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ORIGINAL ARTICLE

Microbial communities reflect temporal changes in cyanobacterial composition in a shallow ephemeral freshwater lake

Jason Nicholas Woodhouse[1], Andrew Stephen Kinsela[2], Richard Nicholas Collins[2], Lee Chester Bowling[3,4], Gordon L Honeyman[5], Jon K Holliday[3] and Brett Anthony Neilan[1]
[1]*School of Biotechnology and Biomolecular Sciences, UNSW Australia, Sydney, New South Wales, Australia;* [2]*UNSW Water Research Centre and School of Civil and Environmental Engineering, UNSW Australia, Sydney, New South Wales, Australia;* [3]*Office of Water, NSW Department of Primary Industries, Elizabeth Macarthur Agricultural Institute, Menangle, New South Wales, Australia;* [4]*Centre for Ecosystem Science, UNSW Australia, Sydney, New South Wales, Australia and* [5]*Office of Water, NSW Department of Primary Industries, Hay, New South Wales, Australia*

The frequency of freshwater cyanobacterial blooms is at risk of increasing as a consequence of climate change and eutrophication of waterways. It is increasingly apparent that abiotic data are insufficient to explain variability within the cyanobacterial community, with biotic factors such as heterotrophic bacterioplankton, viruses and protists emerging as critical drivers. During the Australian summer of 2012–2013, a bloom that occurred in a shallow ephemeral lake over a 6-month period was comprised of 22 distinct cyanobacteria, including *Microcystis, Dolichospermum*, *Oscillatoria* and *Sphaerospermopsis*. Cyanobacterial cell densities, bacterial community composition and abiotic parameters were assessed over this period. Alpha-diversity indices and multivariate analysis were successful at differentiating three distinct bloom phases and the contribution of abiotic parameters to each. Network analysis, assessing correlations between biotic and abiotic variables, reproduced these phases and assessed the relative importance of both abiotic and biotic factors. Variables possessing elevated betweeness centrality included temperature, sodium and operational taxonomic units belonging to the phyla Verrucomicrobia, Planctomyces, Bacteroidetes and Actinobacteria. Species-specific associations between cyanobacteria and bacterioplankton, including the free-living Actinobacteria acI, Bacteroidetes, Betaproteobacteria and Verrucomicrobia, were also identified. We concluded that changes in the abundance and nature of freshwater cyanobacteria are associated with changes in the diversity and composition of lake bacterioplankton. Given this, an increase in the frequency of cyanobacteria blooms has the potential to alter nutrient cycling and contribute to long-term functional perturbation of freshwater systems.
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Introduction

Eutrophication and increased surface and water temperatures, as a consequence of intensive land management strategies and man-made climate change, are having profound impacts on the quality of our inland aquatic ecosystems (Barnett *et al.*, 2004; Füreder, 2012; Winder, 2012; Grafton *et al.*, 2013). The increasing frequency and severity of cyanobacterial blooms is of critical concern both with regards to maintaining ecosystem health as well as ensuring safe and equitable provision of water (Paerl and Huisman, 2009; Paerl and Paul, 2012; Sinha *et al.*, 2012; Michalak *et al.*, 2013). The occurrence of a cyanobacterial bloom is both an indicator of and a contributor to the declining quality of these aquatic ecosystems. The formation of cyanobacterial blooms has largely been linked to excessive eutrophication in the form of high levels of phosphorus (Schindler *et al.*, 2008; Conley *et al.*, 2009 (r11089); Posch *et al.*, 2012; Ho and Michalak, 2015). However, the continuing proliferation of diazotrophic and non-diazotrophic cyanobacterial species in P-limited systems has reinforced the importance of nitrogen species to bloom formation (Paerl *et al.*, 2014). Across the bloom period, the rapid accumulation of high levels of organic matter contributes to numerous changes to chemical properties of the water column. Periods of elevated cyanobacterial biovolume can result in oxygenation, nitrification, phosphorus

Correspondence: Dr BA Neilan, School of Biotechnology and Biomolecular Sciences, UNSW, BABS, Sydney, New South Wales 2052 Australia.
E-mail: b.neilan@unsw.edu.au
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loading of sediments and toxin production, followed by hypoxia owing to mass cell death (Li *et al.*, 2012; Wilhelm *et al.*, 2014). In addition, the natural or induced dispersion of cyanobacterial blooms poses a secondary detriment to the quality of water resources through the release of toxic secondary metabolites, collectively termed cyanotoxins (Carmichael, 2001; Ibelings and Chorus, 2007; Funari and Testai, 2008). The extent to which certain cyanobacterial species exhibit dominance over other phytoplankton, during bloom events or otherwise, has been shown to be dictated by differences in the physiological response of these organisms to prevailing abiotic factors (Paerl, 1996; Davis *et al.*, 2009; Rouco *et al.*, 2011; Neilan *et al.*, 2012).

The Murray–Darling basin, which spans all five of Australia's eastern mainland states, contributes upwards of AUD18.6 billion (40%) annually to gross agricultural production (ABS 2008). Intensive water use places large demands on the basin ecosystem, and although water efficiency measures are being implemented, it is expected that changing climatic conditions will further contribute to reductions in flows within the river system (Kingsford, 2011; Grafton *et al.*, 2013). As an indicator of future conditions, reduced river flows and increased water temperatures in the Murray River during 2009 saw the development of a cyanobacterial bloom spanning 1100 km of the river (Al-Tebrineh *et al.*, 2011; Bowling *et al.*, 2013). During the bloom, two potent cyanotoxins, cylindrospermopsin and saxitoxin, were observed, along with species with the genetic capacity to produce microcystins (Al-Tebrineh *et al.*, 2011). There is an evident difficulty in correlating many environmental parameters with cyanobacterial community composition, toxicity or toxigenicity across such studies (Rinta-Kanto *et al.*, 2009; Al-Tebrineh *et al.*, 2011; Otten *et al.*, 2012; Lee *et al.*, 2014; Ngwa *et al.*, 2014). In part, this lack of correlation can be attributed to genomic variability among closely related strains (Humbert *et al.*, 2013; Sinha *et al.*, 2014), giving rise to differing growth optima and potential to produce a myriad of toxic and non-toxic metabolites (Humbert *et al.*, 2013), changes in the genome copy number throughout the growth phase (Griese *et al.*, 2011) and uncertainty regarding the control of regulatory systems that direct the expression of toxic metabolites (Kaebernick *et al.*, 2000; Alexova *et al.*, 2011; Carneiro *et al.*, 2013; Neilan *et al.*, 2013; Rzymski and Poniedziałek, 2014; Makower *et al.*, 2015).

