Received: 6 May 2022 | Revised: 30 August 2022 | Accepted: 12 September 2022

DOI: 10.1111/fwb.14002

ORIGINAL ARTICLE

Freshwater Biology WILEY

Chemotaxonomic responses of autotrophic periphyton communities to nutrient additions in pools of an intermittent stream

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Funding information
Australian Research Council, Grant/Award Number: LP120200002; Pilbara Corridors Biodiversity Scholarship

Abstract

1. The algal groups present in periphyton communities form an important base of autochthonous food webs in freshwater streams. Nitrogen (N) and phosphorus (P) are key macronutrients in aquatic systems. Excess nutrients benefit some algal groups over others.
2. We paired a nutrient-diffusing substrata limitation experiment with high performance liquid chromatography to (a) identify which nutrient(s) limit periphyton production, and (b) how the periphyton biomass and community structure changes between isolated pools of differing hydrological characteristics along an intermittent dryland stream.
3. Unique peaks for 21 pigments were identified and matched with published values. We then produced a PERMANOVA model using pigment ratios and CHEMTAX analysis to explore changes in community structure resulting from nutrient addition.
4. Periphyton communities in these pools were co-limited by N and P. Nitrogen additions caused the periphyton to shift from diatom- to chlorophyte-dominated community structure and benefited cyanophyta growth. Phosphorus additions reduced the relative proportion of diatoms and also resulted in an increase in pheophoribide-*a*, a pigment indicative of cell lysis, demonstrating a detrimental impact of P additions.
5. Outcomes of this study show that when adding nutrient to a system there may be subtle shifts in community composition which can be telescoped up the food web regardless of the system's nutrient status.

KEYWORDS
algae, intermittent rivers and ephemeral streams, nitrogen, nutrient limitation, phosphorus, pigment analysis

1 | INTRODUCTION

Periphyton are important contributors to primary productivity in aquatic systems. Periphyton communities—also termed "biofilm"—consist of a complex mixture of autotrophic organisms such as algae and cyanobacteria, as well as heterotrophic microorganisms, extracellular matrix, and detritus attached to surfaces in aquatic systems such as sediment, rocks, wood or macrophytes (Larned, 2010; Wetzel, 2001). The biomass and taxonomic composition of the autotrophic component of periphyton communities are shaped primarily by the energy inputs and nutrient status of the water body in which they reside (Townsend et al., 2012), and are thus highly responsive to nutrient enrichment (Fairchild et al., 1985; Tank et al., 2017; Tank & Dodds, 2003). Assessment of the periphyton algae community can provide insight into changes in environmental conditions, and has been used to indicate the onset of eutrophication (Gaiser et al., 2004) as well as to understand periphyton responses to change in hydrological status (Sabater et al., 2016; Townsend et al., 2012), light environment (Hill et al., 2010; Rier et al., 2014) and dissolved gases (Brown et al., 2017). Periphyton can be significant contributors to the autochthonous biomass stock in oligotrophic systems, partly as a consequence of the sediment surface acting as a hotspot for nutrient exchange (McClain et al., 2003), whereas overlying waters may be nutrient-impoverished. Periphyton has long been recognised for its important functional role in the retention of nutrients in aquatic ecosystems, especially of phosphorus (P) (Dodds, 2003; Reddy et al., 1999; Scinto & Reddy, 2003). Retention of nutrients by periphyton in shallow freshwater systems is further enhanced by the settling of nutrient-bearing particles, along with efficient uptake and recycling of nutrients between the autotrophic and heterotrophic component of periphyton (Dodds, 2003). Consequently, we might expect that periphyton would be an important nutrient recycler, and basal food source, in shallow pools of dryland streams. However, despite the ecological significance of periphyton in intermittent rivers and ephemeral streams (IRES) (Sabater et al., 2016), the potential changes in periphyton community structure resulting from shifts in nutrient availability or hydrological status of IRES remain largely unknown for the majority of inland watercourses.

