Climate Change Cluster, Faculty of Science,
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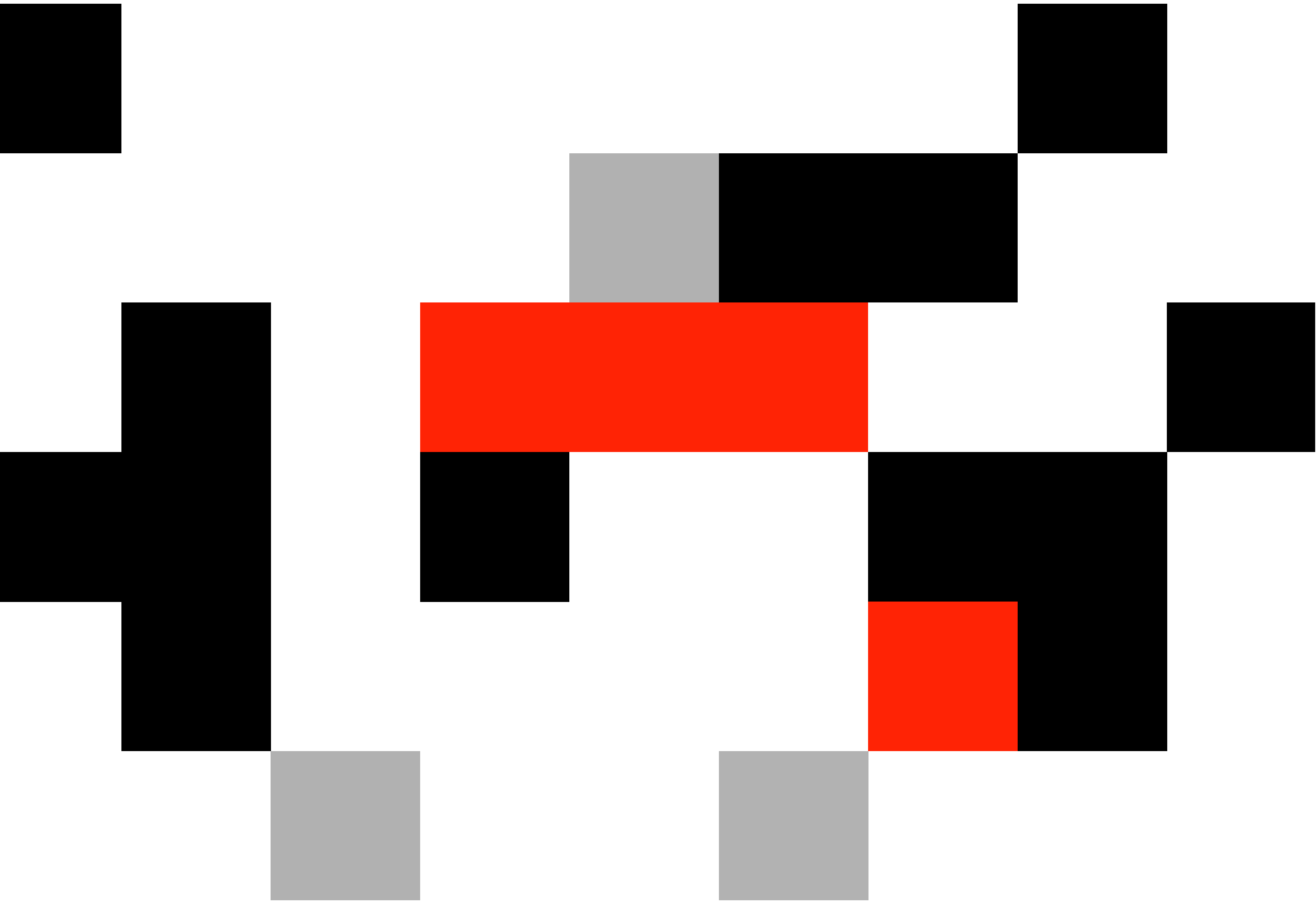
Microbiological Characterisation of the Darling Baaka River System



This Confidential Report has been prepared by the University of Technology Sydney ("UTS") for the NSW Department of Climate Change, Energy the Environment and Water.

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To Cite this report:
Seymour, J.R, Siboni, N., and Korbel, K. (2025) Microbiological Characterisation of the Darling Baaka River System, University of Technology Sydney, June 2025

1.0 BACKGROUND

1.1 Background to project

The work presented in this report is a component of the Darling Baaka River Health Program (DBRHP), which has been designed by the NSW Department of Climate Change Energy, Environment and Water (DCCEEW) Science and Insights Division. The DBRHP is delivered under the NSW Environment Protection Authority's Recovery Program for Water Quality Monitoring in the Darling Baaka, and is funded as a Category D recovery measure under the joint Commonwealth and NSW Government Disaster Recovery Funding Arrangements. The University of Technology Sydney were engaged by DCCEEW to assess and characterise the microbial communities in the lower Darling Baaka river, and to provide potential new indicators of river health.

A primary goal of the DBRHP is to deliver ecological monitoring of a range of river health indicators to inform community understanding of event recovery, and future research and water management of the system. The analyses conducted by UTS as part of this project aims to deliver new information on biotic processes governing riverine ecological function and has the potential to significantly contribute to future monitoring of river health in the lower Darling Baaka river.

1.2. Introduction and Aims

Diverse assemblages of microorganisms play fundamentally important roles in governing the health and function of river ecosystems, whereby they control river primary productivity, govern key nutrient cycling processes and regulate water quality (Davies et al. 2008, Xuan et al. 2022; Toraskar et al. 2022). Indeed, as the foundation of riverine food webs and key mediators of benthic and planktonic biogeochemical processes, microorganisms profoundly shape river ecosystem status (Tiegs et al. 2019; Hildebrand et al. 2020).

Bacterial populations, including phototropic and heterotrophic species play particularly important roles in regulating carbon and inorganic nutrient cycling within river ecosystems. Heterotrophic bacterial growth is limited by the availability of labile dissolved organic carbon (DOC) and inorganic nutrients, including nitrogen (N) and phosphorus (P) (Hitchcock et al. 2010), while phototrophic bacteria (primarily cyanobacteria) are limited by water column light availability and available N and P (Hecky and Kilham 1988; Smith 2006). The organic carbon that fuels heterotrophic bacterial activity within rivers can be provided either from autochthonous DOC derived from phytoplankton primary production or the allochthonous introduction of organic carbon from watershed sources (Cole et al. 2002). During high river flow periods, inputs of allochthonous carbon from runoff can represent the dominant source of carbon (Hitchcock and Mitrovic 2015) with extrinsic factors, including watershed hydrology and frequency and extent of precipitation events, strongly influencing the productivity of lotic systems by shaping microbial community composition, productivity and biogeochemical function (Nicolle et al. 2012; Carney et al. 2016; O'Brien et al. 2023). Shifts in the composition of bacterial assemblages driven by environmental variability have indeed been demonstrated to impact key biogeochemical cycling processes in rivers (O'Brien et al. 2023).

In addition to providing important ecosystem services associated with the control of river productivity and nutrient cycling, lotic bacterial assemblages can also periodically have deleterious effects on river ecosystem health. Blackwater events occur when large amounts of organic material are washed into river ecosystems during flood events, resulting in significantly increased heterotrophic bacterial growth and respiration that leads to hypoxic conditions (Howitt et al. 2007). In addition, periodic blooms of sometimes toxic cyanobacteria can dramatically reduce water quality, which can alter riverine trophodynamics, as well as influencing human and animal health (Havens 2008). Notably, both hypoxic blackwater events and cyanobacterial blooms have had substantial negative effects within the Darling Baaka river system in recent years (Stocks et al. 2021; Ayele 2024).

Assessments of spatial and temporal patterns in the diversity and composition of planktonic bacterial communities can deliver critical insights into the functional status of river ecosystems (Carney et al. 2016; O'Brien et al. 2023). In addition, specific bacterial indicators have been demonstrated to be sensitive sentinels of ecosystem condition within other aquatic environments. For example, when applied within tropical marine ecosystems, microbial indicators have been shown to provide accurate prediction of temperature and trophic conditions (e.g. chlorophyll concentration and turbidity) (Glasl et al. 2019). Given their fundamental importance in governing lotic ecosystem health, bacteria also have great promise as sensitive environmental indicators within river systems.

Due to the critical role of planktonic bacteria in shaping the function of river ecosystems, the assessment of bacterial community characteristics is an important inclusion in any evaluation of river health. Here, we applied DNA sequencing approaches to characterise the diversity and composition of bacterial communities as a component of the Darling-Baaka River Health Project (DBRHP), with the over-arching **objectives** of:

1) Characterising patterns in bacterial community diversity and composition within the Darling Baaka river system across different functional zones and over time.
2) Defining novel microbial indicators for river ecosystem health status.

2.0 METHODOLOGY

2.1 Sampling

Sampling was conducted by the NSW Department of Climate Change, Energy, Environment and Water (DCCEEW) as part of the Darling Baaka River Health Project (DBRHP). Samples were collected during May, June, August, September, October and November of 2024. For this component of the DBRHP, the sample set was divided into 8 discrete functional zones described in Table 1.

At each site, samplers wearing gloves carefully collected 4L of surface water using sterile containers for DNA analysis. These containers were immediately placed in the dark and on ice for transportation. Physico-chemical water quality parameters were then measured using a Xylem EXO2 multi-meter for temperature, pH, dissolved oxygen, electrical conductivity, chlorophyll-a, turbidity and fluorescent dissolved organic matter. Additional water samples were collected, stored either frozen or on ice for a maximum of 28 days prior to analysis at Australian Laboratory Services. These samples were analysed for inorganic nutrients, organic carbon, metals and pesticides. A number of other biotic and abiotic factors were recorded at each site, see DCCEEW (2025) for further details.

TABLE 1: Sampling sites and Functional Zones

Bacteria zone	Site number	River Reach zone
Wilcannia upstream	1-2	1 Wilcannia- Lake Wetherell
Wilcannia downstream	3-6	1
Lake Wetherell	7-9	1
Wilcannia floodplain	10-14	2- Menindee weir pool
Baaka	15-20	3 -Pooncarie North
Lower Darling	21-24 +32	4- Pooncarie South
Great Darling Anabranch	24-28	5 Great Darling Anabranch
Tandou	29-31	5 Great Darling Anabranch

2.2 Sample Processing

Surface water samples were transported on ice to the laboratory, with sample processing occurring within 5 hours of collection along with a field blank consisting of DNA free water. Water was filtered by vacuum filtration (in triplicate) through sterile 0.2-μm mixed cellulose ester membranes (Pall Corporation, Port Washington, NV, USA). The volume of water filtered varied between 200 mL and 1 L per replicate (average 300 mL), depending upon the suspended material within the samples.