Freshwater microbial communities exhibit high compositional and functional variability across spatial and temporal scales (Newton *et al.*, 2011), reflecting changes in water chemistry and nutrient concentrations (Allgaier *et al.*, 2007; Newton *et al.*, 2007; Dennis *et al.*, 2013), hydrodynamic stability of the water column (Salcher *et al.*, 2011) and climatological impacts (Wilhelm *et al.*, 2014). Periods of high cyanobacterial biovolume are associated with elevated rates of denitrification (McCarthy *et al.*, 2007) and carbon sequestration (Becker *et al.*, 2011; Sandrini *et al.*, 2014), applying additional perturbations to the water column that in turn contribute to already highly variable systems (Li *et al.*, 2012). Numerous studies have highlighted the diversity of heterotrophic groups that occur within microbial communities associated with freshwater bloom-forming cyanobacteria species (Eiler and Bertilsson, 2004; Wu *et al.*, 2007; Berg *et al.*, 2008; Cheng *et al.*, 2011; Dziallas and Grossart, 2011; Grossart *et al.*, 2011; Wilhelm *et al.*, 2011; Li *et al.*, 2012; Li *et al.*, 2012; Steffen *et al.*, 2012; Woodhouse *et al.*, 2012; Cai *et al.*, 2013; Xing *et al.*, 2013; Bagatini *et al.*, 2014). Further, such studies have demonstrated that, for cyanobacterial-associated microbial communities, despite the identity of these communities being dependent on the cyanobacterial species present (Bagatini *et al.*, 2014), water chemistry, temperature (Dziallas and Grossart, 2011; Xing *et al.*, 2013) and ultimately the freshwater system within which the experiment is based, the function of these communities is largely conserved across local and continental spatial scales (Steffen *et al.*, 2012; Penn *et al.*, 2014; Steffen *et al.*, 2015). Fewer studies have assessed how the nature of the cyanobacterial-associated microbial community changes over temporal scales (Wu *et al.*, 2007; Tang *et al.*, 2010; Li *et al.*, 2012; Xing *et al.*, 2013) with changes in cyanobacterial species dominance and biovolume likely to impact on both the composition and function of these groups.

The Murray–Darling basin represents a series of interconnected reservoirs, facilitated by construction of dams, and small-to-medium-sized ephemeral water bodies that occur within numerous floodplains (Kingsford, 2000). Yanga Lake represents a shallow ephemeral eutrophic freshwater lake largely representative of many of the water bodies present throughout the floodplains of the Murray–Darling Basin that are utilised for recreation, agriculture and rural drinking water (Kingsford and Thomas, 2004, Kobayashi *et al.*, 2013). Yanga Lake was selected for this study as it is has been the subject of routine monitoring, has a prior history of cyanobacterial blooms (Kobayashi *et al.*, 2013) and, as a consequence of being fed by a single tributary (Yanga Creek), can be readily isolated from the river system, limiting the impact of inflows. In this study, we aimed to utilise a systems framework (Bissett *et al.*, 2013) that encompasses measurement of water quality information, cell enumeration and microbial community data sets to address how abiotic and biotic factors combine and interact during cyanobacterial blooms. We performed routine monitoring over a 6-month period and performed *in situ* measurements, elemental analyses, cyanobacterial species identification and enumeration and 16S rRNA amplicon sequencing at five sites across the lake. This study provides an understanding of how abiotic and biotic factors interact at various stages throughout a cyanobacterial bloom.

Materials and methods

Field data and phytoplankton sample collection
Yanga Lake (34°17′S, 143°36′E) is a shallow ephemeral lake covering 12.5 km^2 with a maximum mean depth of 2–3 m. Yanga Lake receives occasional water inputs as a consequence of overflows from the Lowbidgee floodplains. The lake derives most of its water from the Murrumbidgee River, via Yanga Creek, through a weir located at the northern end of the lake. Water samples were collected at a depth of 0.5 m from Sites 1, 2, 3, 5 and 6 within Yanga Lake (Supplementary Figure S1) on 11 occasions across a 7-month period.

Water temperature, dissolved oxygen, pH, chlorophyll *a* and electrical conductivity were measured *in situ* using a Hydrolab DS5 water quality sonde. No *in situ* data were collected on the 23 April 2013. Samples were collected to provide an approximation of the cyanobacterial community composition and the bacterial community composition at these five sites during and following periods of high cyanobacterial biovolume. Cell enumeration and estimation of cyanobacterial biovolume were estimated using standard procedures (see Supplementary Materials). For the purposes of this study, a bloom was defined in accordance with the Water Quality Research Australia framework, when the biovolume of all cyanobacterial species exceeded 10 $mm^3 l^{-1}$. Given samples were taken from a 0.5-m depth, this value is only with respect to surface waters, with care taken to ensure surface scums were not disturbed during sampling. Nutrient analyses (see Supplementary Materials) was limited to the measurement of major the cations (NO_3^- and NO_2^-) owing to constraints around the availability of infrastructure, storage conditions and time frames necessary to take receipt of samples from the remote Yanga Lake. Elemental analysis (see Supplementary Materials) was performed to provide specific insight into the nature of ions contributing to variation in electrical conductivity (Na, Mg, Al, Si, P, S, K, Ca, Cr, Mn, Fe, Ni, Cu, Zn, Ar, Cd and Pb).

Lugol's persevered samples (0.25 litres) for cell enumerations and molecular analysis and a 0.5-litre sample for elemental analysis and measurement of major cations were collected on each but the first two occasions where only Lugol's preserved samples were obtained. Non-preserved samples were frozen on site at a NSW Office of Water field office (Hay, NSW, Australia) and shipped along with Lugol's preserved samples directly to UNSW Australia. Frozen samples were thawed immediately prior to analysis to limit the dissolution of particulate nutrients. Elemental analysis and measurement of major cations was performed at the Water Research Centre, UNSW Australia (see Supplementary Materials).

Molecular analysis
DNA was extracted from Lugol's preserved samples immediately on receipt at UNSW to prevent any additional introduction of bias that may have arisen owing to the variability in different prokaryotic groups to prolonged storage (Bowers *et al*., 2000). A 100-ml volume of Lugol's preserved sample was filtered through a 0.22-μM MF-Millipore Membrane Filter (Merck Millipore, Darmstadt, Germany). DNA was extracted from filters using the Power Water DNA Extraction Kit (MO BIO Laboratories Inc., Carlsbad, CA, USA) in accordance with the manufacturer's specifications. Amplicon sequencing (2 × 300 bp) of the 16S rRNA gene V1–V3 region was performed using primers 28f (5′-GAGTTTGATC NTGGCTCAG-3′) and 519r (5′-GTNTTACNGCGGC KGCTG-3′) utilising the MiSeq platform at the Ramaciotti Centre for Gene Function Analysis, UNSW Australia. A complete description of the PCR conditions used for this primer set is available elsewhere (see Supplementary Materials). De-multiplexed sequences are available for download via the NCBI short read archive under BioProject PRJNA296748. Quality co-processing was performed using Mothur v 1.34.1 (see Supplementary Methods). Sequences were clustered at a distance threshold of 0.03 using the average neighbour method (Schloss and Westcott, 2011). The taxonomy of each operational taxonomic unit (OUT) was assigned using a combination of the Freshwater Microbial Field Guide (Newton *et al*., 2011) July 2012 release (https://github.com/mcmahon-uw/FWMFG) and the GreenGenes May 2013 release (DeSantis *et al*., 2006) (see Supplementary Materials). Following sequence processing, each sample retained at least 40 000 sequences. Subsampling was performed at this level to ensure consistency across the data set.

Statistical analyses
Permutation multivariate analysis of variance (PERMANOVA), as implemented in Primer v6, on the full set of 11 time points (54 samples), as well as the reduced set of 7 time points (35 samples), was implemented to examine differences in the composition of samples with respect to both cyanobacterial cell counts and 16S rRNA gene OTUs across both spatial and temporal scales. Step-wise distance-based redundancy analysis (dbRDA) was implemented in Primer v6 on a reduced set of 7 time points (34 samples in total) to determine the contribution of each measured environmental parameter to the variation observed within the 16S rRNA gene and cyanobacteria cell count matrices.

