Seagrass productivity peak linked to sediment microbial changes and organic matter peak near stormwater drains

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Abstract

Estuaries support important foundation species, such as seagrasses, which promote biodiversity and contribute to ecosystem functioning. However, growing urbanisation has increased stormwater inputs into estuaries, which can cause physical scour, reduced salinity, increased sedimentation and the introduction of microbial communities and metal contaminants. The impacts are often greatest in sediments surrounding stormwater drains. These factors may affect seagrass performance directly and/or via disruptions of surface-associated and below-ground microbes that influence seagrass performance. This study investigated how seagrass (*Zostera muelleri)* productivity, sediment characteristics and seagrass associated above- and below-ground microbial communities vary with distance from stormwater drains in Lake Macquarie, Australia. We hypothesised that (i) sites closest to stormwater drains scour mark (-1m (inside the scour), 0m (edge of scour), 1m, and 5 m(within seagrass meadow)) would have lower organic matter in sediments, higher metal contamination in sediments and lower seagrass productivity than control sites (200m from stormwater drain scour); and (ii) bacterial and fungal communities on seagrass leaves, roots and associated sediments would differ between sites closest to the drains compared to control sites. We found that sediment nearest to stormwater drains had the highest metal concentrations while seagrass productivity was lowest. Sediment organic matter and seagrass productivity both peaked at intermediate distances (5m). These patterns suggest stressful conditions for the seagrass close to drains, while the organic matter increase 5m away from the drains may help to explain the peak in seagrass productivity. Sedimentary bacterial community structure differed between control sites and all four distances close to stormwater drains with putative nutrient cycling and organic decomposing taxa being less abundant near the drains where organic matter was also lower. This suggests that both sedimentary microbes and sediment organic matter are important in the functioning of seagrass plants however, further investigation is required to disentangle the specific effects of microbial activity from those of organic matter and the mechanisms by which the processes occur.

Introduction

Estuaries play a crucial role in supporting biodiversity and maintaining ecosystem functions (Chilton et al., 2021; Cardoso 2020). However, their proximity to urban areas often results in significant alterations to ecosystem functions (Todd et al, 2019). Urbanisation can lead to the modification of shorelines and the proliferation of impervious concrete surfaces. During rainfall events, these impermeable surfaces generate stormwater runoff that flows directly into estuaries (Freeman et al., 2019; Willis et al., 2017). Stormwater runoff often carries a complex array of chemical, physical and biological stressors in multiple forms (Elliot and Whitman 2011; Chilton et al 2021; Dafforn et al., 2012). The input of urban contaminants such as organic matter, metals and oils, introduces chemical stress to estuaries (Yuan et al., 2023; Liguori et al 2021; Smith et al 2021). Furthermore, under moderate to high flows and within a certain proximity of the stormwater drains, the physical velocity of the discharge creates physical stress by scouring the surrounding sediment and not allowing organic matter to settle (Freeman et al., 2019; Chilton et al 2021). Stormwater runoff can also introduce foreign microbes into the system, which may result in biological stress (Ahmed et al., 2019; Singh et al 2024; Fisher et al., 2015). These stressors can be delivered as a pulse disturbance through runoff events that introduce freshwater, organic materials and waterborne pollutants, or a press disturbance if the contaminants arriving in the stormwater bind to the sediments and persist for months to years creating legacy contamination (Walsh, Fletcher, and Burns 2012). The latter can create hotspots of significant contamination in sedimentary repositories near stormwater discharge points (O'Malley, McDonald and McNamara 2023; Walsh, Fletcher, & Burns 2012; Olds et al., 2018). Individually, these stressors reduce water and sediment quality leading to declines in important estuarine communities such as seagrasses (Freeman et al 2019; Bradley et al., 2023); however, the cumulative impacts of these stressors in stormwater discharges on seagrasses is not well understood (Rees et al, 2023; McMahon et al, 2023).

Seagrass meadows are important estuarine habitats that provide critical ecosystem functions and services which benefit surrounding habitats and organisms including various commercial fisheries species (Heck, Hays, and Orth 2003; Christie, Norderhaug, and Fredriksen 2009). However, populations of seagrass meadows have declined by 19.1% globally since 1880 from anthropogenic disturbances (Dunic et al., 2021; Waycott et al 2009; Orth et al. 2006). Stormwater-associated stressors known to negatively affect seagrass health include heavy metals, physical scour, pathogenic microbes, excess sediment load and freshwater (Li et al., 2023; Pasumpon & Vasudevan. 2021; Fonseca et al. 2019; Zhang et al 2023). The severity of the effects of these stressors are likely dependant on distances from the outlet and on discharge volumes. Under high flow scenarios, the physical force of water discharging from stormwater drains can erode the sediments around the outlet, removing the fine particles that seagrass needs to anchor itself (Jacob et al 2023). This erosion can lead to the formation of bare patches (scour marks) or channels in the seagrass meadow. Additionally, enhanced sediment deposition and influx of contaminants/freshwater can smother seagrass plants leading to these bare patches. Contaminants such as heavy metals can accumulate in the tissues of seagrasses and surrounding environment, damage the cellular structure of seagrass and disrupt their metabolic processes including photosynthesis and nutrient acquisition, leading to stunted growth, increased susceptibility to infections and mortality (Macinnis-Ng & Ralph 2002; Zhong et al 2021; Ngyuen et al 2017). Microbial pathogens commonly associated with stormwater, such as Enterococci, *Salmonella* and *Vibrio*, can lead to disease, reduced photosynthesis and increased mortality of seagrasses (Seymour et al., 2018; Orth et al., 2006; Lamb et al., 2017). Managing stormwater runoff is crucial for protecting seagrass meadows and maintaining coastal ecosystem health (Lamb et al., 2017).

In response to stress, seagrass plants exhibit visible changes in leaf morphology (e.g., decreased leaf size or increased leaf thickness; Viana, Moreira-Saporiti, and Teichberg 2020; York et al 2013). The below ground components of seagrass consist of the rhizomes, roots and rhizosphere (the zone of soil influenced by the plant's root system). These components play a crucial role in anchoring the seagrass meadow, nutrient uptake and interactions with microorganisms (Fahimipour et al 2017; Cucio et al 2016; Jensen et al., 2007; Kristensen, Haese, and Kostka 2005). At the microbial scale, aboveground and belowground environments host different microbial communities (Walker et al. 2024). Within each of these distinct microenvironments, microbes, both bacteria and fungi experience and respond differently to environmental stressors such as increased temperature and nutrient enrichment which may, in turn, affect seagrass performance (Walker et al. 2024; Fuggle et al. 2023). While a pulse stormwater input might have greater influence on aboveground seagrass health and associated biodiversity, the press disturbance occurs as pollutants settle out in the sediments and may have greater impacts on belowground seagrass health.

Microbes can adapt to environmental stressors through rapid genetic changes and horizontal gene transfer, producing stress-related proteins and metabolites quickly (Deveau et al., 2018). Microbes can form beneficial relationships with plants, such as nitrogen-fixing bacteria, and can produce phytohormones to help plants cope with stress (Timofeeva et al., 2024). Fungi rely on complex stress response pathways, including the production of protective compounds like trehalose and melanin, and have robust cell wall structures (Rangel et al., 2015). Fungi often form mycorrhizal associations with plant roots that can lead to enhanced water and nutrient uptake and produce secondary metabolites that can protect plants from pathogens and environmental stress (Meena et al., 2017). Recent studies have shown that seagrass microbial communities vary according to host species, environmental conditions and the host's health status, suggesting that the microbial communities respond rapidly to environmental disturbances and changes (Viana et al., 2017; Tarquinio et al., 2019). Birrer et al (2021) examined correlations between proximity to stormwater drains and estuarine sedimentary bacterial communities and found a clear difference in community composition with increasing distance from the drains; however, this research did not focus on the effects of stormwater contaminants on seagrass and its associated microbial communities.

The aim of the present study was to investigate the correlations between proximity to stormwater drain scour marks, productivity of the seagrass *Zostera muelleri* subsp. *capricorni* (Asch.), the above and belowground microbial community structure and diversity, and the belowground environmental conditions created by stormwater runoff.

We hypothesised that: (1) variation in above and belowground seagrass productivity and microbial community structure will be related to belowground sediment variables such as individual metal concentrations, organic matter content and particle size, and (2) seagrass productivity will peak furthest away from stormwater drains where contamination is lowest. (3) Sediment metal contamination will decrease with distance from stormwater drain while organic matter will be lowest in the scour mark and will increase sharply into the seagrass meadow where organic materials settle out from the water column. (4) Belowground bacterial and fungal communities will differ between the closest sites to the stormwater drain (-1m to 5m from scour marks) compared to control sites (> 200m from scour) as environmental conditions are predicted to be most varied/stressful closer to the drains, while no differences in aboveground communities are expected because the main stressors are belowground.