Filtering was terminated once either 1 L of water had been processed or 2 hours had elapsed, with filters subsequently frozen (−20°C) until DNA extraction. Prior to filtration, all filtration equipment was sterilised using 0.5% sodium hypochlorite, followed by rinsing in sample water.

Prior to extraction, all filter membranes were stored at −80°C at Macquarie University. Membranes were thawed and, under sterile laboratory conditions, cut with sterile blades into pieces (~3 mm^2) into DNA extraction vials (mean weight 0.25 g). DNA was extracted using the DNeasy Power Soil Pro Kit (Qiagen, Australia), with laboratory blanks (consisting of DNA free water) processed at the time of extraction. DNA samples were stored at −20°C for further analysis.

2.3 DNA Sequencing and Analysis

The V3–V4 variable region of the 16S rRNA gene was amplified using the 341f and 805r primer pair (Zheng et al., 2015). Amplicon sequencing was subsequently conducted on the Illumina MiSeq 300 platform at the Ramaciotti Centre for Genomics, with positive and negative controls incorporated into the sequencing run.

Raw demultiplexed sequencing data was analyzed using the QIIME2 pipeline (Caporaso et al., 2010). DADA2 was employed for data denoising (Callahan et al., 2016), and taxonomy was assigned using the classify-sklearn classifier (Pedregosa et al., 2011) against the SILVA v138 database. Sequences identified as mitochondria and chloroplasts, or with fewer than 21 reads (ASV relative abundance < 0.0003%), were removed from the dataset. The remaining sequences were rarefied to 11,000 reads per sample.

Differences in microbial community composition between months and functional zones were tested in R using two-way PERMANOVA with adonis and weighted UniFrac distance. Pairwise analysis of differences between months and functional zones was also conducted using adonis with weighted UniFrac distance. PCoA ordination plots of bacterial communities across different months and functional zones were created using Bray-Curtis distance matrices with 95% confidence ellipses. All alpha-diversity measurements were calculated from the rarefied data. Differences in alpha-diversity between functional zones were tested using one-way ANOVA followed by Tukey multiple comparisons.

2.4 Microbial Indicator Taxa Identification

To identify bacterial genera that serve as reliable indicators of specific environmental conditions and habitat types, we conducted an indicator species analysis at the genus level. The analysis included only genera with a relative abundance of ≥ 0.5%, which together accounted for 83.3% of the total rarefied sequence data. Bacterial indicators were defined according to the IndVal Index, which determines how strongly an organism is associated with a particular sample type or set of locations. The IndVal is defined by the specificity and fidelity of an organism within a sample type or location and is expressed as a percentage, ranging from 0 to 100, where higher values indicate a stronger association with the group.

We performed the bacterial indicator analysis using all available categorical variables derived from the environmental metadata, as well as from figures and tables in the River Condition Index Summary (Chapter 2) of the Darling Baaka River Health Project 2023–2025 report (DCCEEW 2025). The tested categories included: Functional Zone, Geomorphic Condition, Hydrological stress index, Water quality index, Nutrients, Turbidity, Dissolved oxygen, and Chlorophyll a. After incorporating these health-related categories, the analysis was performed using PAST software version 4.12 (Hammer et al., 2001). Only indicator taxa with statistically significant Bonferroni-corrected p-values are reported here.

3.0 Results & Discussion

Characterisation of bacterial community composition using 16S rRNA amplicon sequencing revealed high levels of spatial and temporal variability, with significant differences in bacterial composition occurring between the 8 functional zones, and over the 6 sampling periods ($p < 0.001$, two-way PERMANOVA). More specifically, we observed marked changes in microbial community composition over time, with pairwise analyses revealing significant differences between all months ($p = 0.018$ for August–September and $p = 0.001$ for all other comparisons).

We also observed significant differences in microbial community composition between all of the 8 functional zones ($p < 0.05$), with the single exception of Wilcannia Downstream *vs* Lake Wetherell. Principle Components Analysis generally highlighted clear partitioning of the microbial communities between functional zones during each of the sampling periods (Figure 1).

FIGURE 1

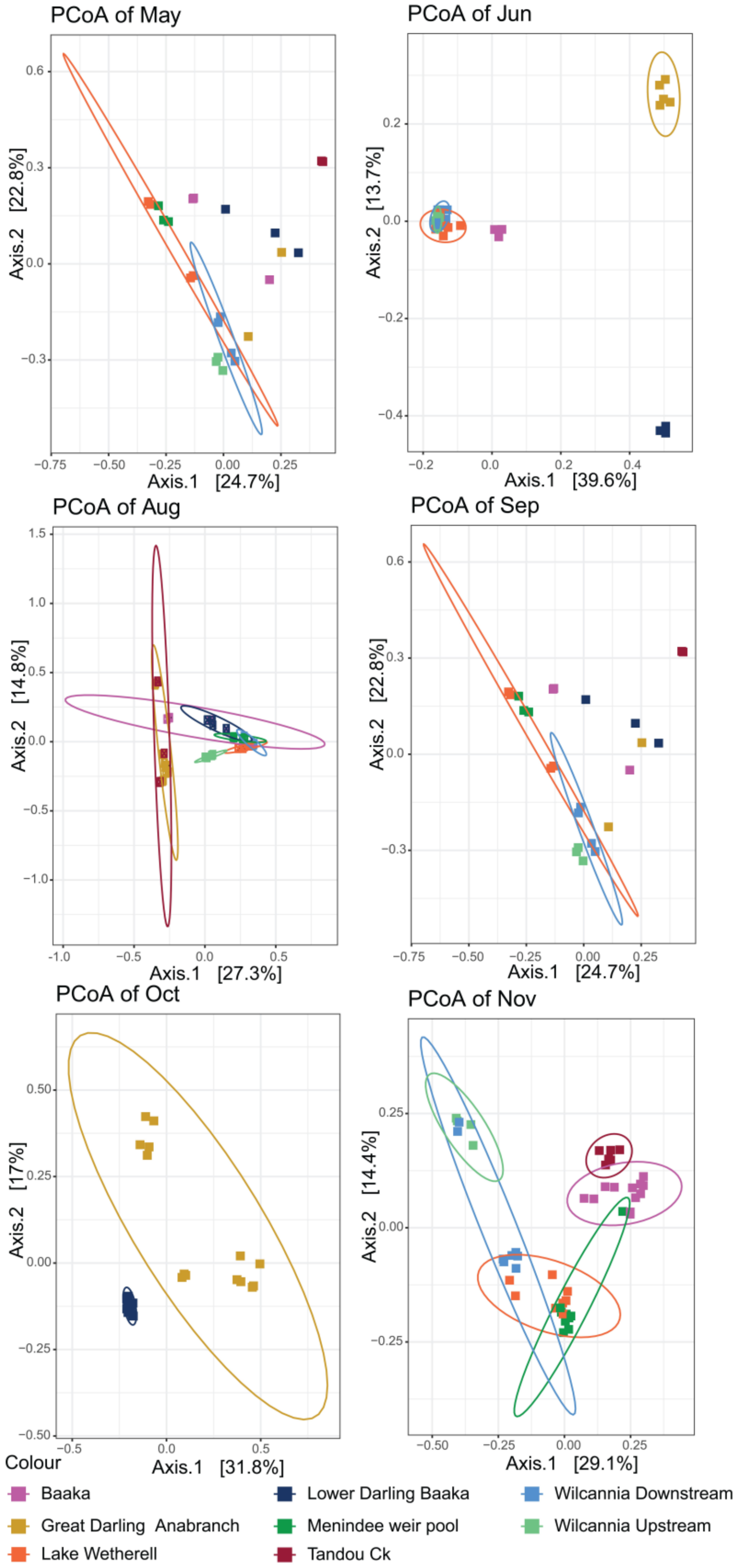

PCoA of May
Axis.2 [22.8%]
Axis.1 [24.7%]
0.6
0.3
0.0
−0.3
−0.75
−0.50
−0.25
0.00
0.25
PCoA of Jun
Axis.2 [13.7%]
Axis.1 [39.6%]
0.2
0.0
−0.2
−0.4
−0.2
0.0
0.2
0.4
PCoA of Aug
Axis.2 [14.8%]
Axis.1 [27.3%]
1.5
1.0
0.5
0.0
−0.5
−1.0
-1.0
-0.5
0.0
0.5
PCoA of Sep
Axis.2 [22.8%]
Axis.1 [24.7%]
0.6
0.3
0.0
−0.3
−0.75
−0.50
−0.25
0.00
0.25
PCoA of Oct
Axis.2 [17%]
Axis.1 [31.8%]
0.50
0.25
0.00
−0.25
−0.50
−0.5
0.0
0.5
PCoA of Nov
Axis.2 [14.4%]
Axis.1 [29.1%]
0.25
0.00
−0.25
−0.50
−0.25
0.00
0.25
Colour
Baaka
Great Darling Anabranch
Lake Wetherell
Lower Darling Baaka
Menindee weir pool
Tandou Ck
Wilcannia Downstream
Wilcannia Upstream

Figure 1: Principal component analysis ordination plots of bacterial community composition structure at each sampling time created using Bray-Curtis distance matrices with 95% confidence ellipses.

The number of observed amplicon sequence variants, which is equivalent to species richness, also varied significantly between functional zones and over time (Figure 2). Pairwise comparisons between discrete functional zones revealed significant differences in this diversity metric in 46 % of cases, with highest levels of richness observed within the Wilcannia Upstream functional zone in June, just after a major influx of water from the upper catchment, and lowest levels observed within the Greater Darling Anabranch functional zone during September, at the end of a managed cease-to-flow event in the GDA (DCCEEW 2025).