Nutrient limitation studies have broadened our understanding of water column and benthic autotrophic production (Francoeur, 2001), and heterotrophic respiration (Burrows et al., 2015). Nutrient limitation also has been investigated utilising a range of approaches including whole lake fertilisation studies (Carpenter et al., 2001), mesocosm experiments (O'Brien & Dodds, 2007) and incubations of *in situ* nutrient diffusing substrates (NDSs; Capps et al., 2011; Fairchild et al., 1985; Tank & Dodds, 2003). Typically changes in biomass or chlorophyll-*a* (Chl-*a*) are measured to assess how primary producer growth may respond to nutrient addition (thus indicating limitation). However, if multiple algal species are present they may not respond uniformly to nutrient addition (e.g., nitrogen [N] vs. P). For example, freshwater cyanobacteria containing heterocysts have the ability to fix atmospheric nitrogen (N_2) under N starvation (Carey et al., 2012). Nitrogen-fixers would be expected to show little response to N additions yet may boom under elevated P (Cottingham et al., 2015). Consequently, studies that also examine shifts in community composition and abundance rather than purely total periphyton production may provide greater insight into overall nutrient limitation in any one system or time (Dalton et al., 2015; Townsend et al., 2012).

The majority of research on nutrient limitation in rivers and streams has focussed on measuring overall changes in autotrophic biomass via Chl-*a* and ash free dry mass (AFDM). Biochemical approaches such as pigment analysis (Tamm et al., 2015) and metagenomics (Bengtsson et al., 2018; Friesen et al., 2017) increasingly are being used to characterise the functional taxonomy of freshwater periphyton. Specifically, chemotaxonomy based on algal accessory pigments via high performance liquid chromatography (HPLC) is an alternative and straightforward method which is compatible with algal nutrient limitation experiments (Dalton et al., 2015). Conventionally NDS experiments measure Chl-*a* pigment as a response variable (Tank et al., 2017). With HPLC the experiment can expand to also measure the response of algal accessory pigments produced by certain taxa, and is capable of detecting algal taxa whose physical features are not well-retained in preservative. Chemotaxonomic analysis was developed and is extensively utilised in marine systems as a means to detect, characterise and monitor phytoplankton communities (Jeffrey et al., 1999; Wright et al., 1991), although this method would be likewise capable for periphyton. Individual pigments of interest may be isolated for analysis, or the relative abundance of algal taxonomic groups in the periphyton can be estimated by factor analysis from the calculated pigment ratios (CHEMTAX; Mackey et al., 1996).

Intermittent streams in arid regions often depend on groundwater sources for pools to persist beyond flood-flow events (Boulton & Hancock, 2006). Groundwater mixing and discharge into these pools via the hyporheic zone during these inter-flood periods thus is critical for maintaining stream productivity (Burrows et al., 2018), determines carbon and nutrient cycling in pools in IRES and helps maintain higher trophic levels (Fellman et al., 2011; Siebers et al., 2016). Pools that are not sufficiently supplemented by ground water will undergo evapo-concentration of solutes during prolonged drought periods with no surface flows (Fellman et al., 2011; Siebers et al., 2016). This difference in carbon and nutrient status among pools (and seasons) probably will alter both the biomass and composition of autotrophic periphyton communities and, in particular, may result in shifts in dominance of green algae versus cyanobacteria. For example, recent studies have revealed that P-iron (Fe) co-limitation can strongly limit N_2-fixing cyanobacteria in aquatic ecosystems even when P is abundant (Larson et al., 2018). Further, greater taxa richness and biomass of N_2-fixing organisms have been observed under treatments of P-Fe addition compared to treatments with only P addition (Larson et al., 2015). However, the relative responsiveness of different taxonomic groups to hydrochemical changes in streams with Fe-rich sediments, and to increased N and P availability, is unknown.