Materials and methods

Survey location and sampling design.

Three sites in Lake Macquarie, NSW, Australia (33° 8'5.69"S, 151°34'6.47"E) where stormwater drains are present were selected to survey seagrass beds of *Z. Muelleri.* Lake Macquarie is permanently open saltwater coastal lake with predominantly urbanised catchments and a tidal range of approximately 0.2 meters and 1.2 meters. Significant metal contamination from industrial activities has previously been identified along the northern shores of the Lake by Roach (2005). As such, three study sites (individual drains at Summerland Point, Nords Wharf North, and Nords Wharf South) were located at the south-eastern end of the lake where the metal signatures from the north were unlikely to influence our study (Fig. 1). Each drain was not of equal size, scour marks varied from 8m radius at Summerland point, 6m radius at Nords Wharf North and 3m radius at Nords Wharf South. Both Nords Wharf north and south are in the Crangan Bay sub-catchment of 2410 ha, while Summerland Points sub-catchment is 2436 ha. All sites were at a minimum distance of 200 m apart (straight line) between 0.5-1 m water depth at low tide within *Z. muelleri* seagrass beds > 50 m parallel to shore (confirmed with aerial imagery) (Fig. 1). Sampling was conducted radially at increasing distances from each stormwater drain, beginning from − 1 m (within the visible scour mark where these is no seagrass), and at 0 m, 1 m, 5 m (from the edge of the scour mark, i.e. all situated within the remaining seagrass meadow). Sampling was also conducted > 200 m from the edge of the scour mark to provide a control for the stormwater inputs. Control sites were healthy meadows where three replicate quadrats were taken 1m apart parallel to shore approximately 10m from the lake edge (approximate distance from shore of the 5m replicates at the storm drains). At each site, three replicate quadrats (50 x 50 cm) were sampled for each sampling distance giving a total of n = 45.

All sampling was conducted in the Austral summer during January 2023, during relatively dry conditions (< 5 mm rainfall/day over previous 14 days, Bureau of Meteorology, 2023). As such, stormwater inputs into the estuary were negligible on the days of sampling (< 0.1 m3/s) (Birch and Rochford, 2010). Sampling included surveys of sediment variables, measures of seagrass productivity, and collection of seagrass sediments and seagrass leaf and root tissues for microbial analyses.

Sampling for above- and below-ground biomass, growth rates, seagrass leaf and sediment microbes, and environmental variables conducted in line with Walker et al (2024), methods are described in short.

Seagrass productivity metrics

Above and below-ground biomass

At each of the three sites, within each quadrat (three samples per distance; 0 m, 1 m, 5 m, and > 200 m), a seagrass biomass sample was collected by 10 cm diameter 15 cm below the surface. All intact seagrass tissue material collected was rinsed with seawater to remove excess sediment and shell grit and placed on ice for transportation and stored at -20°C until processing.

Plant tissue was separated into above and below-ground samples by cutting at the intersection of shoot and rhizome. The separated materials were oven-dried at 60°C for at least 72 h before weighing for dry weight above and below-ground biomasses.

Growth rates

Growth rates of all *Z. muelleri* plants within the centre 10 cm^2 of each quadrat were measured by making two small holes through the sheath of each shoot (at the same time as sampling of other variables) using a sharp probe to create two corresponding reference scars (Short & Duarte, 2001). All plants within each quadrat were resampled after 13–14 days using a trowel for collection and were placed on ice for transport and stored at -20°C until processing.

Each plant was cut at the intersection of shoots and rhizomes to obtain only the marked shoots. Each marked shoot was then cut at the bottom of the lowest corresponding reference scar on the sheath and above the highest reference scar to leave only the new growth between the time of marking and sampling (Short and Duarte, 2001). The new leaf tissue for the shoots was oven-dried at 60°C for at least 72 h and weighed to obtain new growth in grams per shoot per day (Short & Duarte, 2001).

Photosynthetic capacity

Plants were collected for determining photosynthetic capacity using a pulse-amplitude modulated (PAM) fluorometer (Diving-PAM II, Walz GmbH Effeltrich, Germany). The fluorometer was fitted with a 2 mm plastic fibre optic cable and used with the following settings: ML int 8, G 12, SP int 12, SP width 0.8s. Seagrass leaves were dark acclimated for 15 minutes by wrapping in aluminium foil, following which fluorescence measurements were taken from the area located 2 cm from the tip of a mature seagrass leaf to minimise within-leaf variability. The maximum quantum yield of PSII (Fv/Fm) was derived from the measured minimum (Fo) and maximum (Fm) dark-acclimated fluorescence as Fv/Fm = [Fm-Fo]/Fm. Fv/Fm is the maximum proportion of light energy that can be used by PSII reaction centers for photochemistry and is a widely-used indicator of stress in seagrasses (Silva et al., 2009).

Microbial Communities

Microbial sampling

To investigate above and belowground microbial assemblages in seagrass beds, seagrass root and leaf samples were randomly collected aseptically from each replicate quadrat by cutting 1–3 shoots at their base (for leaves) and cutting 1–3 collections of roots at the intersection of the rhizome, rinsing off excess sediments and placing the plant matter in separate sterile 50 ml centrifuge tubes. Sediment microbial samples were collected aseptically from each replicate quadrat via 50 ml tube cored into the top 10 cm of sediment.

Environmental variables

Sediment variables and analyses

Within each replicate quadrats (n = 45), three 50 ml sediment cores were collected from the top 10 cm of the seagrass bed for analyses of particle size, organic matter, and trace metal concentrations. Any visible organic matter (seagrass matter, shells, leaves, sticks, etc.) and excess water collected were removed from the sample before storage on ice for transport to the laboratory. Samples were kept frozen at -20°C until analyses.

Particle size analysis by laser diffractometry was conducted following manufacturer's protocols with minor alterations (Malvern Instruments 2007). Firstly, 30 g (wet weight) sediment subsamples were wet sieved at 2 mm. Each sample was analysed three times in the Mastersizer 2000 laser diffraction particle size analyser (Malvern Instruments, UK) with measurement time of 30 s. The average particle size distribution was used to classify the samples fines (0.01-62 µm) and sand (62.5–2000 µm).

Organic matter (OM) content of sediment samples was analysed with the loss-on-ignition (LOI) method using a Lindberg Blue box furnace. Firstly, 8g wet sample was homogenised in a pre-weighed crucible and dried at 105°C for at least 12 h and weighed again to obtain dry weight. The crucible was then placed in the muffle furnace at 560°C for 12 h. The sample was then weighed immediately to obtain ash dry weight. Weight before entering the furnace minus final weight was used to obtain the percentage of organic matter in the original sample.

To prepare samples for analysis of total recoverable metal (TRM) concentrations in the sediment, ~ 30 g wet sediment samples were wet-sieved at 2 mm and oven-dried at 80°C for at least 24 h. Samples were then pulverised and homogenised into a powder using a Retsch MM 301 Ball Mill machine for 2 mins at 30 oscillations per second. TRM concentrations of the homogenised sediment samples were determined using a handheld Inno-X-Delta-DP-6000 Premium X-ray fluorescence (XRF) instrument (XRF, Olympus Delta Premium 50 kV with Rh anode tube). Samples were placed into XRF plastic cups and covered with 4 µm polypropylene microanalytical film. Each analysis was run according to operational conditions; three beam soil mode, first and second beam at 40 keV; third beam at 16 keV with 30 seconds of analysis per beam. Analytical quality of the sediments was assessed using a certified standard reference material Montana Soil-NIST2711A, as well as a pure silica dioxide blank. Coefficients of variation and detection limits from the XRF analyses are provided in table 1.

Table 1
Metals, abbreviations, detection limits, and coefficients of variations (CV) for XRF analyses.

Parameter	Abbreviation	Detection limit (mg/kg)	CV (%)
Zinc	Zn	4	1.4
Lead	Pb	7	5.6
Copper	Cu	2	1.4
Arsenic	As	1	33.3
Chromium	Cr	2	3.6

DNA extraction

For the leaf, root and sediment samples, DNA was extracted from 0.25 g of plant tissue or sediment, using bead beating and chemical lysis with the DNeasy PowerSoil Pro Kit (QIAGEN, Germany) according to the manufacturer's standard instructions. DNA concentration and quality were evaluated using a Nanodrop-1000 spectrophotometer (Thermo Fisher Scientific, USA).