The bacterial community displayed clear shifts in composition with both location and time (Figure 3). Some notable patterns incluce high relative abundances of Cyanobacteria within all samples in the Baaka, Lower Darling Baaka, Wilcannia Floodplain and Tandou Creek functional zones during May. During this time, the bacteria communities in the Wilcannia Floodplain (i.e. the Menindee Weir pool sites) were distinctly different to other communities on the Darling Baaka River (Figure 1). This period was characterised by prologed stratification of dissolved oxygen concentrations in the weir pool, with a spike in Chl-a observed in mid May (DCCEEW 2025). Cyanobacteria levels were also high in some samples within the Great Darling Baaka Anabranch and Tandou Creek functional zones during August.

Increased relative abundances of *Parcubacteria* were observed during June in both the Lake Wetherell and Wilcannia Downstream functional zones. These peaks conincided with increased water flows in late May and June at Wilcannia, due to water received from rainfall events in the upper Darling Baaka (DCCEEW 2025). These waters had high levels of turbidity, which began in WIlcannia and were transported with flow to Lake Wetherell in roughly 7 days. Notably, *Pacrubacteria* have previously been shown to be prevalent members of the microbiome within waters exposed to soil seepage (Chaudhari et al. 2024), which is consistent with the high turbidity levels observed at this time.

Many of the temporal patterns exhibited in bacterial communities are likely to be linked with flow regime, which are extremely complex in this highly regulated system. A managed cease-to-flow event occurred in the Great Darling Anabranch (GDA) during the study period between August to late October 2024. This event returned the GDA to a more natural intermittent creek, resulting in flow restrictions and the drying out of some river reaches. Notably, the microbial communities at the end of the drying period were distinctly different to the communities on the lower Darling Baaka river. However as the comprehensive flow dataset was not available at the time this report was written, this was not investigated thoroughly. A comparison between flow events, will be essential begin to build knowledge on the impacts of flow, management water releases and cease-to-flow events on the health of the waterways. Further investigation into the relationships between communities and flow will be conducted in the future.

FIGURE 2

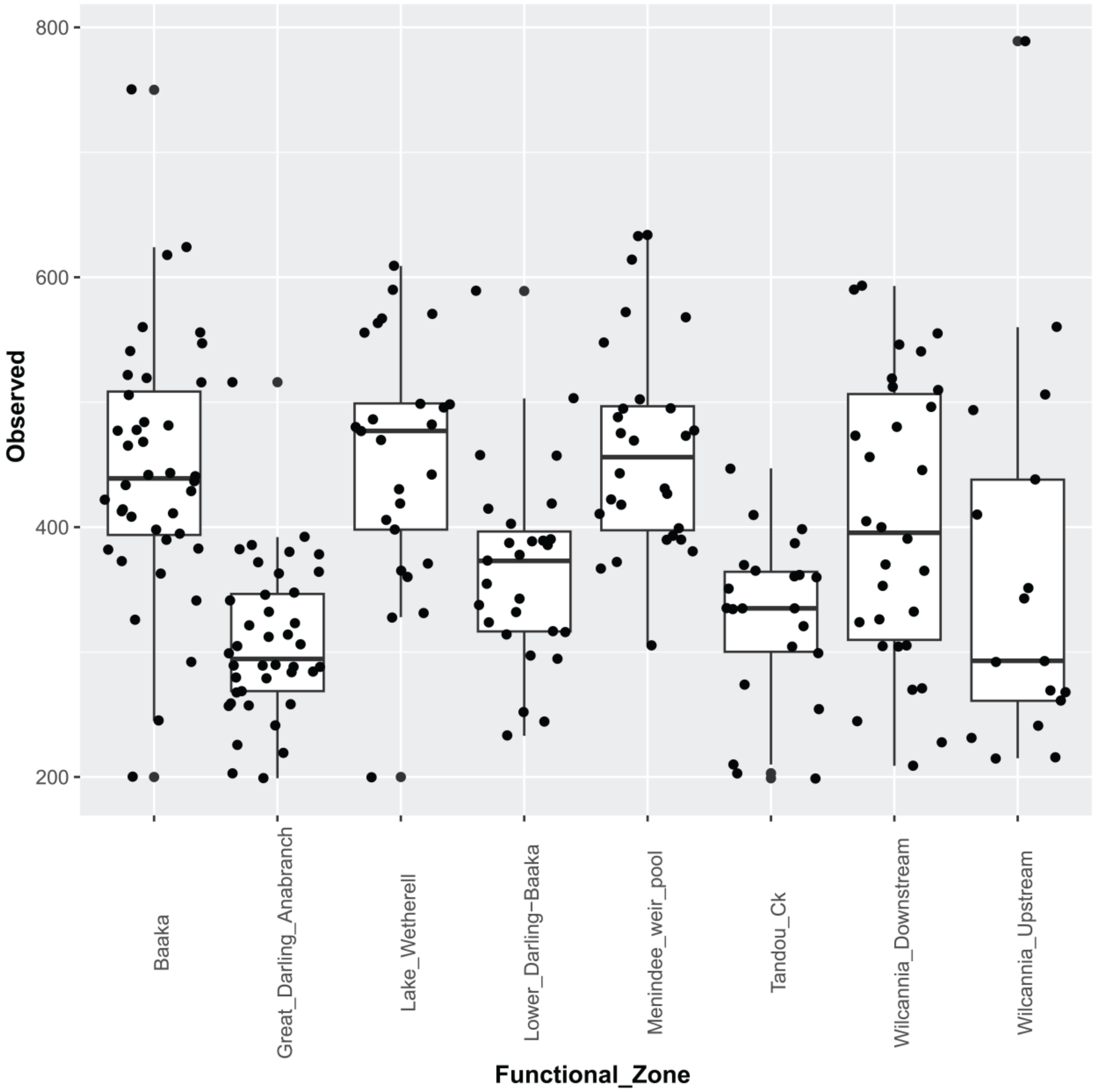
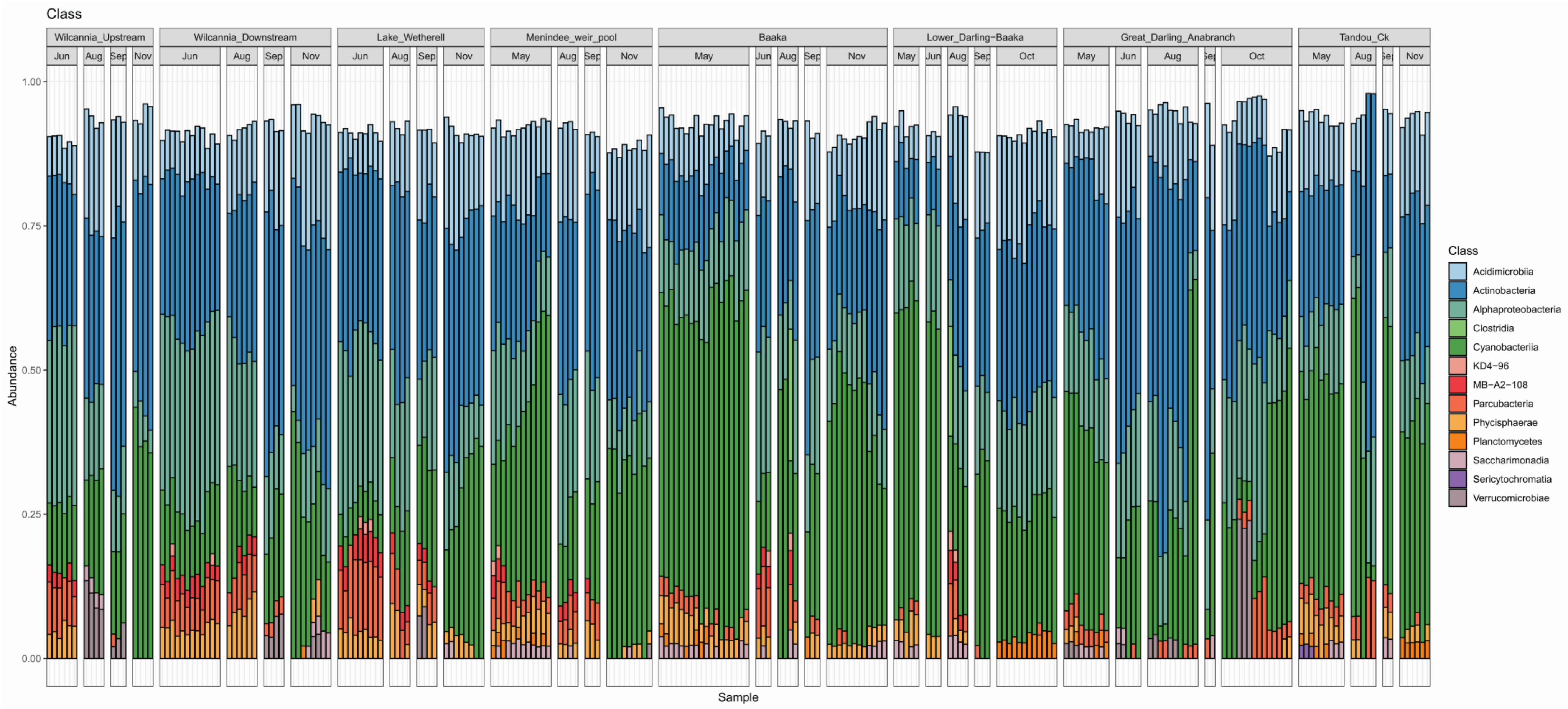


Figure 2: Observed amplicon sequence variants (equivelent to species richness) values across sites.

Figure 3: Patterns in bacterial community composition across sites and time at the Class level of taxonomy.