The degree of association of measured environmental variables, cyanobacterial cell counts and 16S rRNA gene OTUs with respect to one another across the entire bloom period was measured using the Pearson's correlation coefficient (*r*). The number of bacterial OTUs, defined at 0.03-distance threshold, was reduced by retaining only those that occurred within at least three samples and retaining only those that contributed at least 1% to any given sample. Of these OTUs that were retained, no alteration was made to the observed abundance

value within any sample. A Pearson correlation coefficient *r* score and *P*-value were calculated pairwise for each bacterial OTU using the rcor.test algorithm, available from the ltm package (available from http://rwiki.sciviews.org/doku.php?id=packages:cran:ltm) as implemented in R version 3.0.2. *P*-values for each correlation were generated and the false discovery rate was kept below 5% using the Benjamini–Hochberg procedure (Benjamini and Hochberg, 1995). Visualisation of these associations was made with the Cytoscape package version 2.8.3 (available at: www.cytoscape.org). The *r* value was selected to support the generation of an edge-weighted spring-embedded network. Topological and node/edge metrics, including connectivity, density and betweeness centrality, were calculated using the Network Analysis plug-in (Assenov *et al*., 2008).

Results

Cyanobacterial community composition

During the sampling period, the cyanobacterial bloom biovolume remained at levels $>10\,mm^3\,l^{-1}$ for a continuous period of 4 months, from 25 January to 23 April 2013 (Figure 1a). Over this period, 22 morphologically distinguishable cyanobacterial taxa were observed (see Supplementary Table S1). The dominant cyanobacteria during this period were *Dolichospermum circinale* (ex *Anabaena circinalis*), *Microcystis*, *Aphanocapsa*, *Geitlerinema*, *Oscillatoria* and *Sphaerospermopsis* (Figures 1b–e). Bray–Curtis similarities, for cyanobacterial cell counts, revealed a pattern indicative of temporal succession across the samples (Figure 2a). Step-wise distance-based linear modelling (distLM), utilising a reduced sampling set of only seven time points, identified temperature, electrical conductivity (EC), dissolved oxygen (DO) and pH, Si, Mg and Ca as predictor variables for the cyanobacterial community (Table 1), together accounting for 78.23% of the observed variation. Marginal tests further identified nitrate, nitrite, chlorophyll *a*, Na, S, and K as being able to account for the variation in the cyanobacterial community, although their contribution is readily described by environmental variables (pH, Si, Mg, Ca) already identified within the step-wise distLM (Figure 2a).

Microbial community structure

Bacterial community analysis, as assessed by sequencing the V1–V3 region of the 16 rRNA gene, identified 39 606 OTUs defined at 0.03-distance threshold across the bloom period (Supplementary Table S3). Phylotyping of these OTUs revealed a lake microbial community dominated by Actinobacteria and Alphaproteobacteria, when cyanobacterial biovolume were low, and by Actinobacteria, Bacteroidetes and Cyanobacteria when cyanobacterial biovolume were elevated (Figure 3). During periods of high cyanobacterial abundance,

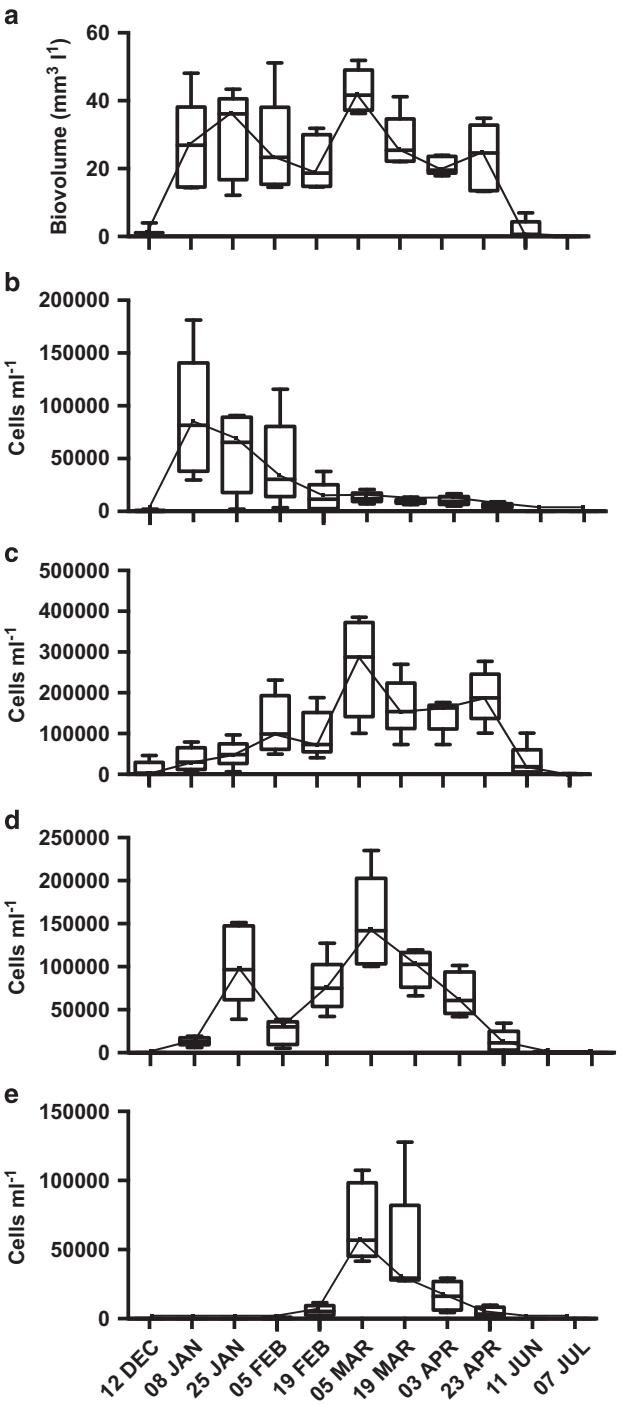

Figure 1 Averaged (**a**) estimated cyanobacterial cell biovolume and (**b**) *Dolichospermum/Anabaena* spp., (**c**) *Microcystis*, (**d**) *Oscillatoria* and (**e**) *Sphaerospermopsis* cell counts across the bloom period as measured at five sites.

Betaproteobacteria, Gammaproteobacteria, Planctomycetes and Verrucomicrobia were also present at substantially greater abundance than at other periods. Cosmopolitan freshwater clades, including acI (Figure 4a), acIV (Figure 4b), alfV (Figure 4c) and bacII (Figure 4d), were the most abundant non-cyanobacterial taxa within Yanga Lake. AcI

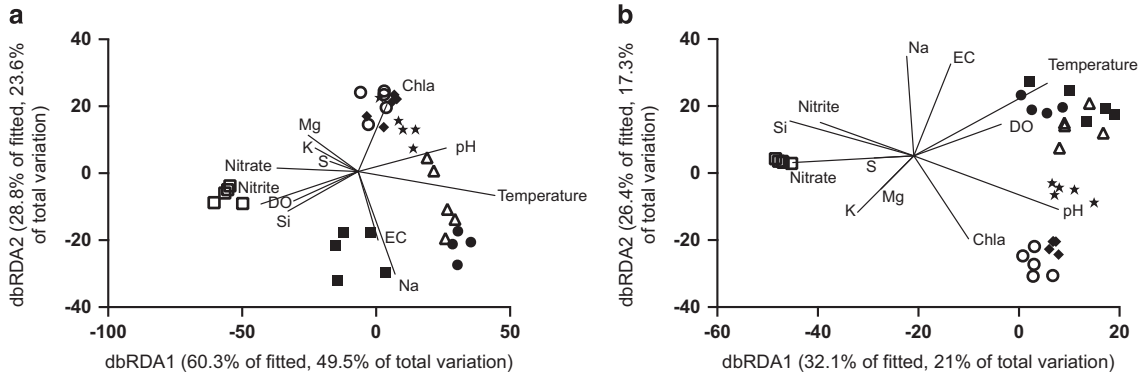

Figure 2 Distance-based linear redundancy visualising the relative contribution of measured environmental parameters on (**a**) cyanobacterial community composition determined by cell enumeration and (**b**) total bacterial community composition determined by 16S rRNA gene amplicon sequencing. Symbols indicate sampling date: 25 January (•), 5 February (■), 19 February (Δ), 5 March (▯), 19 March (♦), 3 April (○), and 9 July (□).