DNA sequencing

To examine bacterial community composition, the V4 hypervariable region of the 16S rRNA gene was amplified with Green GoTaq® Reaction Buffer (pH 8.5, 400μM dATP, 400μM dGTP, 400μM dCTP, 400μM dTTP and 3mM MgCl2; Promega Corporation, USa) and the primers 515F (5'-GTGYCAGCMGCCGCGGTAA-3') and 806R (5-GGACTACNVGGGTWTCTAAT – 3') (Parada et al., 2015). The amplification cycle was 94°C for 2 min, followed by 25 cycles at 94°C for 30 s, 54°C for 30 s, 72°C for 1 min, and a final extension at 72°C for 5 min. To examine fungal community composition, the Internal Transcribed Spacer (ITS) regions of fungal rDNA gene was amplified with the fungal-specific PCR primers ITS1F (5'-GTGYCAGCMGCCGCGGTAA – 3') and ITS2 (5-GCTGCGTTCTTCATCGATGC – 3'). The amplification conditions for ITS region were 94°C for 2 min, followed by 35 cycles at 94°C for 30 s, 52°C for 30 s, 68°C for 30 s, and a final extension at 68°C for 10 min. Positive and no template control PCR reactions were included for both bacterial V4 and fungal ITS amplifications. Resultant amplicons were visualised on a 1% agarose gel stained with GelRed®.

Amplicons were submitted to Ramaciotti Centre for Genomics (UNSW Sydney, Australia) for preparing DNA libraries with the Illumina TruSeq® DNA library preparation kit, and sequencing on the Illumina MiSeq platform. Raw sequences and meta-data have been uploaded to NCBI Sequence Read Archive (SRA) under BioProject ID: PRJNA1191526.

Bioinformatics

Demultiplexed FASTQ files were quality trimmed by truncating at 290 bp (forward) and 260 bp (reverse). The maximum number of expected errors was set at 2 for forward and 6 for reverse reads. Sample inference was made using the core DADA2 denoising algorithm to reconstruct exact Amplicon Sequence Variants (ASVs) and filter out rare ASVs and chimeric sequences. After denoising, paired reads were merged and ASV taxonomic classifications were obtained using the SILVA reference database (v. 138). Quality of sampling and processing was also assessed through the calculation of Good's coverage. Chimera removal was done through a consensus method and with a conservative approach. Reads classified as chloroplast, archaea and mitochondria were removed from the ASV table prior to statistical analysis. All steps of this pipeline were done using the dada2 package (version 1.26.0; Callahan et al. 2016) in R (version 4.3.2; R Core Team 2017).

Statistical analyses

Analyses of seagrass response and environmental variables were performed on untransformed data after first checking data for homogeneity of variance using Shapiro-Wilk test and Levene's test. All statistical analyses were conducted using R Studio (version 3.5.4; R Core Team 2017) with the lme4 (univariate data) (Bates et al. 2014) and vegan (multivariate data) packages (version 2.5–2; Oksanen et al. 2013).

Pearson correlation coefficient was used to test whether any variables were highly correlated ($r > 0.8$); no variables were highly correlated.

To determine differences in seagrass response variables (above and belowground biomass, growth rates and photosynthetic capacity) between distances (fixed factor with 4 levels (0m, 1m, 5m, and > 200m)), a linear mixed-effects models (LMM) was fitted, including site as a random factor.

To examine the relationship between each seagrass response variable and the water and sediment variables, a stepwise model selection procedure and the AIC was used to select the best model. Before fitting models, each abiotic covariate was plotted against each seagrass response variable to check for linearity. No transformations were required as all relationships were linear.

Alpha diversity and richness of bacterial and fungal amplicon data from sediments, seagrass leaf and seagrass root tissue were estimated using observed, Shannon and Inverse Simpson diversity indices on the rarefied datasets. ASVs from each microbial dataset were removed if their total abundance was < 0.01% of the total read count in the dataset using phyloseq package (version 1.50.0; McMurdie and Holmes 2013) in Bioconductor (version 3.20). Each dataset was then standardised for sequencing depth by dividing each cell by total reads per sample. All sedimentary, root and leaf surface tissue microbial community data were compared independently between sites (fixed, 3 levels) and distance from stormwater drains (fixed, 5 levels) also using phyloseq package in Bioconductor. Bray–Curtis similarity matrices were generated using square root-transformed relative abundance (community structure) of ASVs or ASV presence/absence (community composition), using 999 permutations of residuals under a reduced model. Multivariate patterns in bacterial assemblages by site and distance from stormwater drains were visualised using non-metric multidimensional scaling (nMDS) derived from square-root

transformed Bray–Curtis similarity matrices. These similarity matrices was compared across the same two variables via permutational analysis of variance. Where the analyses returned significant differences, Tukey Post-Hoc tests were conducted to determine which distances/sites specifically varied from others. Where specific distances varied, differences in the relative abundances of ASVs at those distances (specifically, -1, 0, 1, and 5m compared 200m) using the linDA function in the MicrobiomeStat package using default parameters (version 1.2; Zhou et al., 2022). Multivariate dispersion among group within each similarity matrix was determined using "betadisper" function in vegan package while relationships between environmental variables and microbial community structure and composition were identified using the "adonis2" function in the same package. (version 2.5–2) (Oksanen et al. 2022).

Results

Differences in seagrass productivity with distance from stormwater drains

Above and belowground biomass ($F_{4,39}$ = 16.923, p < 0.001; $F_{4,39}$ = 19.253, p < 0.001 respectively) increased with increasing distance away from stormwater drains, peaking at 5m from the scour mark and then decreasing at > 200m to levels comparable to 1m (Figs. 2A&B).

Seagrass growth rates ($F_{6,29}$ = 6.256, p < 0.001) depended on site and distance from the outlet, where growth rates at Nords Wharf North differed significantly from Nords Wharf South and Summerland Point but Nords Wharf South and Summerland Point were not different from each other. Growth rates at Nords Wharf North and South increased with distance away from stormwater drains to a peak at 5m past the scour mark before decreasing at > 200m. At Summerland Point growth rates were not significantly different between distances (Fig. 2C). Photosynthetic capacity was higher at > 200 m compared to other distances closer to the scour marks (Fig. 2D).

Microbial assemblages

Community composition and structure

Overall, 12546 bacterial ASVs and 5722 fungal ASVs were obtained from leaf tissues (2772 and 1129), root tissues (4694 and 2243) and sediments (5080 and 2350).

Analyses based on Bray-Curtis dissimilarity showed that bacterial and fungal communities in sediments significantly differed among distances from stormwater drains ($F_{3,48}$ = 8.03; p = 0.00027; $F_{3,48}$ = 2.88; p = 0.01635; Fig. 3A&B; Table S1), while those communities in seagrass leaf and root tissue did not (Fig. 3C, D, E, F). Tukey's HSD post hoc tests revealed that bacterial and fungal sedimentary communities > 200 m from stormwater drains were more homogeneous compared to communities at -1m, 0m, and 1m distances but not 5m from the stormwater drains (Table S2).

Among sites, there were no differences in the bacterial and fungal communities in sediments or seagrass tissue. The one exception was that the fungal communities in seagrass leaf tissue was significantly different between sites ($F_{2,40}$ = 2.02; p = 0.009295; Table S1).

No alpha diversity metrics of sedimentary or leaf bacterial and fungal diversity differed between sites or distances from stormwater drains (Shannon diversity: p > 0.05; inverse Simpson diversity: p > 0.05; observed diversity: p > 0.05; Fig. 4A&B; Table S3). In contrast, all alpha diversity metrics for root bacterial and fungal communities differed between sites but not distances from stormwater drains (Fig. 4C, D, E, F; Table S3). Tukeys HSD post hoc test revealed that alpha diversity differed significantly between communities from Nords Wharf south and Summerland Point only.

Differential abundance of microbes at each distance

The following taxa decreased in relative abundance in -1m communities compared to 200m communities: *Vicinamibacteria Subgroup 17* (o), *Desulfosarcinaceae* (f), *Acidobacteriota Subgroup 21* (c), *Sva0081 sediment group* (g), *Thiohalobacter* (g), *Bathyarchaeia* (c), *Hoeflea* (g), *Candidatus Nitrosopumilus* (g), *Desulfocapsaceae* (f), *Spirochaeta* (g) (Fig. 5). The following taxa increased in relative abundance in 0m communities compared to 200m communities: *Desulfosarcina* (g), *Actinomarinales* (o), *Desulfocapsaceae* (f), *Arcticiflavibacter* (g), *Robiginitalea* (g), *Defluviimonas* (g) while the following decreased in relative abundance of *Vicinamibacteria Subgroup 17* (o), and *Acidobacteriota Subgroup 21* (c).