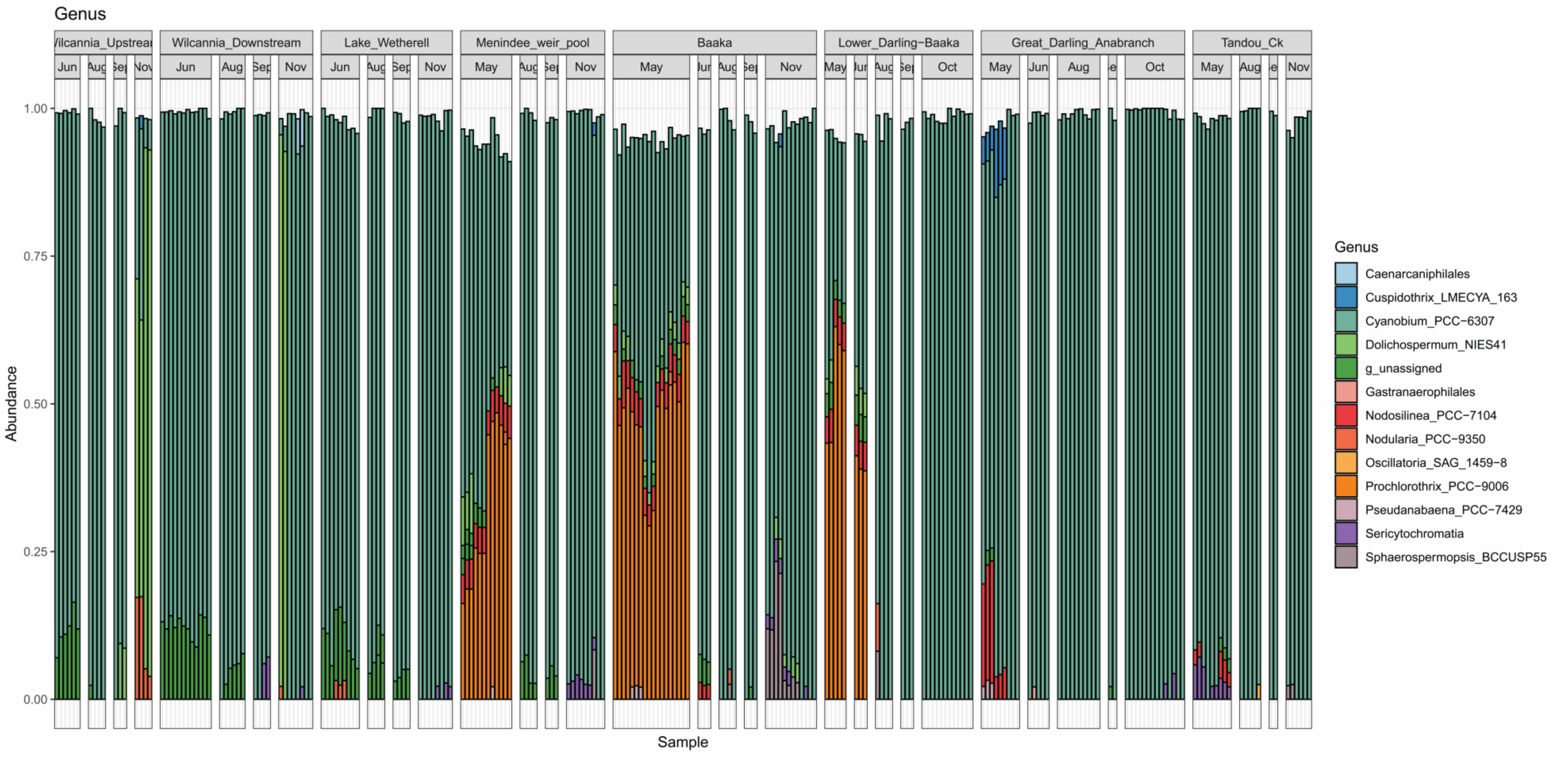


6.2 Patterns in cyanobacteria communities

Within the cyanobacteria community there were also clear spatial and temporal patterns. Across the entire data set the dominant genus of Cyanobacteria was *Cyanobium*, which often comprised 80-90% of cyanobacterial relative abundance. *Cyanobium* is a small but generally abundant cyanobacteria within river ecosystems globally (Salmaso et al. 2024). However, significant blooms of other species were observed during the study period. Notably, a significant bloom of *Dolichospermum* occurred in the Wilcannia Upstream and Downstream functional zones during November, when this organism comprised more than 90% of the cyanobacterial community in some samples.

Dolichospermum is known for its role in freshwater cyanobacterial blooms and is notable for producing cyanotoxins, including saxitoxins (nerve toxin - paralytic shellfish toxin group), lipopolysaccharides (skin irritants), and BMAA (beta-Methylamino-L-alanine; nerve toxin) (Li et al. 2016). It also produces 2-MIB (2-methylisoborneol), a compound that can cause an earthy or musty taste and odor in water (Li et al. 2016; Pham et al. 2020). Another significant, yet smaller, bloom of Prochlorothrix occurred in the Wilcannia Floodplain, Baaka and Lower Darling Baaka functional zones during May, when this organism comprised up to 50% of the cyanobacterial community. Prochlorothrix is not known to be toxic.

Figure 4: Patterns in cyanobacterial community composition across sites and time at the Genus level of taxonomy.



6.3 Potential indicators or river conditions

Discrete bacterial indicator taxa were found to be representative of each of the 8 river functional zones (Figure 5). In total, 19 different bacterial genera were identified as statistically significant indicator taxa for different functional zones, with between 2 – 4 indicator taxa identified for each functional zone (range: 0 for the Lower Darling Baaka functional zone – 5 for the Great Darling Anabranch functional zone). Notable genera identified as indicator taxa included *Cyanobium* and *Prochlorothrix*, which are widespread and often numerically important cyanobacteria within river ecosystems (Salmaso et al. 2024), several bacteria that are often associated organic-rich freshwater habitats or soil, including *Sporichthyaceae*, *Terrimicrobium* and *Xanthobacteraceae* (Qiu et al. 2014; Xiao et al. 2023; Yu et al. 2024), as well as common planktonic bacteria within aquatic habitats, such as *Rhodobacteraceae* (Simon et al. 2017).

FIGURE 5

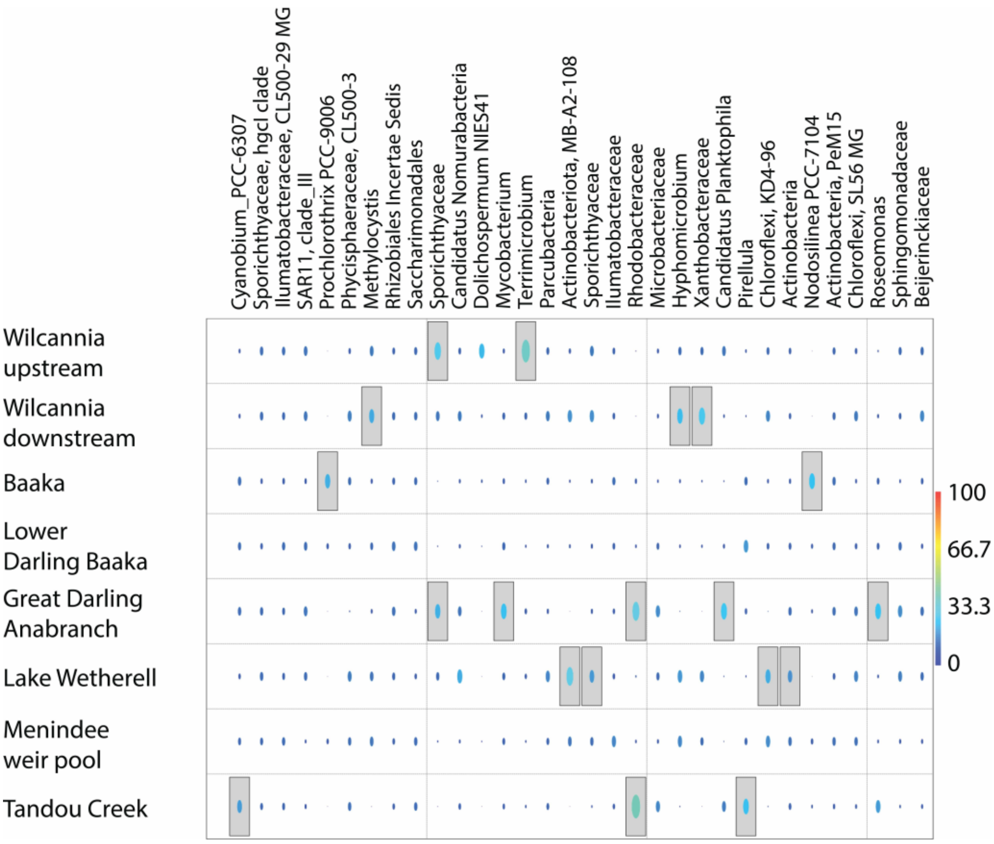


Figure 5: Indicator species analysis across functional zones at the genus level. The color and size of the ellipses represent IndVal (%) values, indicating the strength of association between each genus and a specific functional zone. Gray boxes highlight statistically significant differences. Higher IndVal values reflect a stronger association.

A total of 24 statistically discrete bacterial indicator taxa were identified across the 5 categories of geomorphic condition determined in Chapter 3 of the DBRHP report (Figure 6). Bacterial indicator taxa for poor geomorphic condition included *Actinobacteriota* MB-A2-108, which is generally known to occur in soil habitats (Lan et al. 2022), *Hyphomicrobium*, which are denitrifying and methane oxidizing bacteria found in both soil and aquatic habitats (Martineau et al. 2015), and *Nodosilinea*, which is a terrestrial cyanobacterium (Nuryadi et al. 2024). The strongest indicators for "very Good" geomorphic condition were *Rhodobacteraceae* and *Roseomonas*, which are common planktonic bacteria within aquatic ecosystems (Simon et al. 2017; Zheng et al. 2020).