Table 1 Marginal tests of abiotic variables to both cyanobacterial and microbial community composition as determined by PERMANOVA and Distance-based linear modelling (DistLM)

	Cyanobacteria		*Bacteria*	
	Pseudo-F	P	*Pseudo-F*	P
Spatial	31.5	<0.001	6.84	<0.001
Temporal	1.87	0.074	1.31	<0.001
Temperature	22.4	<0.001	7.44	<0.001
Electrical conductivity	8.74	<0.001	5.01	<0.001
Dissolved oxygen	1.84	0.133	2.43	0.005
pH	16.3	<0.001	8.13	<0.001
Nitrate	17.5	<0.001	7.19	<0.001
Nitrite	18.6	<0.001	6.40	<0.001
Chlorophyll *a*	6.67	<0.001	4.88	<0.001
Na	7.85	<0.001	4.56	<0.001
Mg	6.24	0.004	3.52	<0.001
Al	0.76	0.501	0.89	0.54
S	4.71	<0.001	2.77	<0.001
Si	18.6	<0.001	7.57	<0.001
K	8.20	<0.001	4.19	<0.001
Ca	0.86	0.466	2.30	0.01
Fe	2.33	0.124	1.86	0.018
P	2.39	0.063	2.47	0.006

Abbreviation: PERMANOVA, permutation multivariate analysis of variance.

(Figure 4a) and alfV (Figure 4c) were distinct in that they were enriched during periods of low cyanobacterial biovolume, whereas acIV (Figure 4b) and bacII (Figure 4d) were most abundant during periods of high cyanobacterial biovolume. Several additional cosmopolitan clades were present at lower abundance, yet still exhibited distinct profiles in that they were enriched in accordance with periods of varying cyanobacterial biovolume (Figures 4d–h).

Bray–Curtis similarities, calculated across the 39 606 OTUs (Figure 2b), were significantly correlated with the ecophysiological parameters (Rho statistic = 0.697) and cyanobacterial cell counts (Rho statistic = 0.809). PERMANOVA, across all 11 time points, supported both a significant temporal (Pseudo-F = 8.3551, *P*(perm) = 0.001) and, albeit less substantial, spatial (Pseudo-F = 1.4565, *P*(perm) = 0.001) succession (Supplementary Figure S2). Similarly, PERMANOVA supported a temporal and spatial separation of samples using the reduced data set of seven time points (Table 1). Step-wise distLM, utilising a reduced sampling set of only seven time points, identified pH, temperature, DO and EC as predictor variables for the bacterial community, accounting for 46.17% of the observed variation (Figure 2b). Marginal tests further identified, nitrate, nitrite (Pseudo-F = 6.3983), chlorophyll *a*, Na, Mg, Si, P, S, and K as being able to account for variation within the total bacterial community (Table 1, Figure 2b).

The Chao1 richness and InvSimpson diversity indices for the microbial community varied across the sampling period (Supplementary Figure S3). Both the diversity and richness indices tended upwards with total cyanobacterial biovolume (Figure 1a), peaking around mid-March, coinciding with a decline in cyanobacterial biovolume.

Covariance analysis of biotic and abiotic lake components

Of the 147 measured variables, 144, including 104 16S rRNA gene OTUs that contributed >1% to any sample, were shown to form a single interconnected network. Of the 10 731 tested correlations, only 1835 were considered significant (Supplementary Table S4). Edge-weighted spring-embedded visualisation of the network, utilising the *r* score as the edge-weight, revealed the arrangement of the variables along three axes (Figure 5). By scrutinising the distribution of measured environmental parameters and cyanobacterial cell counts, it was apparent that these three axes mirrored that observed within the dbRDA plots (Figure 2). The summer sampling period (January–February) was associated with high temperature, DO, EC, Al, Na, the cyanobacteria *Dolichospermum circinale* and *Phormidium* and 47 16S rRNA gene OTUs. The autumn period (March–April) was

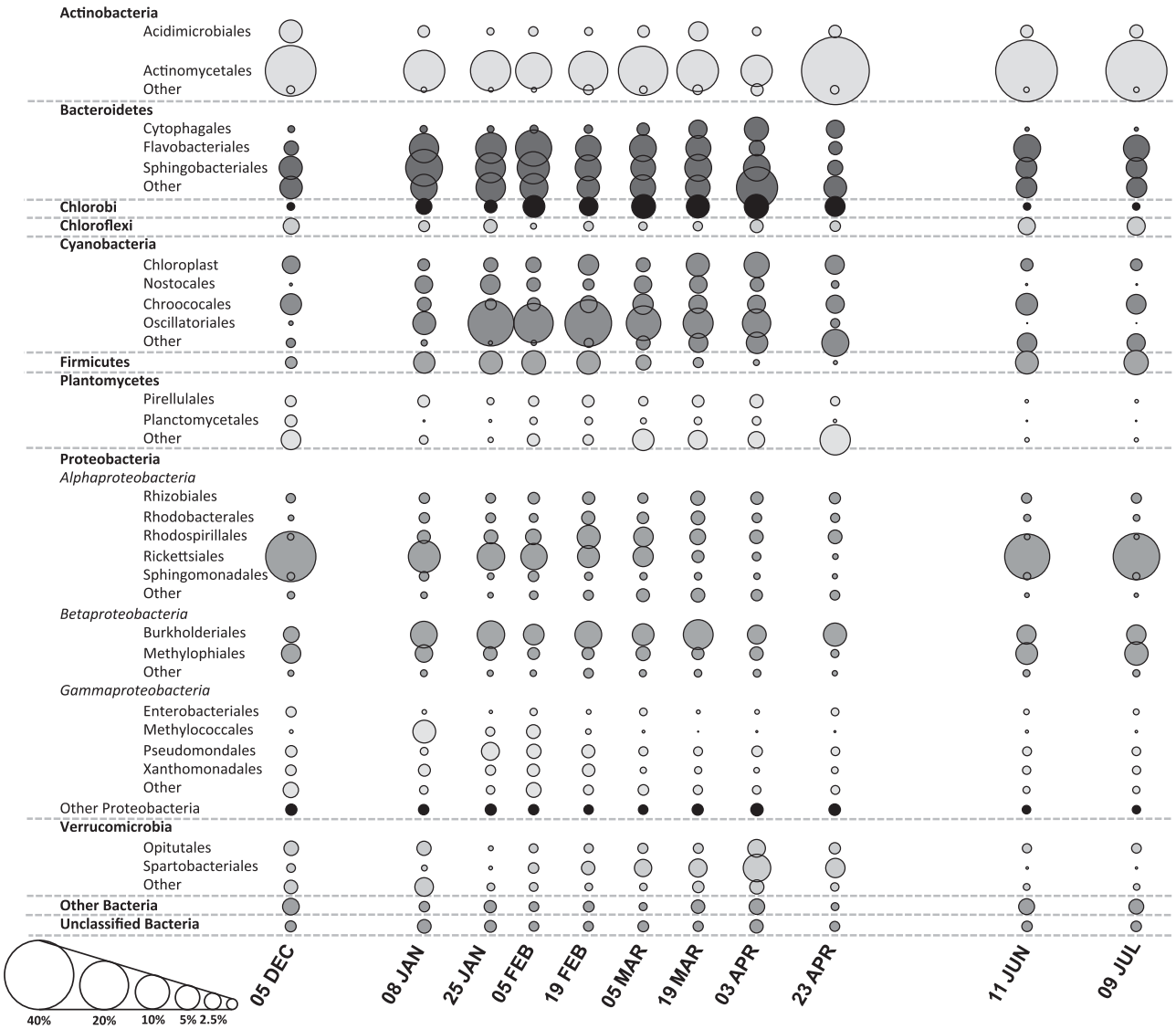

Figure 3 Relative abundance of 16S rRNA gene phylotypes across the sampling period. The area of each circle reflects the relative abundance of that phylotype averaged across the five samples collected at each time point. The colour of each bubble is indicative of the phylum to which each phylotype belongs. A full colour version of this figure is available at the *ISME Journal* journal online.