This study investigated the chemotaxonomic response of autotrophic periphyton to nutrient additions on NDS and how this response varied among pools of contrasting connectivity to the alluvial

ground water under field conditions. Firstly, Chl-*a* biomass was measured to identify the extent to which N and P availability limit periphyton production. Given that grazing on algae can influence overall periphyton responses to nutrient additions (Eckert & Carrick, 2014; Jones et al., 2000), we compared the biomass of periphyton on nutrient-enriched substrates between open and caged experiments. Secondly, photosynthetic and accessory pigments of the periphyton were quantified and Chl-*a*:pigment ratios were used to determine how autotrophic periphyton composition changed in response to nutrient additions and how this varied with hydrological characteristics. The expectation was that autotrophic biomass would be limited by both N and P, and that individual nutrient additions would favour particular periphyton groups, leading to distinct changes to community composition. We also expected that both community compositional changes and biomass responses to nutrient additions would be most apparent in the most hydrologically isolated pools.

2 | METHODS

2.1 | Site description

Coondiner Creek is an intermittent—and extremely ephemeral—dryland stream situated in the Upper Fortescue River catchment of the Pilbara region of northwestern Australia (Figure 1). Persistent and ephemeral pools (albeit ones generally recurring annually in the same position) are most concentrated within the semi-confined gorge section of the creek. Duration of water retention after cessation of surface flow is based on a combination of pool hydrological regime, aspect/position, and channel substrate. The following experiments were conducted at Coondiner Creek during a dry phase in July–August 2016. At this point in time surface flows had ceased ~18 months previously and the stream had retracted to a series of isolated pools along the main channel. Pools were classed by their hydrological status as either predominantly "persistent" or "ephemeral", based on previous studies of pool–alluvium connectivity within the study catchment (Fellman et al., 2011; Iles, 2019). Pool hydrological status was nevertheless confirmed for this study from water stable isotope ratios measured at the time of sampling.

2.2 | Nutrient limitation experiments

Nutrient diffusing substrate were constructed from 70-ml polypropylene containers (Sarstedt) with a glass fibre filter (Whatman GF/F) acting as a growth surface (Fairchild et al., 1985; Tank & Dodds, 2003). A round opening (ø42 mm) was cut from the cap to expose the growth substrate. The containers were filled with 2% agar solutions amended with 0.5 M NH_4NO_3 ("N" treatment), 0.5 M KH_2PO_4 ("P" treatment), 0.5 M NH_4NO_3 + 0.5 M KH_2PO_4 ("NP" treatment), or unamended ("C" control treatment). To test whether grazing by fish and macroinvertebrates had a significant impact on the biomass and composition of algal development on the NDS, the nutrient design was duplicated with matching NDS covered in 5-mm HDPE mesh. Five replicates of each nutrient treatment and grazing experiment were attached to wire racks with the growth surface face-up and positioned in either persistent or ephemeral pools. Samplers were left *in situ* to incubate for 28 days, during which time there was no surface flow. After 28 days, all samples were retrieved and the glass fibre filters removed. Filters and attached periphyton were placed immediately in 5-ml 90% acetone (AR grade; Chem-Supply), wrapped in foil and refrigerated for transportation back to the laboratory in Perth. The pigment acetone extract was filtered through a 0.22-µm nylon filter (Thermo Scientific) into 1-ml glass HPLC vials (Waters). Vials were capped and placed in a −80°C freezer until HPLC analysis was carried out. A further 2 ml of sample