FIGURE 6

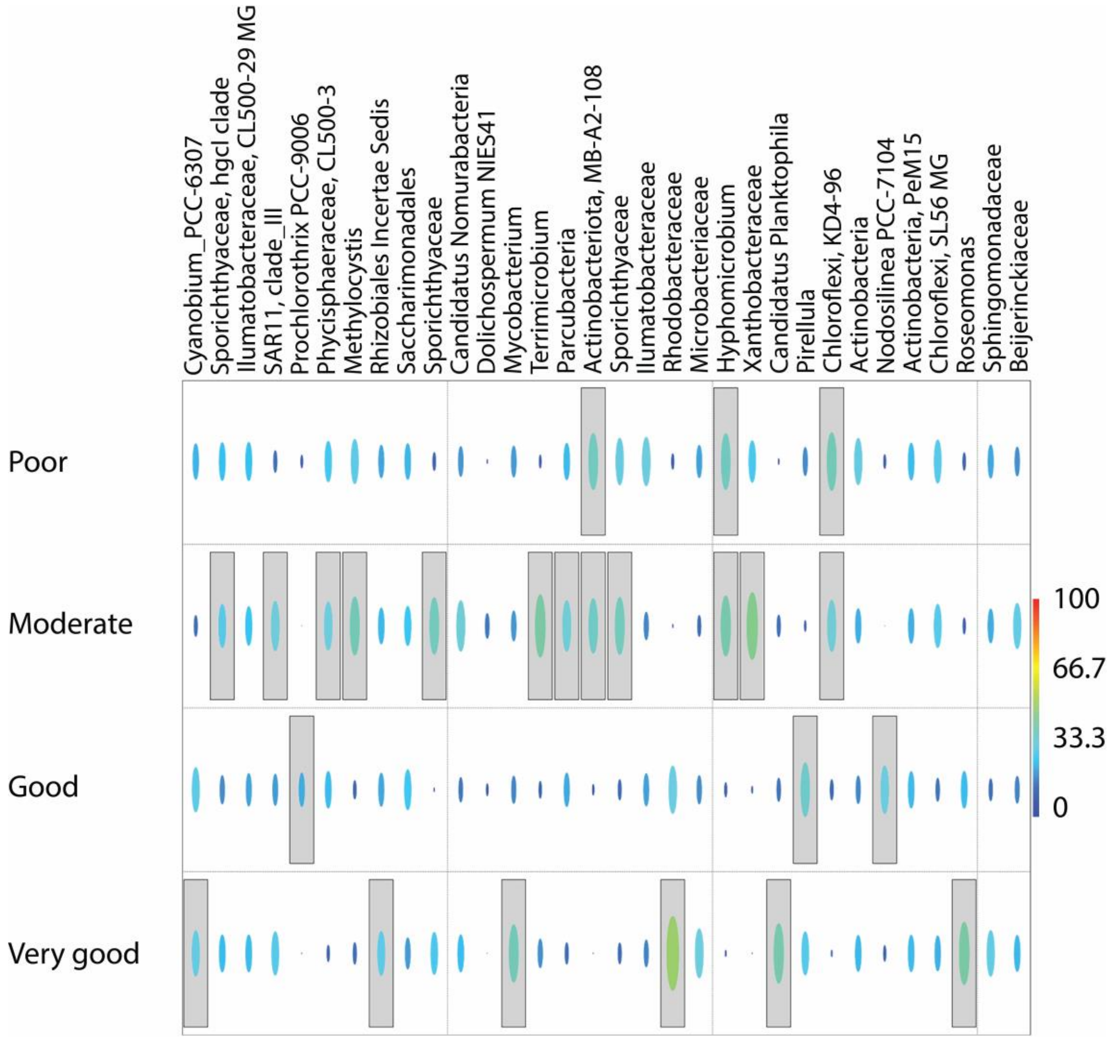


Figure 6: Indicator species analysis across geomorphic condition at the genus level. The color and size of the ellipses represent IndVal (%) values, indicating the strength of association between each genus and a specific functional zone. Gray boxes highlight statistically significant differences. Higher IndVal values reflect a stronger association. See Appendix figure A1 for regional distribution of geomorphic condition taken from Chapter 3 of the Darling Baaka River Health Project report.

Only one statistically significant bacterial indicator linked to the Water Quality Index (DCCEEW 2025) was detected, with *Phycisphaeraceae* indicative of “Poor” water quality. *Phycisphaeraceae* are known to be common members of the freshwater microbiomes and have previously been shown to be an indicator of free-flowing sites within dam-fragmented rivers (Serrana et al. 2021), but they have also been found in soil environments and wastewater microbiomes, with their ecological role in natural ecosystems currently unclear (Dedysh et al. 2020; Lenferink et al. 2024).

FIGURE 7

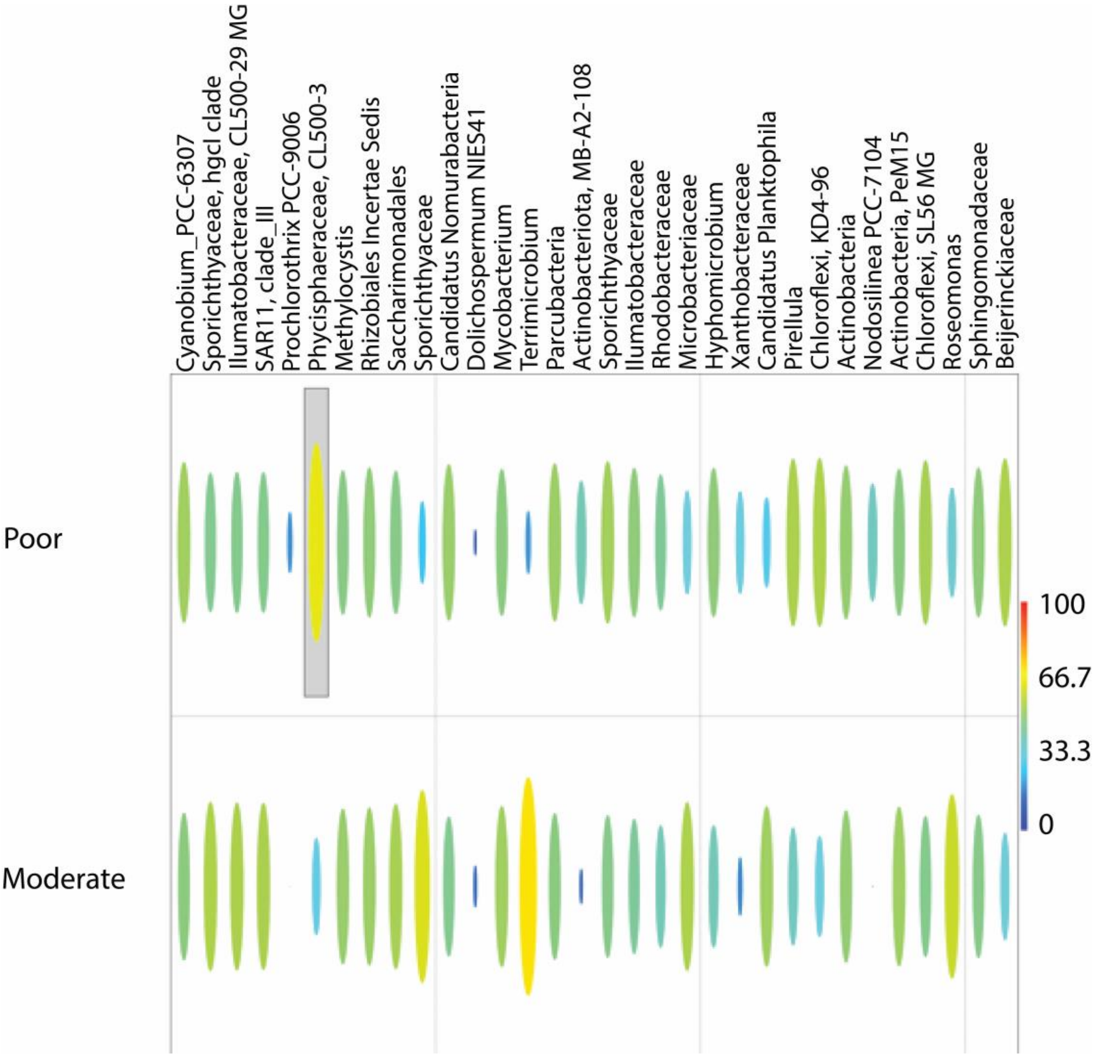


Figure 7: Indicator species analysis across water quality index at the genus level. The color and size of the ellipses represent IndVal (%) values, indicating the strength of association between each genus and a specific functional zone. Gray boxes highlight statistically significant differences. Higher IndVal values reflect a stronger association. See Appendix figure A2 for regional distribution of water quality index values taken from Chapter 4 of the Darling Baaka River Health Project report (DCCEEW, 2025).

Seven bacterial indicator taxa for hydrological stress condition were identified (Figure 8). “Poor” hydrological stress condition (DCCEEW 2025) was indicated by the presence of *Cyanobium* PCC6307 and *Pirellula*. *Pirellula* are aquatic bacteria that are often associated with low oxygen levels (Glöckner et al. 2003). “Good” hydrological stress condition was indicated by the presence of *Dolichospermum* and *Terrimicrobium*, both of which are common members of river microbiomes (Farkas et al. 2022).

