0.544, p = 0.001), class β-Proteobacteria (r = 0.801, p < 0.001) and δ-Proteobacteria (r = 0.731, p < 0.001) were positively correlated with pH in the surface sediment. Actinobacteria (r = 0.404, p = 0.018) was positively correlated with ammonium concentrations.

In the deep sediment, pH also explained the greatest proportion of the variation in assemblage structure (r² = 0.33, p = 0.001) with electrical conductivity explaining a smaller amount of variation (r² = 0.05, p = 0.012) (Fig. 7). Again, Acidobacteria (r = −0.469, p = 0.009) and class α-Proteobacteria (r = −0.670, p < 0.001) were positively correlated with pH, as was Planctomycetes (r = −0.534, p < 0.001). Bacteroidetes (r = 0.787,
p < 0.001), Actinobacteria (r = 0.738, p < 0.001) and class β-Proteobacteria (r = 0.571, p < 0.001) were positively correlated with pH. Planctomycetes also had a weak positive correlation with electrical conductivity (r = 0.368, p = 0.035).

The separation of bacterial assemblage samples in the nMDS (Fig. 7) along the same axis as the pH vector, supports the findings of the DistLM result that pH explained the greatest proportion of the variation in the bacterial assemblage structure. The few cases where an urbanised catchment swamp was clustered with those from the non-urbanised sites can be explained by the sample having a low sediment pH.

Discussion

Applying next generation sequencing techniques to the high conservation value Temperate Highland Peat Swamps on Sandstone of the Sydney Basin, we have found catchment urbanisation coincides with shifts in the bacterial assemblage and the relative abundance of functionally important taxa. Despite relatively small towns situated in the catchments of these swamps (e.g. Blackheath (population 5,000), Wentworth Falls (6,000), Katoomba (8,000) and Leura (4,600) (ABS 2019)), the patterns of change in
the microbial assemblages were similar to those observed in highly urbanised or disturbed aquatic environments elsewhere (Hosen et al. 2017; Jani et al. 2018; Saxena et al. 2018; Wang et al. 2018). This highlights the sensitivity of bacteria and the potential of microbial measures as indicators of function, trophic status and ecosystem health (Fierer et al. 2007; Hartman et al. 2008; Sims et al. 2013; Ho et al. 2017; Urakawa and Bernhardt 2017).

As hypothesised, in non-urbanised catchment swamps we found greater relative abundance of taxa associated with oligotrophic traits, such as Acidobacteria (Fierer et al. 2007). While Acidobacteria are a diverse and ubiquitous phylum (Janssen 2006; Jones et al. 2009; Kielak et al. 2016), they are generally competitively advantaged in low resource (Smit et al. 2001; Eichorst et al. 2007; Fierer et al. 2007; Ward et al. 2009; Ho et al. 2017) and low pH environments (Sait et al. 2006; Hartman et al. 2008). Along the same lines, studies elsewhere have found greater relative abundance of Acidobacteria in streams from forested catchments compared with urbanised catchments (Hosen et al. 2017) and in natural wetlands compared with artificial wetlands (Peralta et al. 2013). The taxa that were identified as key contributors to the dissimilarity between the catchment types and were relatively more abundant in the non-urbanised catchment swamps, also had oligotrophic traits. For example, Candidatus Koribacter (Acidobacteria) is a genus adapted to low resource conditions and slow growth (Ward et al. 2009). The genus Roseicarcus (class α-Proteobacteria) has been characterised as slow growing and moderately acidophilic (Kulichevskaya et al. 2014). The family Isosphaeraceae (phylum Planctomyces) have been commonly found in boreal peatlands and are slow decomposers and includes members that may be capable of anaerobic ammonium oxidation (Youssef and Elshahed 2014; Fuerst 2017; Ivanova et al. 2017), which is associated with low nutrient levels (Burgin and Hamilton 2007; Urakawa and Bernhard 2017).