associated with high pH and chlorophyll *a* content, 12 cyanobacterial taxa and 45 16S rRNA gene OTUs. The winter period (July) was associated with high Ca, Fe, K, Mg, P, Si, NO_3^-, NO_2^- and 23 other bacterial 16S rRNA gene OTUs. The weak association of variables, such as temperature, within a single module suggests variables may be associated with more than one stage, with temperature-associated community shifts in summer and autumn stages when contrasted with the winter period. A sub-network, comprising only correlations between *Microcystis* spp., *Dolichospermum circinale*, *Sphaerospermopsis*, *Oscillatoria*, *Aphanocapsa*, *Geitlerinema*, *Phormidium* and *Cuspidothrix* cyanobacterial cell counts and either 16S rRNA gene OTUs or abiotic environmental parameters, revealed variability in the number and nature of unique and shared correlations between each of the cyanobacteria and other variables, including Na, and the taxa bacII, bacIII, bacIV, acIV and betII, within the network (Figure 6).

Network analysis supported the conclusions drawn from distLM that temperature (centrality = 0.050) was strongly correlated with changes in bloom microbial composition. EC (centrality = 0.027) and Na (centrality = 0.037) were highlighted owing to an average decline of 30 $\mu S\,cm^{-1}$ and 10 p.p.m., respectively, between the early and mid bloom periods associated with 29.4 mm of rainfall in surrounding areas. Ten 16S rRNA gene OTUs exhibited betweeness centrality >0.02. Firmicutes (centrality = 0.041), Saprospirales (centrality = 0.036), *Luteolibacter* (centrality = 0.036), *Planktothricoides* (centrality = 0.024) Bacilliaceae (centrality = 0.022) and Pirellulaceae (centrality = 0.021) OTUs were associated with the summer period.

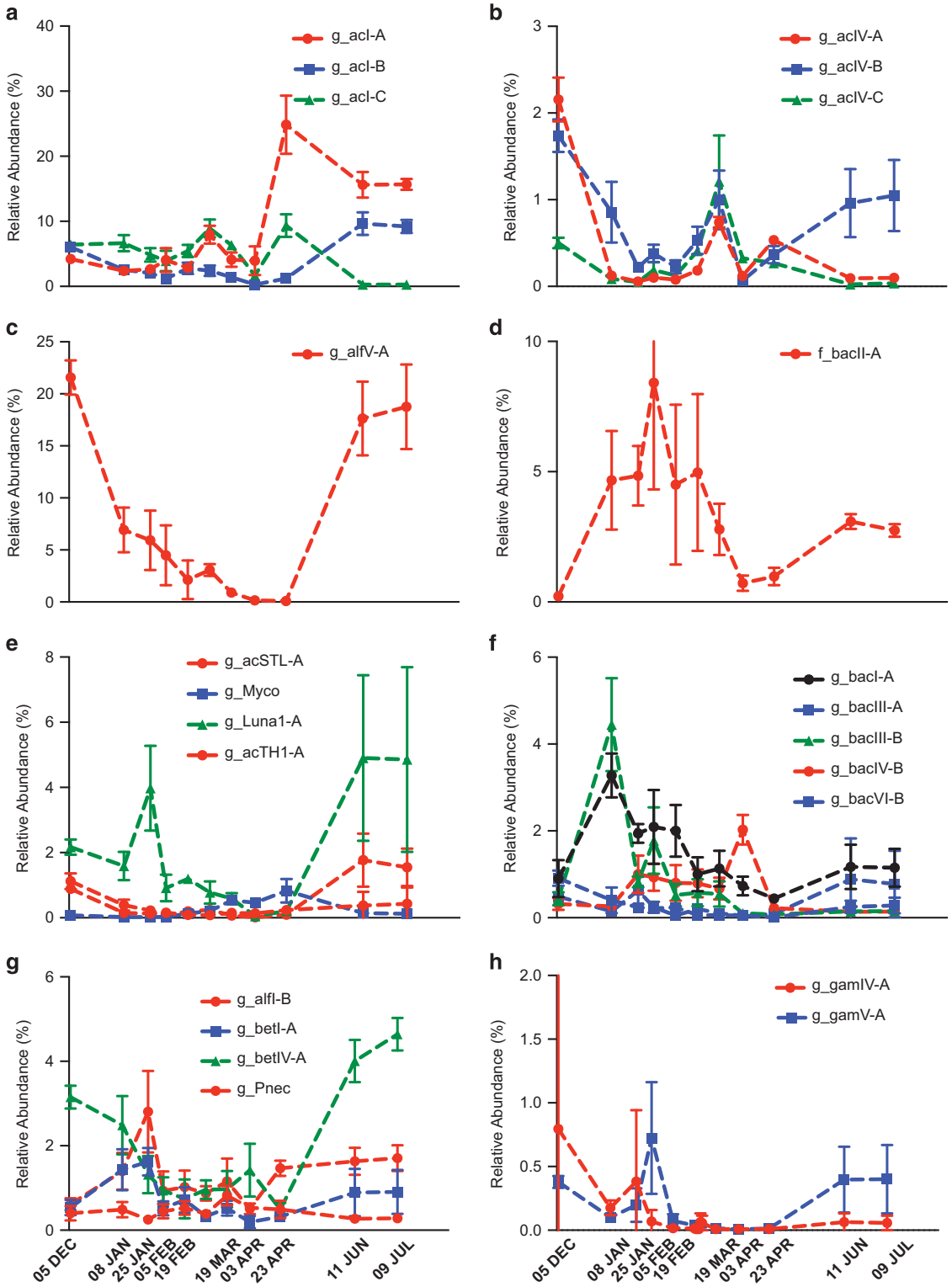

Figure 4 Relative abundance of cosmopolitan freshwater (**a**) acI, (**b**) acIV, (**c**) alfV, (**d**) bacII, (**e**) actinobacterial, (**f**) bacteroidetes, (**g**) alphaproteobacterial and betaproteobacterial and (**h**) gammaproteobacterial clades identified at the genus level.

The taxon *Myco* (centrality = 0.039) and bacVI (centrality = 0.021) were highlighted during the autumn period, whereas betII (centrality = 0.042) and Luna1-A (centrality = 0.0314) were only associated with the winter period.