FIGURE 8

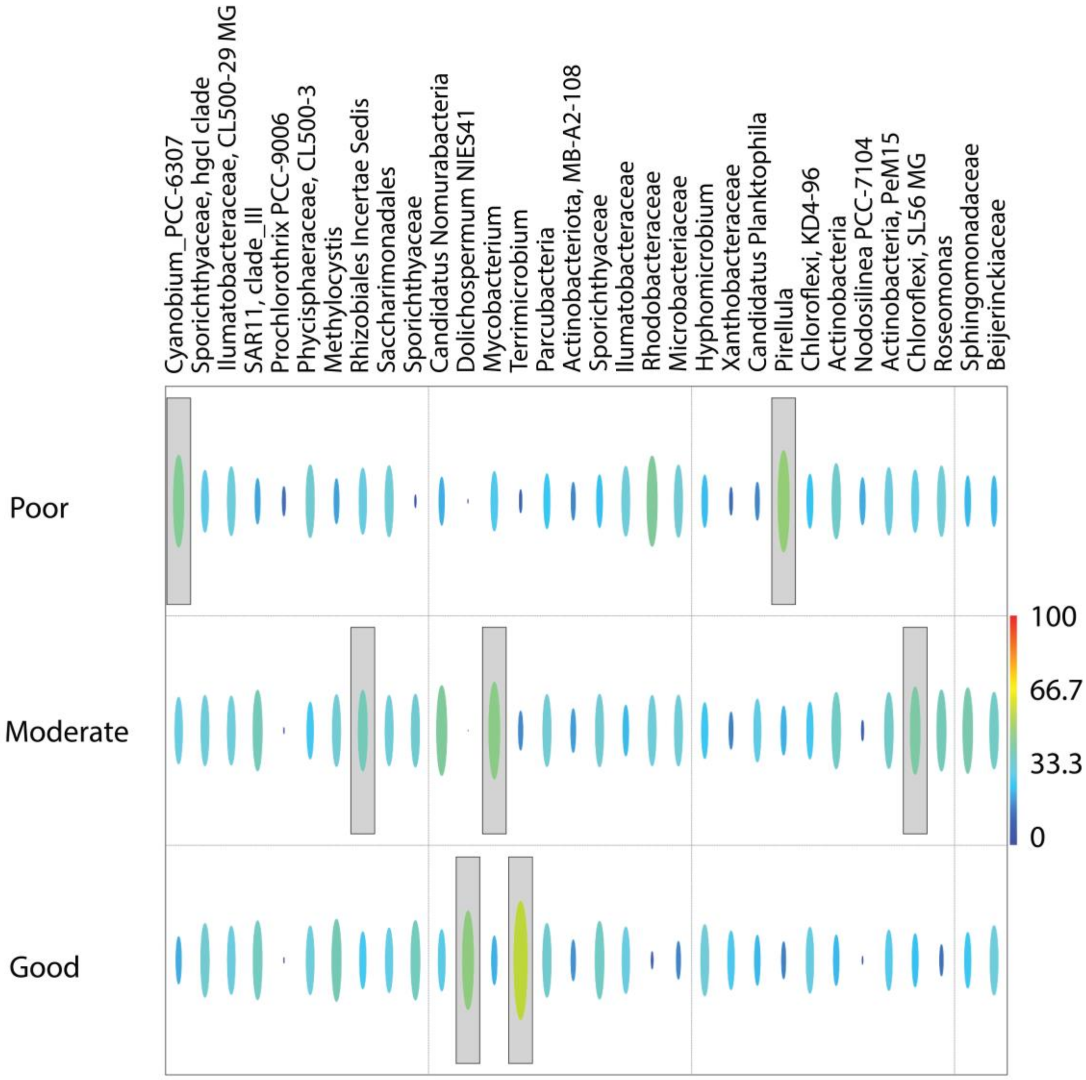


Figure 8: Indicator species analysis across hydrological stress index at the genus level. The color and size of the ellipses represent IndVal (%) values, indicating the strength of association between each genus and a specific functional zone. Gray boxes highlight statistically significant differences. Higher IndVal values reflect a stronger association. See Appendix figure A3 for regional distribution of hydrological stress index values taken from Chapter 7 of the Darling Baaka River Health Project report.

Thirteen significant bacterial indicator taxa for nutrient score categories were identified (Figure 9). The strongest indictors for “Very Good” nutrient condition were *Actinobacteriota* MB-A2-108, *Hyphomicrobium* and *Chloroflexi.* Elsewhere, *Actinobacteriota* have been shown to be prominent within restored rivers following nutrient removal (Zhang et al. 2023), while *Hyphomicrobium* are recognised as important denitrifiers that are responsible for the removal of excess nitrogen from aquatic habitats (Martineau et al. 2015). The two significant bacterial indicators for poor nutrient scores were *Phycisphaeraceae* and *Dolichospermum*, with *Dolichospermum* known to bloom in nutrient rich rivers (Kelly et al. 2021), while *Phycisphaeraceae* occurs in ecological associations with algae (Rambo et al. 2019) that often bloom in high nutrient conditions.

FIGURE 9

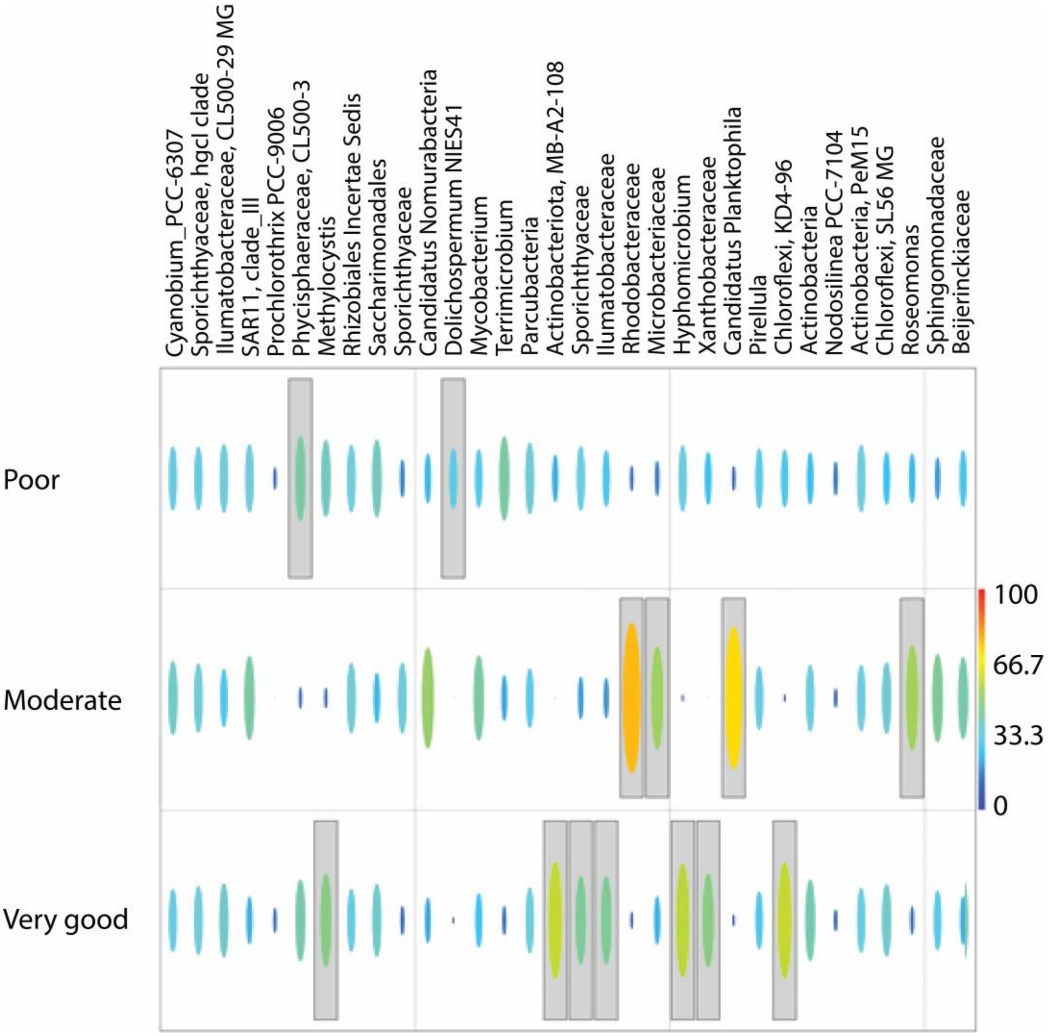


Figure 9: Indicator species analysis across nutrient score categories at the genus level. The color and size of the ellipses represent IndVal (%) values, indicating the strength of association between each genus and a specific functional zone. Gray boxes highlight statistically significant differences. Higher IndVal values reflect a stronger association.

Fifteen bacterial indicator taxa associated with “Poor” and “very poor” turbidity scores were identified (Figure 10). The strongest indicators for “very poor” turbidity scores included *Hyphomicrobium*, *Chloroflexi* KD4-96, *Candidatus Nomurabacteria*, *Methylocystis* and *Sporichthyaceae*. Notably, all of these organisms are commonly found in soil and anoxic freshwater sediment environments (Davis et al. 2011; Xu et al. 2021; Tikhonova et al. 2021), which is consistent with their occurrence within turbid river waters.

FIGURE 10

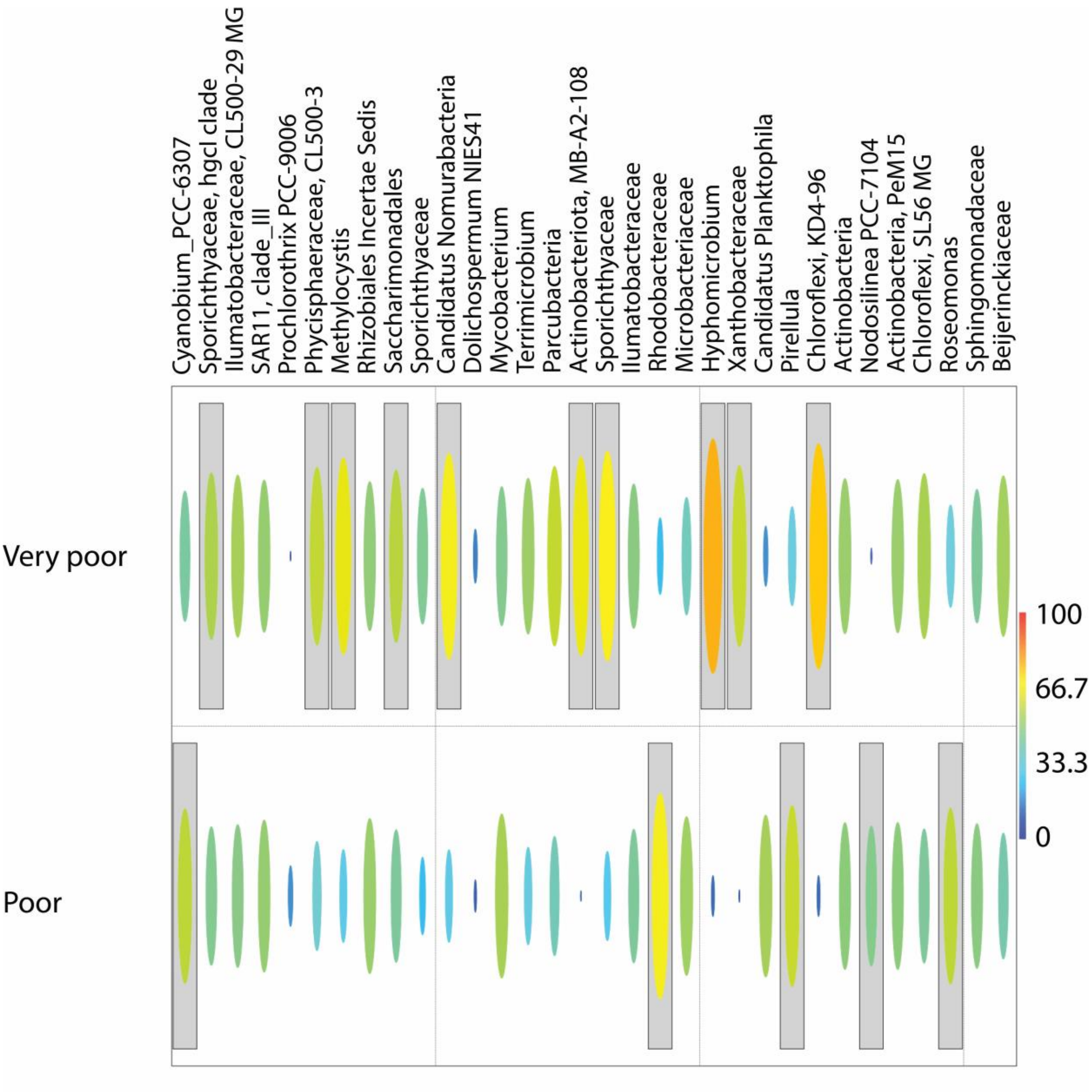


Figure 10: Indicator species analysis across turbidity score categories at the genus level. The color and size of the ellipses represent IndVal (%) values, indicating the strength of association between each genus and a specific functional zone. Gray boxes highlight statistically significant differences. Higher IndVal values reflect a stronger association.