The following taxa increased in relative abundance in 1m communities compared to 200m communities: *Bathyarchaeia* (c), *Sulfurovum* (g), *Actinomarinales* (o), *Saprospiraceae* (f), *Arcticiflavibacter* (g), *Latescibacteraceae* (f), while the following bacterial taxa decreased: *Thiohalobacter* (g), *Alphaproteobacteria* (c), *Vicinamibacteria Subgroup 17* (o), *Desulfosarcinaceae* (f).

The following taxa decreased in relative abundance in 1m communities compared to 200m communities: *Gammaproteobacteria* (c), *Xenococcaceae Pleurocapsa PCC-7319* (g), *Limnobacter* (g), *Microtrichaceae* (f), *Woeseia* (g), *Kiloniellaceae* (f), *Desulfosarcinaceae* (f), *Alphaproteobacteria* (c), *Vicinamibacteria Subgroup 17* (o), *Desulfocapsaceae* (f) and fungal taxa *Agaricales* (o) while fungal taxa *Paralulworthia* (g) decreased.

Environmental variables with distance from stormwater drain

Sediment variables

Sediment temperature and sediment grain size did not differ significantly with distance from the stormwater drain (Fig. 6A and 6C; Table S4). Organic matter (OM) tended to increase from − 1m to 5m away from scour edge with OM at 5m being significantly higher than at control plots (> 200m, Fig. 6B). Whilst heavy metal concentrations did not differ significantly at plots between − 1m and 5 m from the stormwater drain, As, Cr, Pb and Zn were significantly lower at

the control plots compared to some plots at other distances. Notably, As and Zn at the control plots were lower than at -1m from the stormwater drain. Control plots also had considerably lower Cr compared to plots at -1m and 5m, and lower Pb than those at the stormwater drain (0m; Fig. 6D, E, F, G, H).

Relationship between seagrass productivity and environmental variables

Stepwise model selection indicated that aboveground seagrass tissue biomass was best predicted by organic matter content alone (AIC = 190.77; Table 2), with aboveground biomass increasing as organic matter content increased. Similarly, variation in belowground biomass was best explained by and increased with organic matter content and proportion of fines (AIC = 278.61; Table 2).

Seagrass leaf tissue growth rates positively correlated to increased organic matter content, lead concentration, and proportion of fines (AIC = 52.23; Table 2). Photosynthetic capacity increased as sediment temperature and chromium decreased (AIC = -113.64; Table 2).

Table 2
Outputs and AIC values of linear models for environmental variables accounting for seagrass productivity variables

Variable	Std. Error	T-value	P-value
Above ground biomass: AIC = 190.77			
OCC	0.1860	2.504	0.016
Below ground biomass: AIC = 278.61			
Sed temp	0.4221	-2.641	0.011
OCC	0.4237	3.489	0.001
fines	0.4146	-2.749	0.008
Growth rates: AIC = 52.23			
OCC	0.06698	3.720	0.0007
Lead	0.14918	-3.256	0.002
fines	0.08726	-2.071	0.045
Photosynthetic capacity: AIC = -113.64			
Sed temp	0.010307	-2.477	0.017
Chromium	0.010222	-2.610	0.0125

Relationships between microbial communities and environmental variables

The dbRDA's for sedimentary bacterial and fungal community structure indicate separation of communities along gradients of increasing sediment temperature and OM (bacterial community only), decreasing zinc, grain sizes (bacterial community only) and copper (Fig. 7A&B; Table 3). Similar patterns were obtained for the leaf and root microbial communities. For leaf bacterial community structure, the dbRDA shows separation of communities of distances by gradients of increasing sediment temperature, chromium, zinc, and grain sizes (Fig. 7C&D; Table 3). The dbRDA for root bacterial community structure shows separation of communities of distances by gradients of decreasing sediment temperature, zinc, copper (fungal only) and grains size (fungal only; Fig. 7E&F; Table 3).

Table 3
Output of dbRDA for sedimentary, leaf, and root sediment and fungal communities

Bacterial						**Fungal**					
Sediment		Root		Leaf		Sediment		Root		Leaf	
Sediment temperature	D_f = 1, 39 R^2 = 0.07 F = 3.38 **p = 0.001**	Sediment temperature	D_f = 1, 33 R^2 = 0.06 F = 2.35 **p = 0.001**	Percentage of fine particles	D_f = 1, 31 R^2 = 0.06 F = 2.43 **p = 0.001**	Sediment temperature	D_f = 1, 39 R^2 = 0.04 F = 1.67 **p = 0.001**	Sediment temperature	D_f = 1, 33 R^2 = 0.04 F = 1.46 **p = 0.001**	Sediment temperature	D_f = 1, 31 R^2 = 0.07 F = 2.52 **p = 0.001**
OCC	D_f = 1, 39 R^2 = 0.04 F = 1.98 **p = 0.001**	Zinc concentration	D_f = 1, 33 R^2 = 0.6 F = 2.35 **p = 0.001**	Sediment temperature	D_f = 1, 31 R^2 = 0.09 F = 3.84 **p = 0.001**	Chromium concentration	D_f = 1, 39 R^2 = 0.03 F = 1.15 **p = 0.039**	Zinc concentration	D_f = 1, 33 R^2 = 0.04 F = 1.44 **p = 0.001**	Zinc concentration	D_f = 1, 31 R^2 = 0.04 F = 1.70 **p = 0.001**
Zinc concentration	D_f = 1, 39 R^2 = 0.05 F = 2.53 **p = 0.001**			Zinc concentration	D_f = 1, 31 R^2 = 0.06 F = 2.49 **p = 0.001**	Copper concentration	D_f = 1, 39 R^2 = 0.03 F = 1.31 **p = 0.001**	Percentage of fine particles	D_f = 1, 33 R^2 = 0.03 F = 1.25 **p = 0.012**	Chromium concentration	D_f = 1, 31 R^2 = 0.04 F = 1.44 **p = 0.007**
Percentage of fine particles	D_f = 1, 39 R^2 = 0.03 F = 1.54 **p = 0.022**			Copper concentration	D_f = 1, 31 R^2 = 0.05 F = 2.04 **p = 0.009**	Zinc concentration	D_f = 1, 39 R^2 = 0.03 F = 1.22 **p = 0.01**	Copper concentration	D_f = 1, 33 R^2 = 0.03 F = 1.15 **p = 0.027**	Percentage of fine particles	D_f = 1, 31 R^2 = 0.03 F = 1.24 **p = 0.038**

Discussion

Urban runoff introduces multiple stressors that threaten estuarine ecosystems such as seagrass meadows, yet the mechanisms linking these stressors to seagrass decline remain poorly resolved (Rees et al., 2023; Suzzi et al., 2022; Dafforn et al., 2012). Our study investigated patterns in seagrass meadows with distance from stormdrains in a coastal Lake and found changes in potential sediment stressors that were linked to spatial gradients in seagrass productivity and sedimentary microbial communities. Seagrass was entirely absent in scour zones immediately adjacent to stormwater drains (-1m), potentially due to physical disturbance and/or elevated contaminants (e.g., zinc, chromium). Beyond this zone, seagrass productivity peaked at 5m from drains, however, above- and belowground biomass declined at 200m and was comparable to the biomass at 1m. This peak in biomass mirrors sedimentary organic matter enrichment, which also peaked at 5m. The peaks in these two variables at 5m did not align with our hypothesis of a linear increase in seagrass biomass although multiple studies in the past have shown these strong links between increased OM resulting in increased seagrass biomass (Fraser & Kendrick., 2017; Trevathan-Tackett et al., 2017). Patterns in the sediments with distance from stormwater drains suggest initial scour resulting in coarser grain sizes with some of these organic materials settling out at around 5m from the drain causing a nutrient pulse. The increase in seagrass productivity at 5m may be directly linked to the greater availability of organic matter in the sediments. We also observed differences in sedimentary microbes that may have supported mineralisation of the organic material and also contributed to the increased seagrass productivity (Vieira et al., 2022).