In contrast to the swamps in non-urbanised catchments, the swamps in urbanised catchments had higher proportions of taxa associated with copiotrophic life strategies, Bacteroidetes and β-Proteobacteria (Fierer et al. 2007). While these taxa are common in freshwater environments, Bacteroidetes respond positively to pollution and β-Proteobacteria abundances are often associated with nutrient enriched environments (Wobus et al. 2003; Hartman et al. 2008; Zhang et al. 2012; Saxena et al. 2018). Members of Bacteroidetes can degrade high molecular weight organic compounds (Thomas et al. 2011; Martin et al. 2012) and have been reported to increase in disturbed urban waters including River Ganges, India (Jani et al. 2018), at industrial outfalls in the Nanxi River, China (Lu and Lu 2014) and in urban streams in Chicago, USA (Drury et al. 2013). Members of β-Proteobacteria are nitrogen cyclers and include ammonia oxidisers in the family Nitrosonomonadaceae, (Garrity 2005), which was among the top contributors of dissimilarity of the swamp surface sediment assemblages between the catchment types in this study. High relative abundances of ammonia oxidising bacteria (AOB) indicate nutrient enrichment (Sims et al. 2013; Urakawa and Bernhardt 2017; Korbel et al. 2022), which is consistent with the correlations between ammonia levels and AOB in the surface sediments of the swamps across an urbanisation gradient (Christiansen et al. 2019).

In the swamps from urbanised catchments, Actinobacteria, δ-Proteobacteria and Nitrospirae were also relatively more abundant. Species in the genus Geobacter (class δ-Proteobacteria) are capable of oxidising organic compounds and metals and are important for biogeochemical processes and bioremediation (Lovley et al. 2011; Cologgi et al. 2014). They had greatest relative abundance in the urbanised catchment swamps and were highlighted as a key taxon explaining the dissimilarity between the catchment types. Geobacter have been associated with contaminated environments (Lovley et al. 2011) including polluted river systems (Liao et al. 2019). Thus, the increased Geobacter abundance may indicate contamination is occurring in swamps found in urbanised catchments, perhaps caused by runoff from roads. Nitrospirae, which are capable of nitrite oxidation (Daims 2014) are favoured in higher nitrogen conditions and have been shown to increase in abundance around the outfalls of wastewater treatment plants (Drury et al. 2013). However, we found no correlation between ammonium levels and Nitrospirae. Alternatively, higher relative abundances of Nitrospirae may be a result of members from the genus Leptospirillum (Order Nitrospirae), which are capable of iron (II) oxidation (Daims 2014), and reflect conditions suitable for iron cycling. In nearby swamps, evidence of bacterial iron oxidation has been linked to groundwater inputs from the local iron-rich sandstone and historical disturbance of the sediment profile through excavation (Hardwick 2019). Supporting this, Katoomba Falls Road (KF) swamp had the highest relative abundance of Nitrospirae, and we observed iron flocs indicating active iron cycling at the time of sampling.

In accordance with previous studies (Fierer and Jackson 2006; Hartman et al. 2008; Lauber et al. 2009), sediment pH was again important in determining the composition of the bacterial assemblage, with significant correlations with several phyla and to the assemblage overall. The swamps in this study are naturally acidic and nutrient poor environments (Keith and Myerscough 1993; Pemberton 2005), while the impacts of urbanisation are locally associated with augmented nutrients and higher pH (Belmer et al. 2015; Lane 2016; Christiansen et al. 2019). It is not surprising that we found greater relative abundance of Acidobacteria in the swamps in non-urbanised catchments and a negative correlation with pH. Conversely, relative abundance of
β-Proteobacteria and Bacteroidetes were most strongly positively correlated with pH. The higher pH in the urbanised catchment swamps is likely due to high alkalinity inputs from concrete stormwater infrastructure and the impervious catchment area (Walsh et al. 2005; Christiansen et al. 2019; Carroll et al. 2020). Stormwater contains sediment, nutrients and other pollution and contaminants (Barbosa et al. 2012) to which bacteria may also be responding.