Thirteen bacterial indicator taxa associated with “Poor” and “Very Poor” dissolved oxygen score categories were identified (Figure 11). The strongest indicators for a “Poor” dissolved oxygen score included Actinobacteriota, Chloroflexi KD4-96, Microbacteriaceae and Hyphomicrobium. Notably, all of these groups include facultatively anaerobic species (Meiberg & Harder 1978; Lipko & Belykh 2021; Zhang et al. 2023).

FIGURE 11

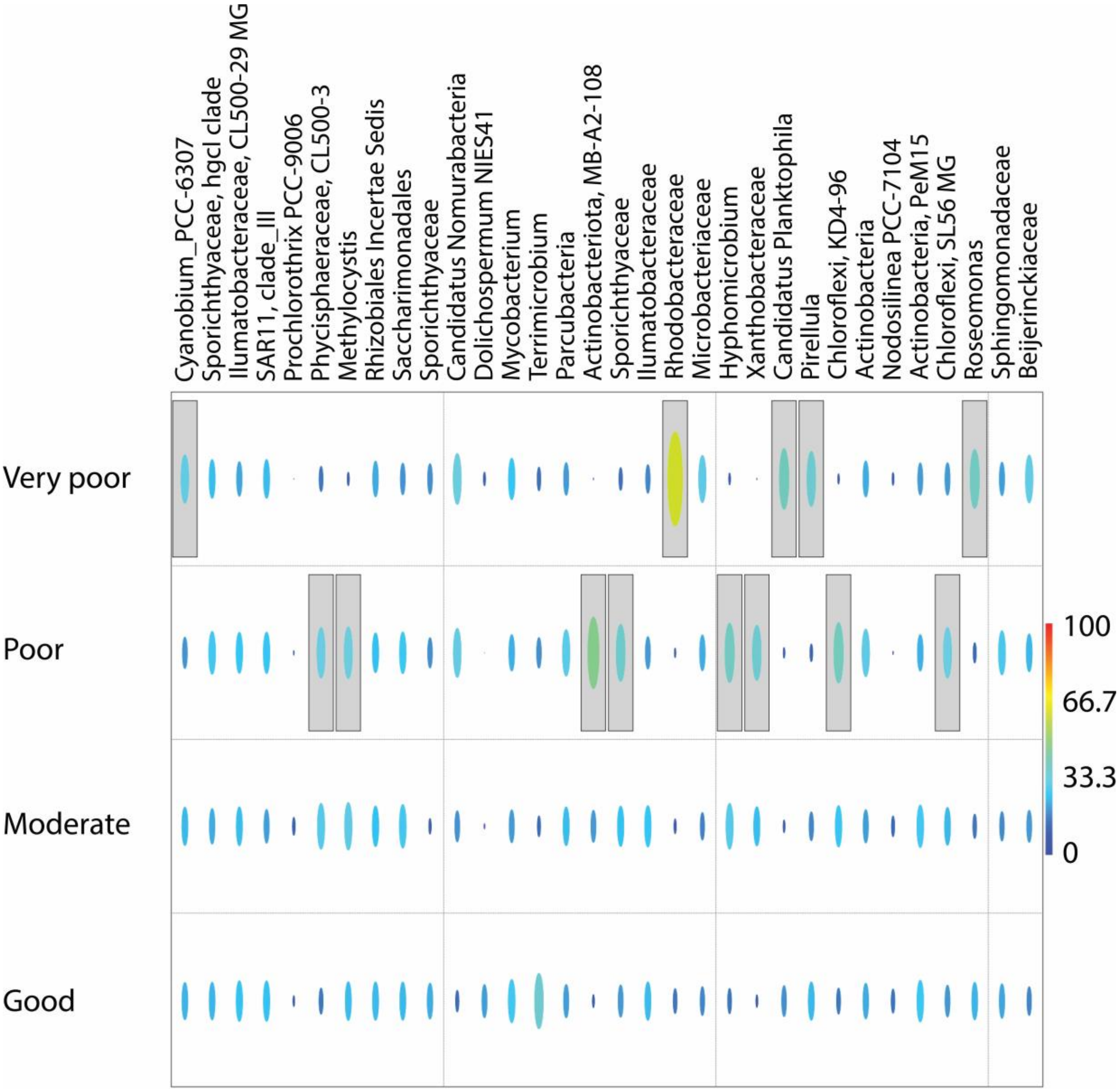


Figure 11: Indicator species analysis across Dissolved oxygen score categories at the genus level. The color and size of the ellipses represent IndVal (%) values, indicating the strength of association between each genus and a specific functional zone. Gray boxes highlight statistically significant differences. Higher IndVal values reflect a stronger association.

4.0 Summary & Conclusions

Microorganisms are a fundamental biotic element within river ecosystems, where they define ecosystem productivity, biogeochemical cycling and water quality. Here, as part of the Darling Baaka River Health Project (DBRHP), we employed 16S rRNA amplicon sequencing approaches to characterise the diversity and composition of bacterial assemblages within the Darling Baaka River system.

Significant spatial and temporal variability in bacterial diversity and assemblage structure was observed. Bacterial species richness was highest in the Wilcannia Upstream functional zone in June, coinciding with inflows from the upper catchment, while lowest levels were observed within the Greater Darling Anabranch functional zone during September, coinciding with the managed cease-to-flow event. The composition of the bacterial community differed significantly between the 8 functional zones, which is consistent with observations of marked bacterial community heterogeneity in other river systems (Yadav et al. 2021; Geng et al. 2024) and the substantial spatial variability in diverse biotic parameters recorded in other components of the DBRHP. Shifts in bacterial community composition sometimes pointed towards potential functional shifts in microbiological processes. For example, elevated levels of *Parcubacteria* observed in the Lake Wetherell and Wilcannia Downstream functional zones are notable, given previous evidence that these bacteria are prevalent members of the microbiome within waters exposed to soil seepage (Chaudhari et al. 2024). Some of these spatial differences are potentially linked to flow regimes and the highly regulated nature of the system, and are worthy of further investigation.

A significant bloom of the cyanobacteria *Dolichospermum* also occurred in the Wilcannia Upstream and Downstream functional zones during November, when this organism sometimes comprised more than 90% of the cyanobacterial community. This high abundance of *Dolichospermum* is notable given its potential for producing cyanotoxins and negatively impacting river ecosystems during bloom events (Li et al. 2016).

The observed heterogeneity in bacterial community composition across the DBRHP sampling program provided an opportunity to link specific bacterial “indicator taxa” to river condition characteristics. In other aquatic environments, bacteria have been demonstrated to be sensitive indicators of ecosystem condition (Glasl et al. 2019). Here, we identified 19 different bacteria that were classified as significant indicators of different river functional zones, with between 1-5 unique indicator organisms identified for each functional zone. We also identified bacterial indicators for a suite of river condition characteristics including geomorphic condition, water quality, hydrological stress, nutrient condition, turbidity, and dissolved oxygen.

Notably, the metabolic and/or ecological characteristics of these indicator bacteria were often very well aligned with their indicator status for specific river conditions. For example, strongest indicators for “Very Poor” turbidity scores included bacteria that are commonly found in soil and anoxic freshwater sediment environments, while the strongest indictors for “Very Good” nutrient condition included bacteria that are prominent within restored rivers following nutrient removal, and denitrifiers that play important roles in the removal of excess nitrogen from aquatic habitats. Cyanobacteria (e.g. *Dolichospermum*) that regularly bloom in nutrient rich rivers were identified as indicators for “Poor” nutrient scores. We suggest that, with further confirmation and optimisation, the bacterial indicators identified here have the potential to augment river ecosystem assessment efforts.

In summary, the results of this study demonstrate that the composition of microbial assemblages within the Darling Baaka River system is highly heterogenous in space and time, with evidence that specific regions of the system are vulnerable to impacts from toxic cyanobacterial blooms and shifts in river microbiology caused by seepage of soil bacteria. Specific “indicator bacteria” associated with discrete river functional zones as well as a suite of physicochemical and biotic river condition characteristics were identifiable. This work highlights the dynamic nature of microbiological processes within the Darling Baaka River system, which is likely reflective of the ecological importance of microorganisms within this ecosystem. Furthermore, this research has defined a set of potentially sensitive bacterial indicators of ecosystem state and paves the way for their integration into future river monitoring efforts.

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Appendix: Spatial Organisation of river ecosystem assessment categories used in the bacterial indicator analyses.