Organic matter accumulation near drains provides a substrate for microbial decomposition, converting complex organic compounds into bioavailable nutrients (Chen et al., 2024; Liu et al., 2022). At intermediate distances (5m), enriched microbial taxa such as *Saprospiraceae* (carbohydrate/polysaccharide degraders), *Bathyarchaeia* (organic matter mineralisation), and *Rhodobacteraceae* (denitrifier) drive nitrogen and phosphorus mineralisation (Lu et al., 2023; Wang et al., 2024; Ruiz-Blas et al., 2024; Zhou et al., 2024; Rajeev and Cho, 2024). These taxa may break down organic matter and increase the bioavailability of dissolved inorganic nutrients (e.g., NH_4^+, PO_4^{3-}) to seagrasses (Kirchman, 2018), potentially explaining the coupling of seagrass productivity and sedimentary organic matter. Additionally, organic matter enrichment at 5m may buffer the impacts of contaminants (e.g., heavy metals) by fostering

microbial taxa that enhance nutrient availability. For instance, *Hyphomicrobium*, linked to nitrogen fixation and organic acid production, was abundant in these zones, potentially increasing bioavailable nitrogen and phosphorus for seagrass roots (Kloos et al., 1995). Conversely, the decline in organic matter and microbial functional diversity at 200m may have contributed to reduced seagrass biomass, indicating metal contamination and nutrient limitation as stressors that fluctuate with distance.

The trends in seagrass productivity are also tightly coupled to shifts in sedimentary microbial communities. Proximity to drains drove significant divergence in bacterial and fungal assemblages, with communities near drains (-1m to 5m) differing from control sites (> 200m). Additionally, microbial taxa associated with nutrient cycling, organic matter decomposition, and sulfur metabolism were more abundant in sediments containing seagrass plants (0–5m) compared to sediments at > 200 m. For example, *Sulfurovum* (sulfur oxidation), *Rhodobacteraceae* (denitrification), and *Bathyarchaeia* (organic matter degradation) dominated near drains, coinciding with the seagrass productivity peak at 5m (Alamoudi et al., 2025; Rajeev and Cho., 2024). In contrast, scour-zone sediments (-1m) hosted depauperate communities enriched in stress-tolerant taxa (e.g., Gammaproteobacteria AT-s2-59) but depleted in functional groups critical to nutrient mobilisation, such as *Candidatus Nitrosopumilus* (ammonia oxidation) and *Desulfobulbaceae* (sulfate reduction; Gao et al., 2023; Abby et al., 2023; Kamal et al., 2021). These disparities in microbial community structure suggest that stormwater disturbances disrupt sediment biogeochemistry, impairing processes that otherwise sustain seagrass growth.

The shifts in microbial community structure holds functional implications within these seagrass meadows. In scour zones, the loss of taxa like *Acidobacteriota Vicinamibacteria Subgroup 17* (cellulose degradation) and *Desulfosarcinaceae* (sulfate reduction) likely reduces organic matter turnover, exacerbating nutrient limitation in already barren sediments (Štursová et al., 2012; Rolando et al., 2024). Root-associated microbes maintained consistent abundances of phosphate-solubilising taxa (e.g., Pseudomonas), which could mitigate metal toxicity by reducing the availability of zinc or chromium (Monica & Harshada., 2015; Ahemad 2015). Within seagrass meadows, however, enriched decomposers (e.g., *Anaerolineae SBR1031*) and sulfur cyclers (e.g., *Thiohalobacter*) likely enhance porewater nutrient fluxes (Xia et al., 2016; Sun et al., 2024). The peak in seagrass biomass at 5m aligns with these microbial dynamics, suggesting that stormwater-derived organic matter fuels a mutualistic loop: microbial decomposition releases nutrients, stimulating seagrass growth, which in turn supplies new organic matter to sediments. Beyond 5m, declining organic matter and shifts in microbial communities may disrupt this loop, leading to reduced productivity.

Root and leaf microbial communities did not mirror sedimentary community trends, challenging assumptions that belowground seagrass microbes are strongly influenced by sediment conditions. This decoupling implies that seagrass selectively recruits or maintains microbial symbionts critical to health, such as *Defluviimonas* (associated with stress resistance) and *Arcticiflavibacter* (organic acid production; Liu et al., 2017; Lee et al., 2023). Such host control may explain why photosynthetic capacity improved with distance from drains despite biomass declines, as seagrasses prioritise microbial partnerships that optimise light use over nutrient acquisition under low-contaminant conditions (Martin et al 2019; Vieira et al 2022). This stability suggests microhabitats within roots and rhizosphere of seagrass plants buffer microbial communities and thus nutrient cycling perturbations.

These findings highlight the complex interactions between nutrient enrichment, contaminant exposure, and microbial community dynamics in effecting seagrass productivity. The initial nutrient influx 5m from stormwater drains which promotes seagrass growth, the subsequent decline in nutrients and seagrass biomass at greater distances and distinct microbial communities at these different distances indicates a potential link between microbial functionality and their role in nutrient remineralisation enhancing bioavailability and the potential negative impacts of contaminants. The resilience of seagrass-associated microbial communities and their potential role in nutrient cycling suggest that these microbes may act as mediators of seagrass performance, buffering against environmental stressors and supporting seagrass health in dynamic estuarine environments.

Conclusion

This study investigated the links between seagrass health, microbial community structure, and legacy sediment contamination associated with stormwater runoff along a distance gradient from stormwater drains. Microbial community structure in seagrass sediments, organic matter and seagrass performance differed with distance from stormwater drains, indicating that stormwater runoff significantly influences these ecosystems through the introduction of physical disturbance, contaminants and altered nutrient dynamics. Overall, these findings provide initial insights into the complex dynamics between stormwater runoff and seagrass ecosystems. The observed patterns highlight the need for targeted management strategies to mitigate the adverse effects of legacy effects of stormwater disturbance on seagrass health and associated microbial communities. Future research should focus on understanding the impacts of stormwater-related contaminants on marine ecosystems, particularly on seagrass meadows. Additionally, investigating the cumulative effects of multiple stressors on these ecosystems will provide a more comprehensive understanding of the challenges they face.

Declarations

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Author contributions statement

LW: Data curation; Formal analysis; Investigation; Methodology; Project administration; Validation; Visualization; Writing - original draft;

KD: Funding acquisition; Methodology; Supervision; Resources; Writing - review & editing

PG: Supervision; Writing - review & editing

TG: Methodology; Supervision; Writing - review & editing

ZM: Supervision; Writing - review & editing

DP: Formal analysis; Supervision; Writing - review & editing

All authors contributed to the study conception and design of the study. Material preparation, data collection and analysis were performed by Luke Walker. Bioinformatics was performed by Luke Walker with assistance from Deepa Varkey. The first draft of the manuscript was written by Luke Walker and all authors commented on previous versions of the manuscript. Primary supervision was provided by Katherine Dafforn who reviewed and editied multiple previous versions. All authors read and approved the final manuscript.

Competing interests:

The authors have no relevant financial or non-financial interests to disclose.

Data Availability Statement

Data will be made available upon reasonable request.