Given that we observed patterns of taxonomic shifts similar to other studies, we propose that microbial indicators can be applied to analysis and assessment of these swamp’s ecosystem health. The use of microbes as biological indicators for assessing ecosystem health and restoration efficacy has received increased attention lately (Watts et al. 2008; Sims et al. 2013; Aylagas et al. 2017; Urakawa and Bernhard 2017; Reumer et al. 2018; Simonin et al. 2019; Hilderbrand et al. 2020; Ritson et al. 2021). The ratio of Proteobacteria to Acidobacteria has been suggested as a microbial indicator using the principle that these taxa broadly represent copiotrophic and oligotrophic life strategies (respectively) and so a change in the ratio indicates a trophic shift (Smit et al. 2001; Hartman et al. 2008). Although we did not find the ratio of Proteobacteria to Acidobacteria to be significantly different between the catchment types, we found ratios of taxa with more reliable copiotrophic traits (Bacteriodetes or β-Proteobacteria) (Fierer et al. 2007) were significantly different between the catchment types. Given that Proteobacteria is a large and diverse phylum including taxa with varied traits (Ho et al. 2017), it may not be best suited to represent the copiotrophic taxonomic group. Indeed, in the current study, and in other nutrient poor, low pH systems, Proteobacteria often makes up the greatest overall proportion of the sequencing reads (Too et al. 2018). As discussed above, both Bacteriodetes and β-Proteobacteria made up a significantly higher proportion of the bacterial community in swamps in urbanised catchments than in swamps in non-urbanised catchments, whereas there was no clear relationship between Proteobacteria and the catchment types.

Several studies have also recommended that analysis and assessment of ecosystem health should focus on taxa that are responsible for important biogeochemical processes such as nitrogen cycling or measures of diversity and richness (Sims et al. 2013; Urakawa and Bernhard 2017). Sims et al. (2013) highlighted ammonia oxidizing bacteria (AOB) as indicative of disturbance and nutrient enrichment. In accordance with that, we have found that the Family Nitrosomonadaceae, which includes AOB (Garrity 2005), was a defining taxon in the surface sediments of swamps found in urbanised catchments. Potentially Nitrosomonadaceae could represent an indicator taxon for which qPCR assays could be developed. We also found measures of diversity and richness differed between catchment types. Importantly, bacterial diversity is related to functionality and ecosystem services (Bell et al. 2005; Delgado-Baquerizo et al. 2016). While high diversity is often associated with healthy ecosystems (Urakawa and Bernhard 2017), the swamps in non-urbanised catchments had lower diversity and richness. Contamination and disturbance can negatively influence diversity (Baker and Banfield 2003; Lenart-Boroń and Boroń 2014), however, in naturally acidic and nutrient poor systems, such as those in the THPSS in non-urbanised catchments, the inverse can be true (Griebler and Lueders 2009; Horton et al. 2019). Any shift in diversity associated with degradation, whether positive or negative, indicates a departure from the natural condition and a potential shift in function.

Here, we have shown bacteria assemblage shifted in ways that are consistent with ecosystem degradation. A change from greater oligotrophic taxa to copiotrophic taxa under urbanised catchment conditions suggests carbon is being metabolised and exported from the system providing a microbial mechanism for the increased carbon exports reported by Cowley and Fryirs (2020). Altered processes threaten the peat formation processes and therefore the viability of these unique ecosystems in the landscape. The relationship between sediment conditions and bacterial assemblages found in this study highlights the importance of natural ecosystem pH levels for the maintenance of microbial function. Given that THPSS, wetland and peatland monitoring programs are investigating new and more innovative ways of assessing ecosystem function, this study demonstrates the potential for microbial indicators as a meaningful biomonitoring tool.

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Availability of data and material Sequencing data is publicly accessible through the European Nucleotide Archive (ENA) https://www.ebi.ac.uk/ena/browser/view/PRJEB23135

Declarations

Ethics approval Not applicable.

Consent to participate Not applicable.

Consent for publication Authors give consent for publication.

Conflicts of interest/competing interests Authors have none to declare.

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