Discussion

Cyanobacterial species composition

A bloom, defined by a combined biovolume of >10 $mm^3 l^{-1}$ of all cyanobacteria, was first observed in

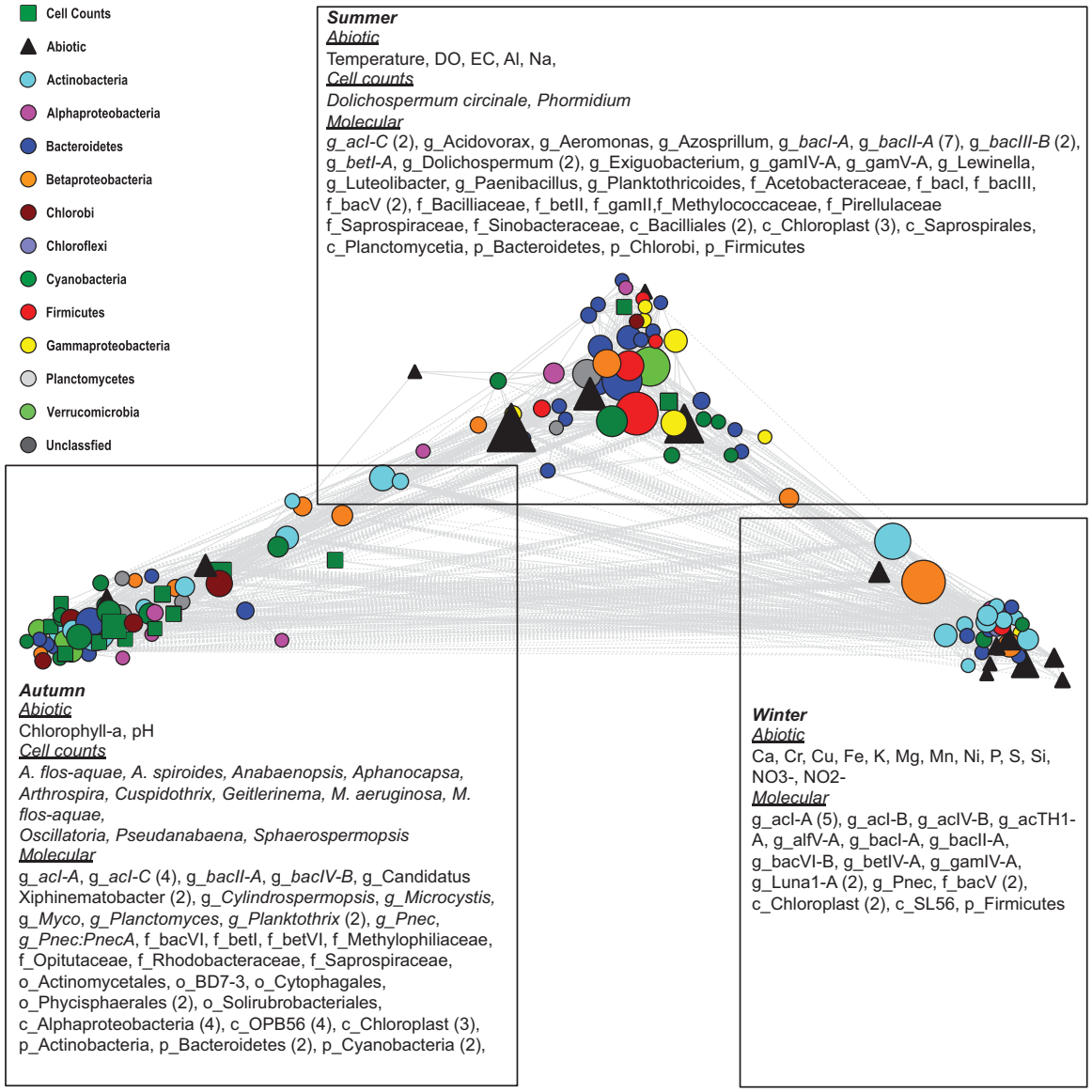

Figure 5 Pearson correlation coefficient edge-weighted Spring-embedded network visualising significant correlations between measured biotic and abiotic variables. Node size is reflective of the betweeness centrality of the variable. Line types (solid = positive and dashed = negative) are indicative of the Pearson correlation coefficient.

Yanga Lake on 8 January and persisted till 23 April 2013. During this period, it was evident that this single event was highly dynamic, with 22 morphologically distinguishable cyanobacteria observed across the period. A dbRDA model incorporating temperature, pH, EC and DO (Figure 2a) was able to describe much of the variability in the cyanobacterial community.

Measured abiotic factors, however, were less able to explain variability among individual taxon. It is worth mentioning that, owing to the remote location of Yanga Lake, samples were frozen on site, shipped and stored for several days. This process limited the analyses that could be performed on these samples and transformed an unquantifiable proportion of nutrients between soluble and particulate phases, which may account for these observations. *Dolichospermum circinale*, which was initially dominant, could only be directly correlated to Mg, K and Na and was the only cyanobacterial taxon correlated with temperature (Figure 6). *D. circinale* dominance of the surface waters during hotter months is consistent with an ability to persist in the euphotic zone, through regulating buoyancy, during periods of persistent stratification (Mitrovic *et al.*, 2001), providing a competitive advantage over other taxa that, unlike *Dolichospermum*, exhibit even rather than distinct distribution through the water column. Decline of this species likely arose owing to a combination of multiple factors, either measured or otherwise, within this study, including decreasing temperature, changes in light availability