Ethical Approval

This is not applicable

Consent to Participate

This is not applicable

Consent to Publish

This is not applicable

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Figures

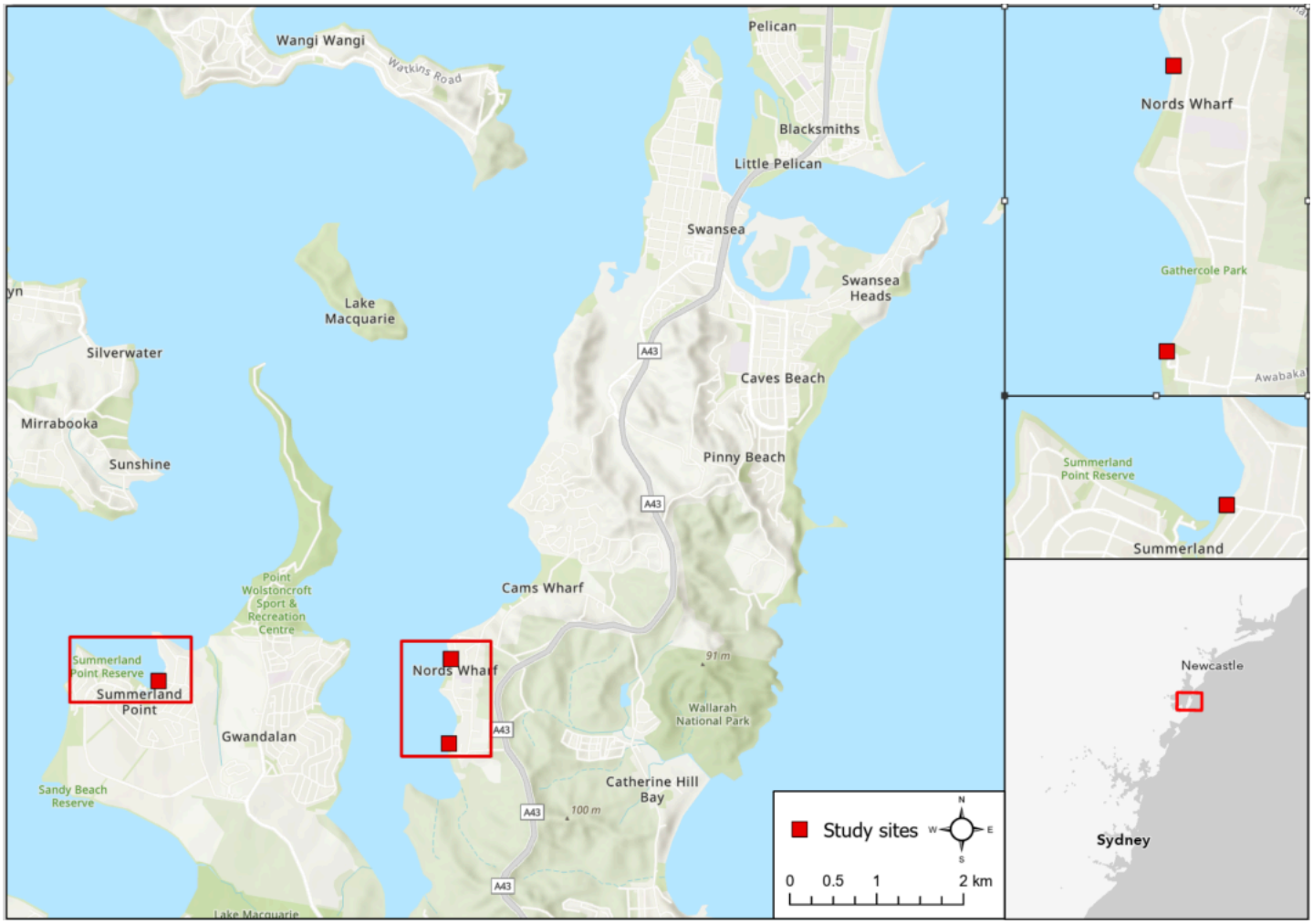

Figure 1

Location of study sites within Lake Macquarie, Australia.

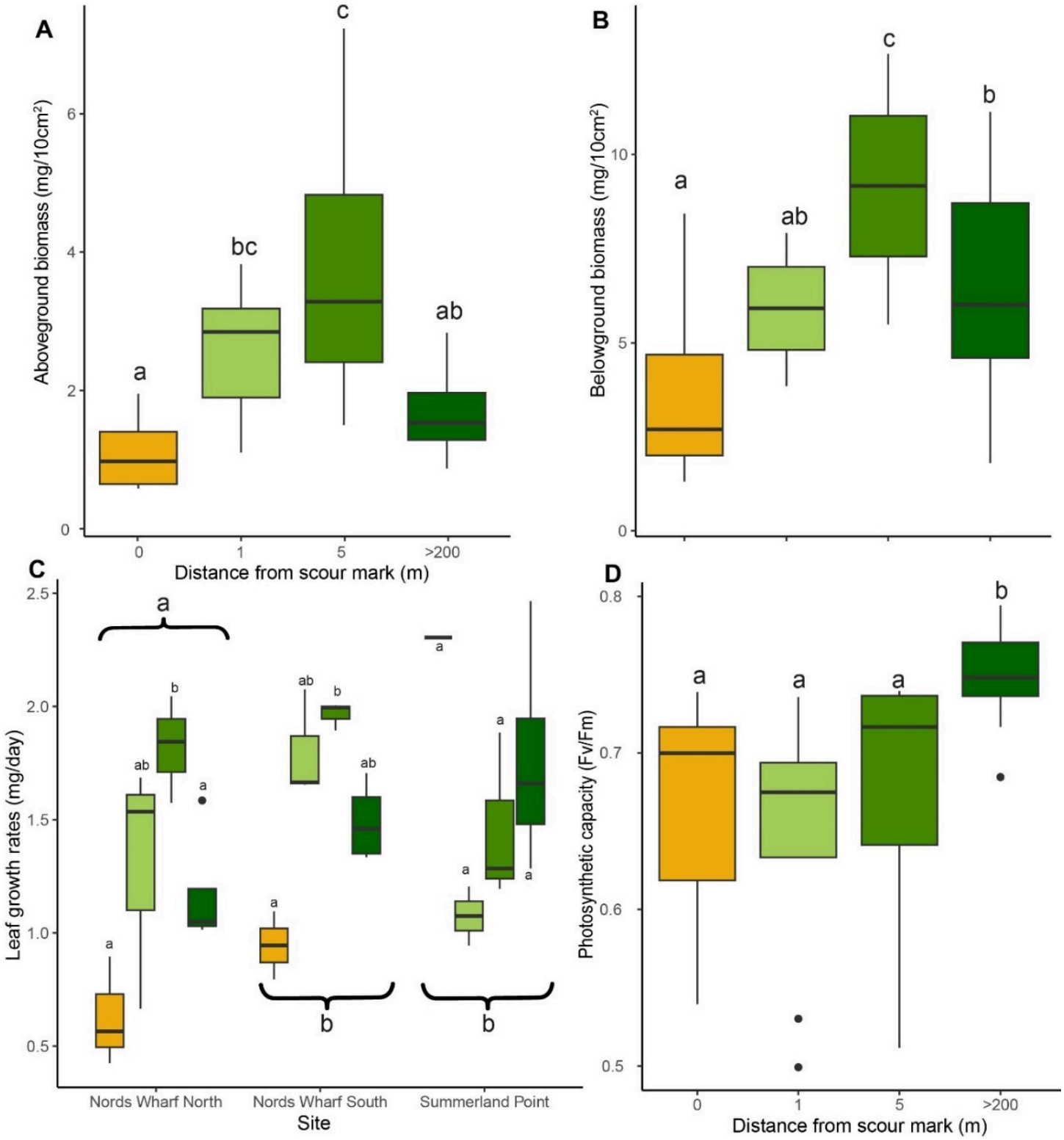

Figure 2

Boxplots of(A) Above-ground biomass (n=9), (B) below-ground biomass (n=9), (C) seagrass leaf tissue growth rates (n=3 except for Summerland Point*0m for which n=1), and (D) photosynthetic capacity(Fv/Fm) (n=9) varying distances from stormwater drain scour mark. Site is included for leaf growth rates because the interaction of site and distance was significant.

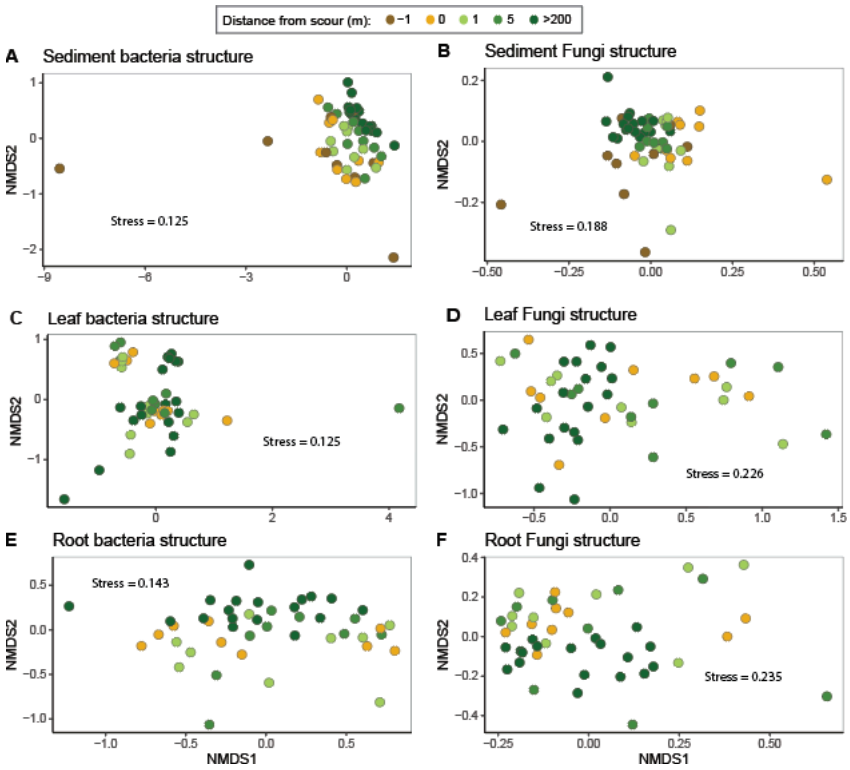

Figure 3

non-metric multidimensional scaling (nMDS) of leaf tissue, root tissue, and sedimentary bacterial assemblages at various distance from stormwater drains sampled from seagrass meadows in Lake Macquarie (N=35). A) Structure of sediment bacteria communities (square root transformed) B) Structure of sediment fungal C) Structure of leaf tissue bacterial communities D) Structure of leaf tissue fungal communities E) Structure of root tissue bacterial communities, F) Structure of root tissue fungal communities. Data was standardised by total ASV abundances and then nMDS plots were constructed with square-root transformed Bray-Curtis dissimilarity matrices. Note that seagrass was not present in the scour (-1 m), hence this distance is not represented on plots for leaf or root microbes.