Figure A1

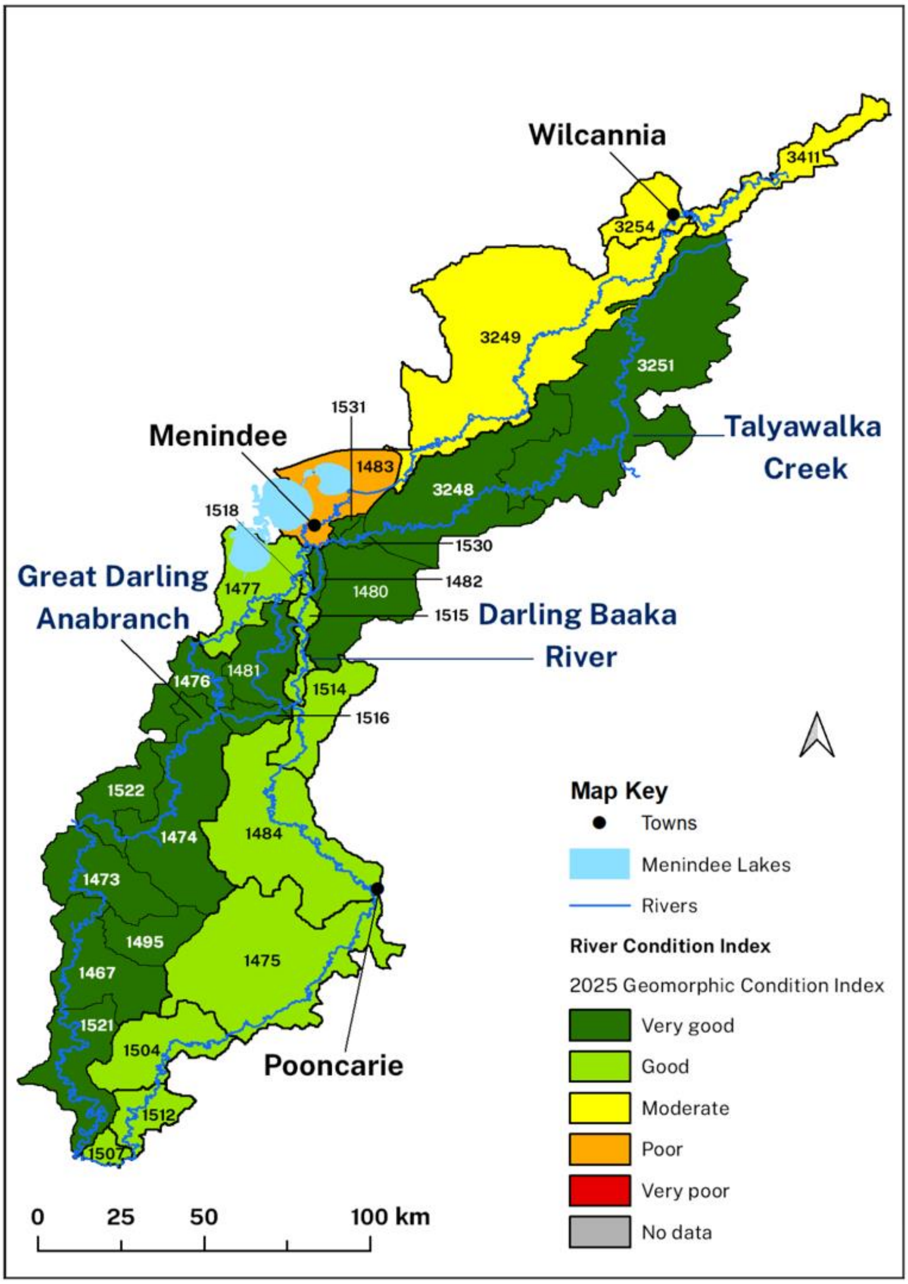

Figure A1: Geomorphic Condition Index grades, obtained from Chapter 3 of the Darling Baaka River Health Project report (DCCEEW, 2025) and used for bacterial indicator analysis

Figure A2:

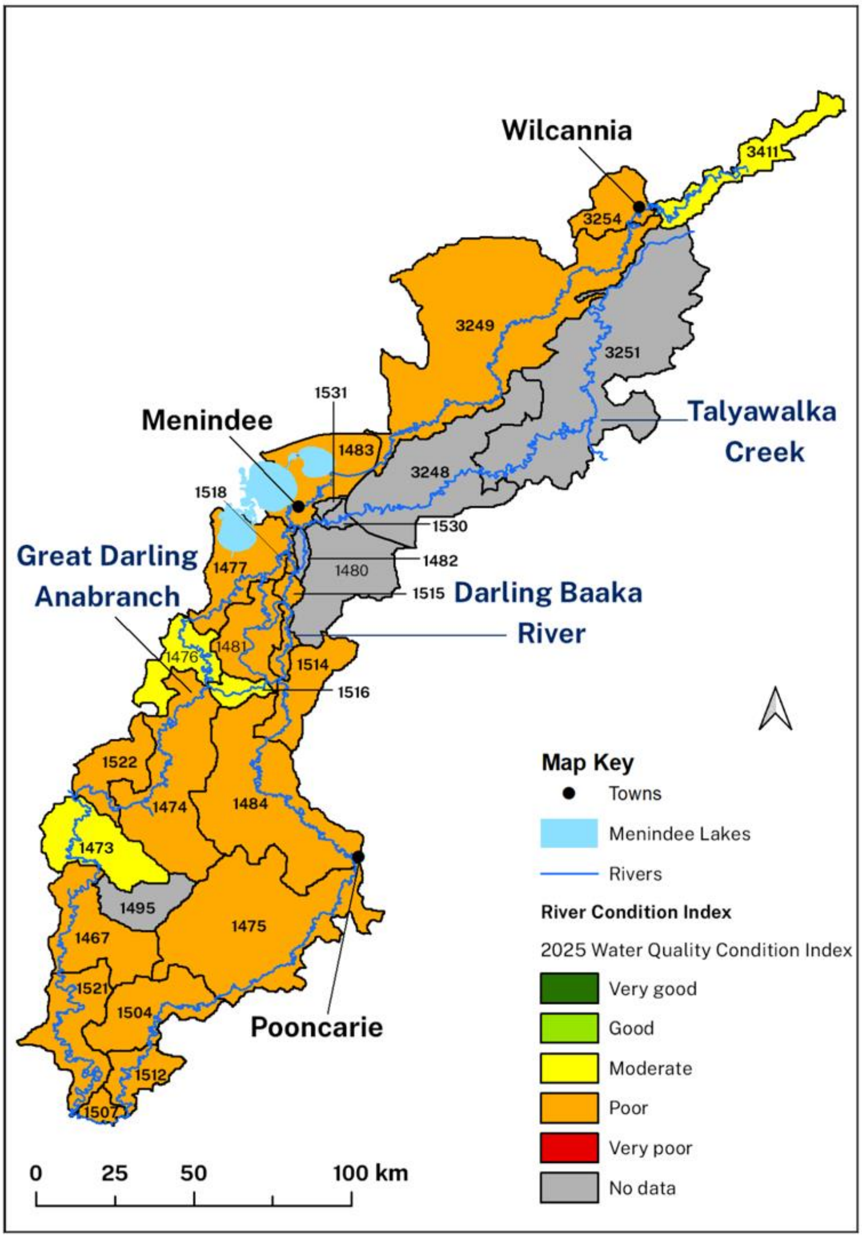

Figure A2: Water Quality Index grades, obtained from Chapter 4 of the Darling-Baaka River Health Project report (DCCEEW, 2025) and used for bacterial indicator analysis

Figure A3

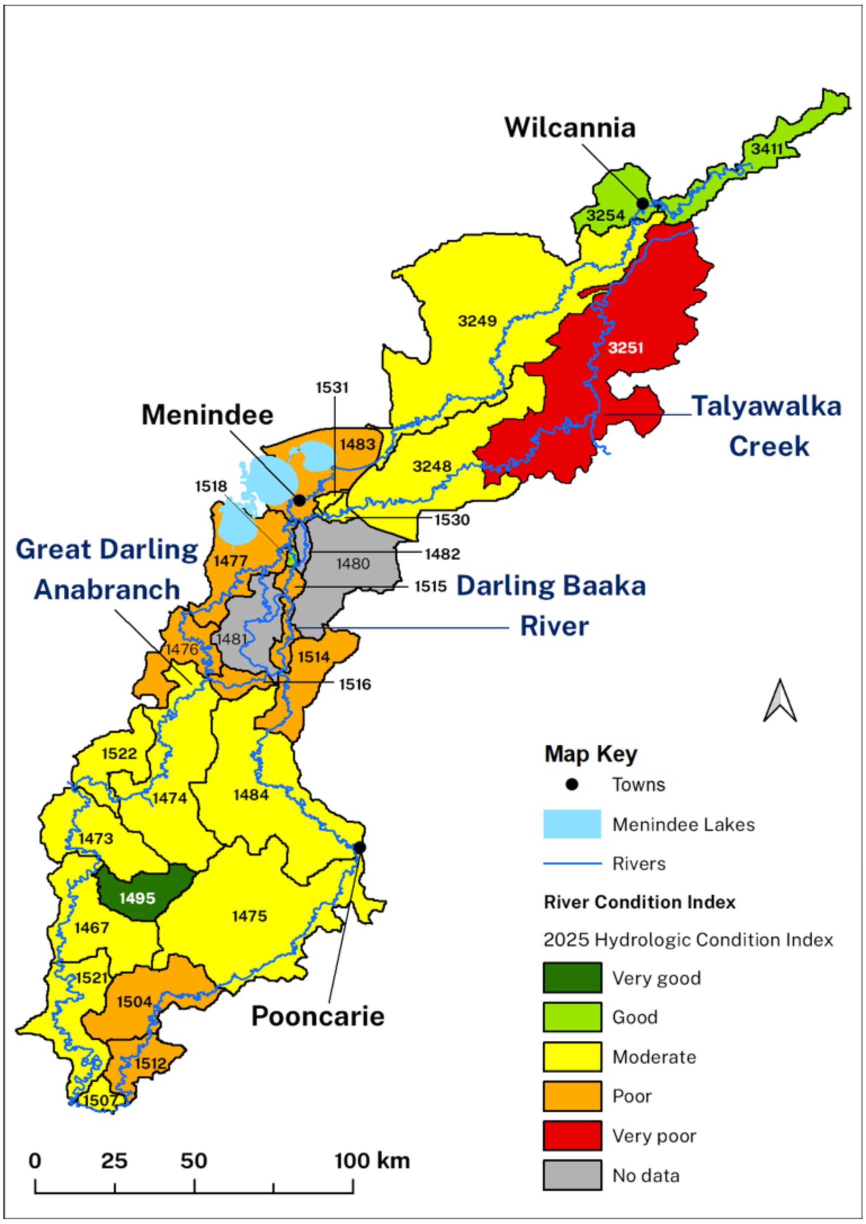

Figure A3: Hydrological Stress Index grades, obtained from Chapter 7 of the Darling-Baaka River Health Project report (DCCEEW, 2025) and used for bacterial indicator analysis