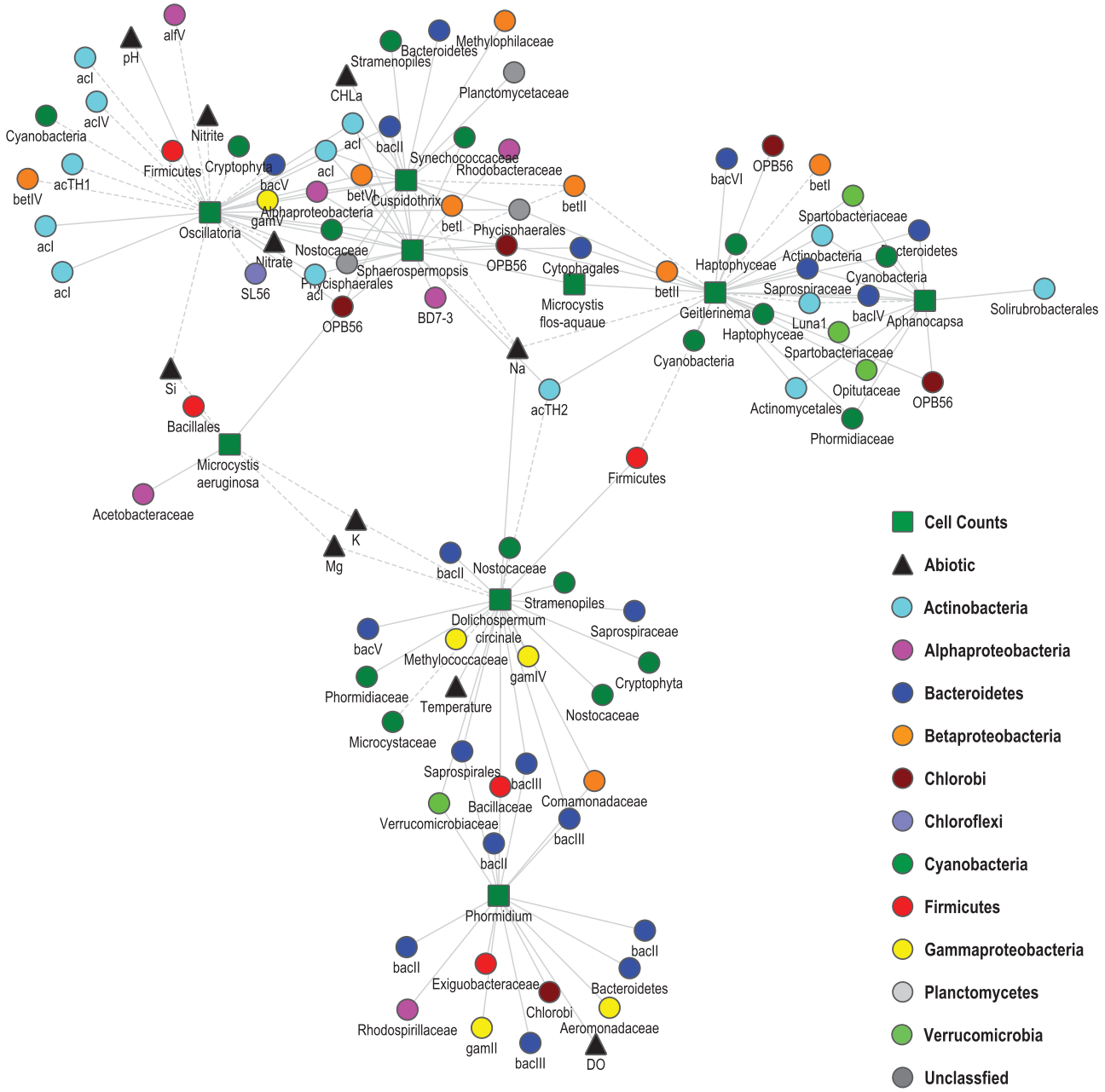

Figure 6 Organic correlation network visualising pairwise correlations between cyanobacteria and 16S rRNA gene OTUs and abiotic parameters. Line types (solid = positive and dashed = negative) are indicative of the Pearson correlation coefficient.

(Brookes, 1999; McCausland *et al.*, 2001; McCausland *et al.*, 2005), P-limitation and dissolved N concentrations (Supplementary Table S2). The sudden collapse in the *D. circinale* population strongly implicates an external biological effector, such as viral lysis or bacterial predation, neither of which was addressed at length within the scope this study. In this regard, numerous studies have demonstrated biotic mechanisms for the rapid dispersal of cyanobacteria from surface waters that may explain this observation, including phage-induced lysis (Proctor and Fuhrman, 1990; Williamson *et al.*, 2002; Matteson *et al.*, 2011; Steffen *et al.*, 2015), bacteria-induced lysis (Rashidan and Bird, 2001), grazing by protists (Ger *et al.*, 2014), algicidal compounds (Luo *et al.*, 2013; Shao *et al.*, 2013) and cyanobacterial programmed cell death (Franklin, 2014).

Eubacterial diversity and composition

To the best of our knowledge, this study represents one of only a few studies to examine the total bacterial population of a freshwater lake on the Australian continent (Dennis *et al.*, 2013) and the first Australian study to do so in the context of a multi-site time series comparable to those conducted elsewhere (Allgaier and Grossart, 2006; Wu *et al.*,

2007; Tang *et al*., 2010; Dziallas and Grossart, 2011; Eckert *et al*., 2012; Paver *et al*., 2013; Wilhelm *et al*., 2014). During periods of low cyanobacterial biovolume, the microbial community present within the surface water was dominated by the taxa acI (-A and B) (Figure 4a) and alfV-A (Figure 4c). In the absence of cyanobacteria, these three lineages alone accounted for approximately 50% of the total sequences obtained from the five sites within Yanga Lake. During periods of elevated cyanobacterial biovolume, it was clear that acI (Figure 4a), alfV-A (Figure 4c), acTH1-A, Luna1-A (Figure 4e), betIV-A (Figure 4g), gamIV-A and gamV-A (Figure 4h) decreased as a proportion of the total sequence reads observed (Figure 4). Overall, the bacterial community composition could only partially be explained by those factors that defined cyanobacterial species composition. Increased heterogeneity between samples (Figure 2b) during periods of elevated biomass is consistent with high levels of variability reported among freshwater microbial communities during periods of increasing cyanobacterial biovolume (Wu *et al*., 2007; Li *et al*., 2012).

AlfV-A, sister group to the marine *Pelagibacter* (SAR11), represents a free-living ultramicrobacterium that was largely thought to be oligotrophic with its small cell size enabling it to persist at low nutrient concentrations. Consistent with the presence of this taxon in the eutrophic Yanga Lake (Kobayashi *et al*., 2013), an analysis of the literature, demonstrating that the abundance of alfV-A can be linked to increased surface water temperatures and high nutrient concentrations, concluded that there was clear evidence that functional heterogeneity occurs within this lineage (Newton *et al*., 2011). A caveat to this is that the absolute abundance of alfV is overwhelmingly dependent on water turbidity, with phytoplankton blooms drastically decreasing its abundance (Salcher *et al*., 2011) in a manner akin to that observed in this study. The emergence of acI, in Yanga Lake, subsequent to the bloom (Figure 4e) is consistent with the genomic potential of these organisms to take advantage of cyanobacterial exudates, specifically cyanophycin, following the lysis of cyanobacteria (Ghylin *et al*., 2014). Albeit contributing a smaller proportion of the total community, methylotrophs betIV and gamI (Figures 4g and h) exhibited similar distributions consistent with observations that the heterotrophic decomposition of cyanobacterial biomass leads to an accumulation of acetate, selecting for taxa capable of acetoclastic methanogenesis under either oxic or anoxic conditions (Bogard *et al*., 2014). The late emergence of methylotrophs following cyanobacterial blooms is also implicated as contributing to the accumulation of methane derived from archaeal methanogenesis in surface waters (Grossart *et al*., 2011).

During the summer and autumn periods, the total bacterial community was dominated by a number of OTUs assigned to the Nostocales, Chroococcales and Oscillatoriales cyanobacterial orders (Figure 3), consistent with the presence of these cyanobacteria taxa. Actinobacteria remained a major part of the bacterial community during periods of high cyanobacterial biovolume, with clear increases in the relative abundance of the acIV taxon (Figure 4b). Studies have pointed to a number of actinobacteria as associated with particles, with several demonstrating the acI-C and acIV lineages form species-specific associations with cyanobacteria (Allgaier *et al*., 2007). Similar increases were observed for the particle-associated betI and betIV (Figure 4g), which have been noted for their attachment to prokaryotic (Bagatini *et al*., 2014) and eukaryotic phytoplankton (Paver *et al*., 2013), as well as the metabolism of the cyanobacterial toxin microcystin (Mou *et al*., 2013). Among the Bacteroidetes, bacI-A, bacIII-B, bacIV-B and bacVI-B (Figure 4f) were more dominant during periods of high biovolume. BacI-A and bacIII-B were most abundant during the summer period, suggesting that their presence is linked to the same factors that influenced the initial proliferation of *D. circinale* cell numbers or to the cyanobacterium itself. BacII-A (Figure 4g) contributed upwards of 10% of the total microbial community during the first 2 months of the bloom period. It is unclear whether the sharp decrease in the relative abundance of bacII-A or other taxa during the summer period arose simply owing to displacement by other taxa or on account of some external factor, coinciding with the proliferation of additional cyanobacterial taxa during the autumn period.