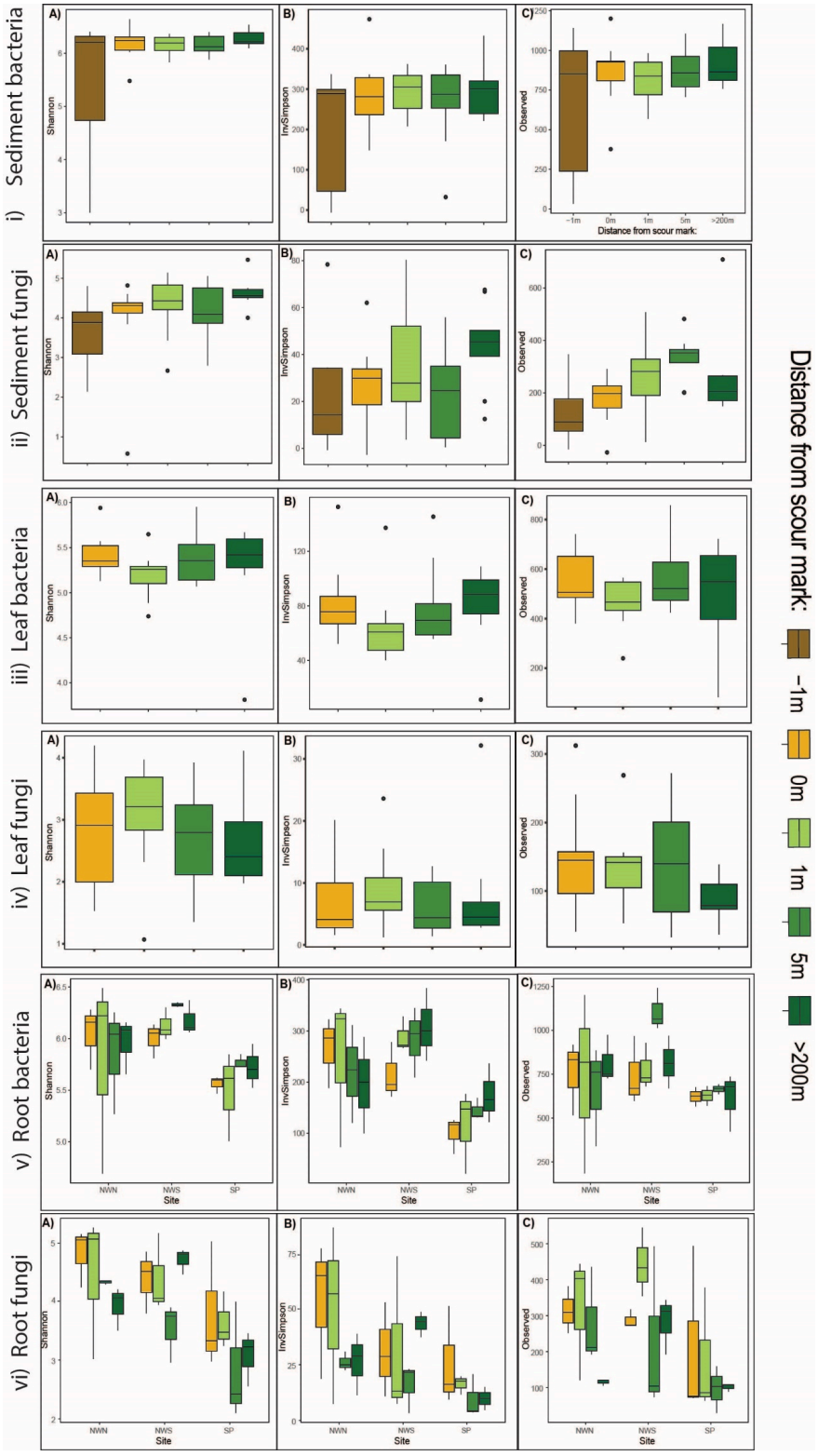

Figure 4

Alpha diversity of bacterial and fungal communities associated with Z. muelleri sediments (n=9), leaves (n=9) and roots (n=9) at varying distances from stormwater drains within Lake Macquarie. Mean species richness estimated as (A) -Shannon index, and (B) inverse Simpson diversity and (C) observed diversity. Note: -1m sampling was only possible for sediments because there was no seagrass growing in the scour marks.

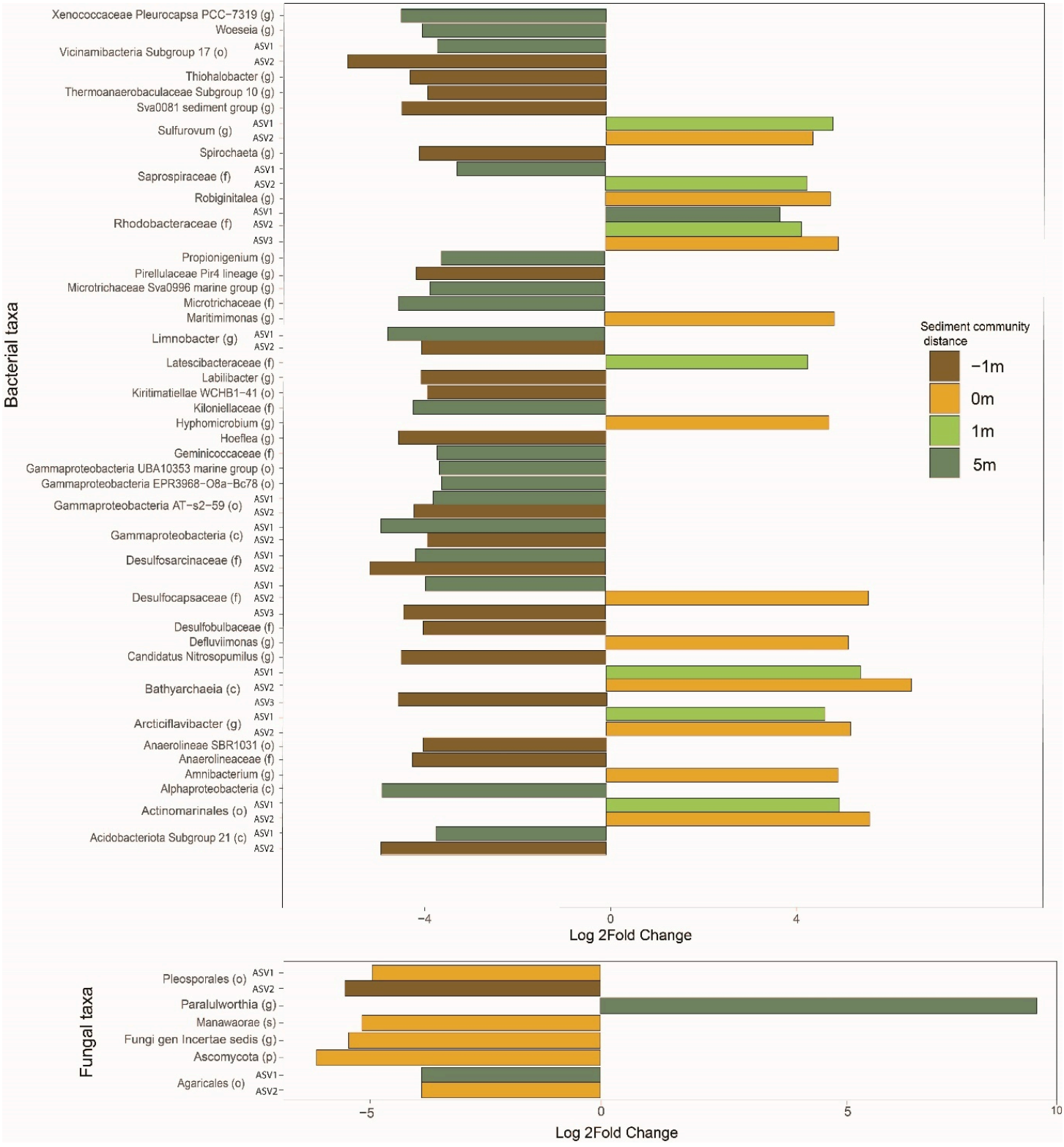

Figure 5

Mean log fold change in relative abundance of sedimentary bacterial and fungal ASVs with most significant log fold change between distances -1m, 0m, 1m, 5m relative to >200m. n = 9.