Covariance of biotic and abiotic factors

A covariance approach was applied to visualise how biotic and abiotic factors interact to define patterns within the cyanobacterial and bacterial communities using an edge-weighted spring-embedded network (Figure 6). In contrast with other studies, examining soil and sediment-bound microbial communities (Bissett *et al*., 2013; Sun *et al*., 2013), which have observed a lack of correlations between absolute abiotic measurements and 16S rRNA OTU relative abundances, numerous correlations were observed between biotic and abiotic factors. Although consideration should be made that such approaches, wherein relative abundances are used to derive correlations between species, may overestimate the numbers of true correlations (Friedman, 2012; r14933), this analysis was effective at classifying factors characteristic of the three observed periods, namely summer, autumn and winter periods, also observed in the dbRDA analysis (Figure 2). In this context, with cyanobacterial cell counts as the driver, 16S rRNA gene OTUs, cell counts and abiotic data were considered to be associated with each of the distinct periods when the observed variables exhibited strong positive correlations to either the cell counts or to one another (Figure 5).

The summer period was defined by high cell numbers of the cyanobacteria *Dolichospermum*

circinale and *Phormidium*, elevated temperature, DO and EC, a value influenced by elevated Na and Al levels. Recruitment of bloom-forming cyanobacteria from littoral zones (Kononen *et al.*, 1996) has been demonstrated to be significantly enhanced by high water temperatures and disturbance of these sediments (Rengefors *et al.*, 2004). Although littoral recruitment cannot be ruled out, the shallow ephemeral nature of the lake suggests that mixing is not critical for the recruitment of cyanobacteria from the sediment. Similarly, it is plausible that major inflows arrived from upstream and that the rapidly moving water was sufficient for recruitment of cyanobacteria and suspension of nutrients from river and creek sediments. The closing of the northern weir, following the initial identification of the bloom on 8 January suggests that a similar mechanism was not responsible for the succession of the bloom from one dominated by *Dolichospermum* to the one dominated by *Microcystis* and *Oscilltoria*.

A difference in the number and nature of variables correlated with *Dolichospermum* and *Phormidium* and the low proportion of heterotrophic taxa that are correlated to either genus was evident (Figure 6). *Dolichospermum* was the only cyanobacterial taxon positively correlated with temperature and was negatively correlated with Na, Mg and K, while *Phormidium* was positively correlated with DO. The bacII and bacIII OTUs, as well as several γ-proteobacterial OTUs, were overrepresented in both species-specific and shared correlations (Figure 6). The presence of particle-associated Bacteroidetes lineages following bloom events have led to the assertion that postbloom conditions provide favourable conditions for proliferation of heterotrophs (Li *et al.*, 2012; Shao *et al.*, 2013). Consistent with this, bacII reached its relative abundance maxima in the period following peak *Dolichospermum* cell counts. Peak levels of bacIII corresponded with peak *Dolichospermum* cell counts, indicating that their levels are more explicitly linked to viable cyanobacterial numbers, rather than the concentration of cyanobacterial exudates.

The autumn period was defined by multiple cyanobacterial genera, with *Microcystis*, *Oscillatoria*, *Sphaerospermopsis*, *Cuspidothrix*, *Aphanocapsa* and *Geitlerinema* present at levels exceeding 10^5 cells ml^{-1}. High levels of these cyanobacteria were associated with high chlorophyll *a* and pH levels during the mid bloom period. Decreased dissolved oxygen in the mid bloom compared with the early bloom period indicated that heterotrophic metabolism, established following the dispersal of *Dolichospermum*, continues despite the proliferation of multiple cyanobacterial species. Covariance analysis showed that the Na concentration was an important associating factor in the cyanobacterial community composition, with *Dolichospermum* positively correlated to Na, whereas *Cuspidothrix*, *Geitlerinema* and *Sphaerospermopsis* were negatively correlated. Na levels and EC were only slightly higher in the summer bloom period (average [Na] = 28.2 p.p.m., average EC = 399 μS cm^{-1}) than in the autumn period (average [Na] = 18.9 p.p.m., average EC = 361 μS cm^{-1}). Although it is unlikely that Na concentration was sufficient to suppress the growth of any cyanobacterial species, several studies have demonstrated a link between ionic flux and toxin production (Pomati *et al.*, 2004; Wilhelm *et al.*, 2011; Carneiro *et al.*, 2013), presenting a credible route through which subtle changes in Na concentrations may influence, via secondary and tertiary mechanisms, the cyanobacterial and bacterial community composition (Wilhelm *et al.*, 2011; Mou *et al.*, 2013).

In the autumn period, patterns of covariance between cyanobacterial cell counts and bacterial OTUs were not distinct for each taxa. The observed bacterial community associated with *Aphanocapsa* and *Geitlerinema* was similar to that associated with *Phormidium* and *Dolichospermum*, being overrepresented by OTUs identified as unclassified bacteria and Bacteroidetes. However, the bacterial community associated with *Aphanocapsa* and *Geitlerinema* differed to that of the autumn period with a greater number of OTUs identified as belonging to the Verrucomicrobial orders Opitutales and Spartobacteriales. The Verrucomicrobia have previously been reported in cyanobacterial blooms and metagenomic analysis, suggesting that they possess numerous pathways for the assimilation of cyanobacterial extracellular polymeric substances, as well as exudates (Mou *et al.*, 2013; Bagatini *et al.*, 2014). The bacterial groups associated with the cyanobacteria *Microcystis*, *Oscillatoria*, *Sphaerospermopsis* and *Cuspidothrix* were overrepresented by particle-associated groups, including betI, acI-C, acIV-C (Allgaier *et al.*, 2007; Paver *et al.*, 2013; Bagatini *et al.*, 2014) and the methylotrophic taxon betIV, and Methylophiliaceae (Grossart *et al.*, 2011).

The winter period was defined by the absence of cyanobacteria, high levels of nitrate/nitrite, likely as a consequence of a lack of cyanobacterial nitrate reduction and an increased relative abundance of numerous acI(-A and B), alfV and betIV OTUs. Two OTUs annotated as *Luna1*-A and *Pnec* were positively correlated with variables from both the early bloom and postbloom period (Figure 5), suggesting that their abundance is more closely linked to the decomposition of cyanobacterial organic matter rather than the other postbloom-associated OTUs. Endemic lake members, including acI(A-B), betIV and alfV, were very strongly ($r < -0.94$) correlated with pH, which mirrors the findings of similar studies (Newton *et al.*, 2007). Cyanobacterial carbon fixation, which increases pH, may contribute to the observed seasonality of acI, particularly its decreased abundance between the late spring and early autumn maxima. AcI is incapable of metabolising carboxylic acids, the primary product of cyanobacterial carbon fixation (Ghylin *et al.*, 2014). However, their capacity to utilise the cyanobacterial pigment cyanophycin (Ghylin *et al.*, 2014) and the action of particle-

associated heterotrophic groups during the dispersal of the cyanobacterial biomass may ensure sufficient dissolved organic carbon for the rapid proliferation of both acI and alfV in the periods following a decline in cyanobacterial biovolume.

Conclusion

This study highlights the dynamic nature of freshwater microbial communities, during periods of elevated cyanobacteria cell numbers, with regard to both the cyanobacterial species composition and the composition of particle-associated and free-living lake bacteria. The proliferation of cyanobacteria in Yanga Lake was shown to enrich in a strain-specific manner for particle-associated, opportunistic heterotrophs. Despite the identification of microbial taxa, whose relative abundance within surface waters was substantially altered during periods of high cyanobacterial abundance and the evident strain-specific nature of this, additional research is required to identify the nature of the effects that specific particle-associated or free-living microbial have on the cyanobacteria and nutrient cycling in freshwaters. A continued effort to establish the dynamics of microbial organisms in freshwater systems, particularly with an emphasis of cyanobacteria blooms, over the mid and long term is necessary to determine the impact of rising surface water temperatures and increased eutrophication on nutrient cycling and the security of these critical resources.

Conflict of Interest

The authors declare no conflict of interest.

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