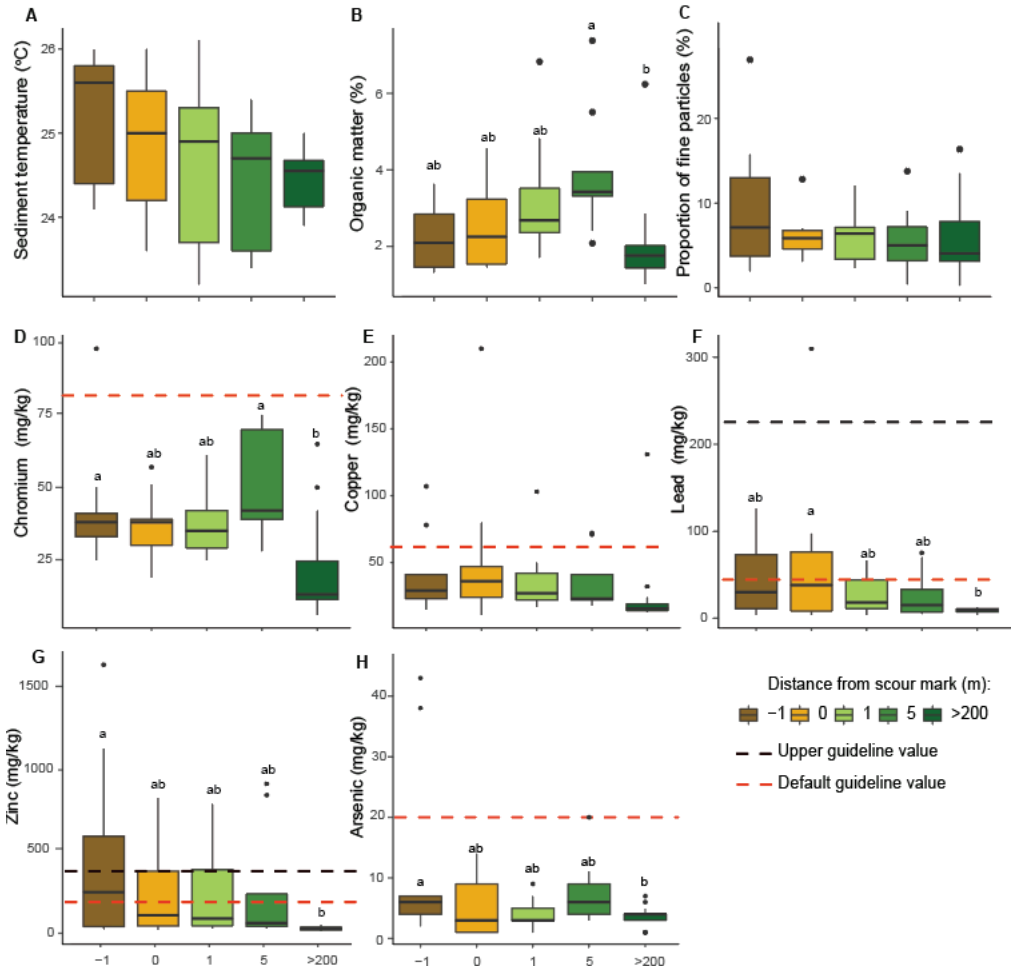

Figure 6

Boxplots of sediment variables and metal contaminants at each fixed distance away from stormwater scour marks n=9. AB used to indicate statistical significance. Dashed lines used to indicate ANZECC toxicant default guideline values and upper guideline values for sediment quality.

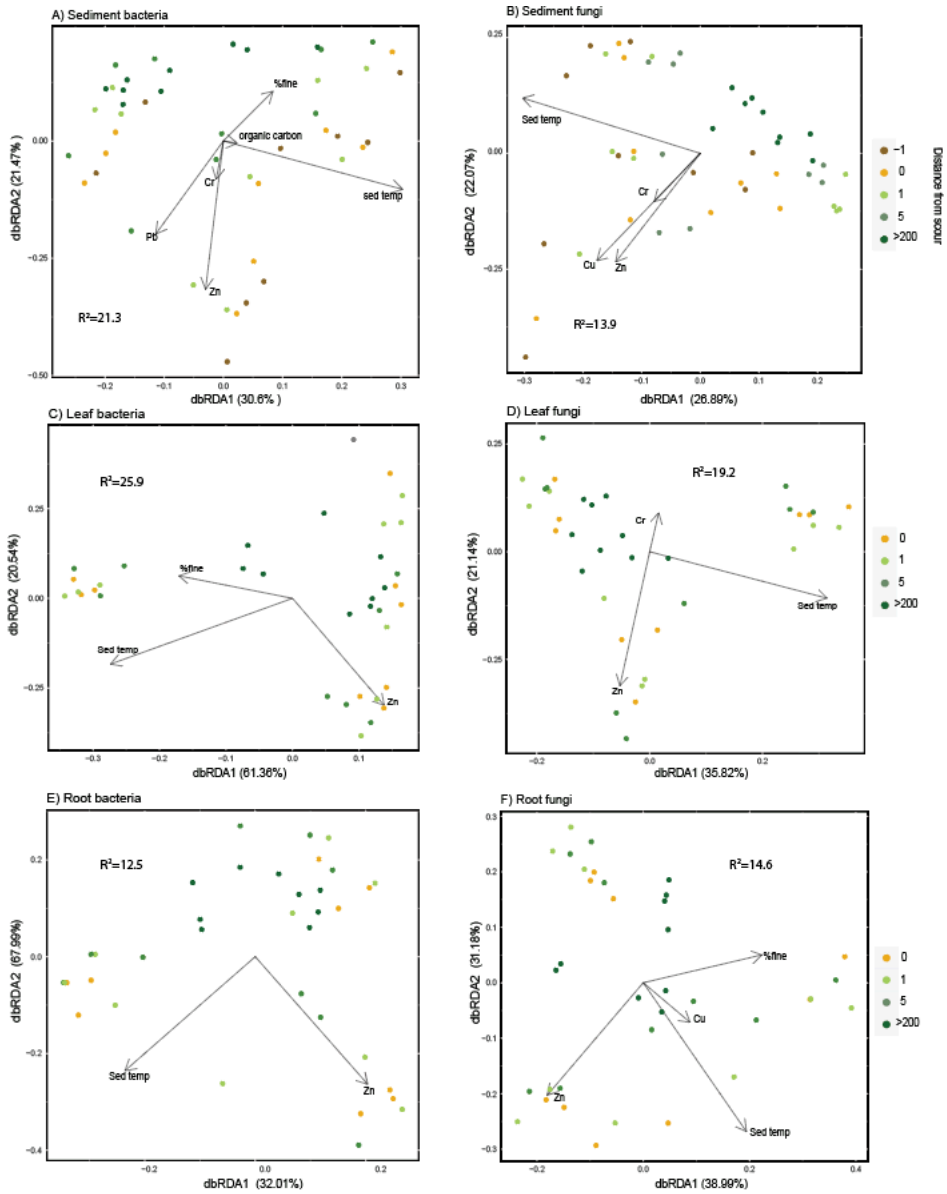

Figure 7

Distance-based redundancy analyses of square-root transformed Bray-Curtis dissimilarity matrices with vectors overlayed for variables selected by model selection for A) Structure of sediment bacteria communities (square root transformed) B) Structure of sediment fungal C) Structure of leaf tissue bacterial communities D) Structure of leaf tissue fungal communities E) Structure of root tissue bacterial communities, F) Structure of root tissue fungal communities. OM = Organic matter, DO = Dissolved oxygen.

Supplementary Files

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- GraphicalAbstract.png
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- 66412df28be74205b569a133fb20c4d5.jfif
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- SupplementaryMaterials.docx