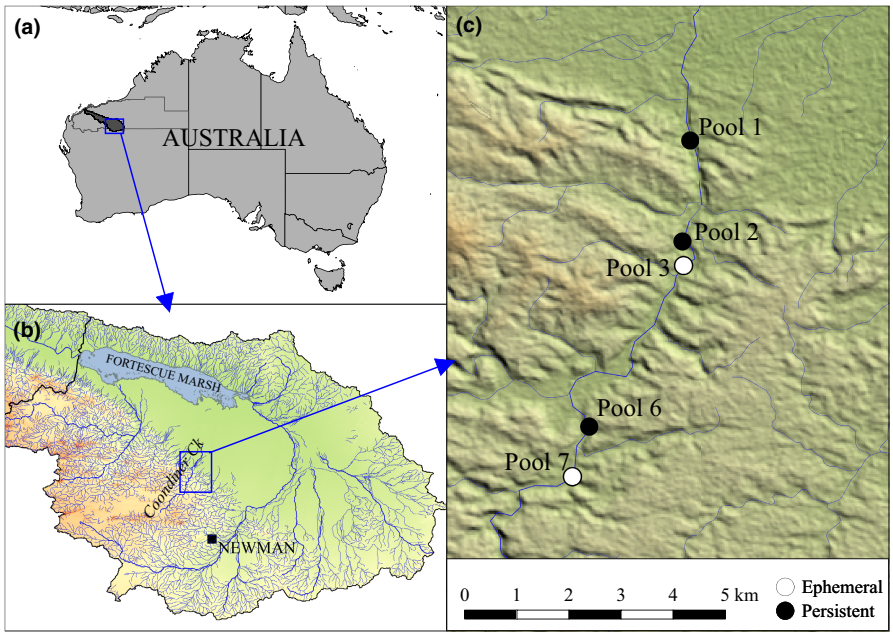

FIGURE 1 Location of study pools in Coondiner creek, Fortescue catchment, northwestern Australia. Symbols denote pools with "persistent" (black circle) and "ephemeral" (white circle) hydrological status. Flows had ceased and all pools were isolated from each other at the time of the experiment.

was filtered and diluted to 20 ml in 90% acetone for fluorometric determination of Chl-*a*. Sample extracts were measured on a Trilogy fluorometer (Turner Designs) using the non-acidification method (EPA 445.0: Arar & Collins, 1997).

2.3 | HPLC pigment analysis

A subset of NDS filters were selected from the nutrient limitation experiment for pigment analysis via HPLC. The 60-sample subset consisted of three replicates of each nutrient treatment per site from the grazed treatment. A mix of standards and reference materials was used to build up a pigment library and to calibrate pigments extracted from the samples. A mixed phytoplankton standard PPS-MIX-119 (DHI Group) also was injected once for each 10 samples in order to evaluate drift in retention time throughout the experiment. Peak areas were calibrated against a Chl-*a* reference standard (DHI Group). Algal reference material extracted from pure cultures of *Dunaliella tertiolecta* (chlorophyte), *Tetraselmis suecica* (chlorophyte), *Chaetoceros muelleri* (bacillariophyte) and *Tisochrysis lutea* (haptophyte) also were run. Method blanks of 90% acetone were processed identically to samples and passed through the entire extraction process.

Pigments were quantified on a Waters HPLC system (600 controller, 217 autosampler; Waters) with a reverse-phase C18 column (Spherisorb ODS2, 250 mm × 4.6 mm, 5-μm particle retention). Our solvents and elution scheme were modified from Tamm et al. (2015). Solvent A consisted of 80% methanol: 20% 0.5 M ammonium acetate (pH 7.2) (v/v). Solvent B consisted of 80% methanol:20% acetone (v/v). The elution scheme consisted of solvents A and B initially in a 50:50 mixture, switched to 100% solvent B at 30 min, then returned to the 50:50 mixture at 50 min. Column flow rate was set at 0.7 ml/min and column temperature was set at 22°C for the duration of the experiment. Peaks were detected with a 996 photodiode array (PDA) detector with scanning range 310–750 nm at a resolution of 1.2 nm. PDA peaks were integrated at a quantification wavelength of 450 nm. Eluent then flowed through a 470 scanning fluorescence detector (excitation: 440 nm, emission detection: 660 nm). Chromatographs were processed in EMPOWER2 software. Peaks were identified by comparison with standard reference material, and documented peak retention times and absorbance characteristics––elution order and peak shape (Tamm et al., 2015; Wright et al., 1991). Peaks then were integrated and peak area obtained using the software.

2.4 | Stream pool hydrochemistry

Water samples were collected at the beginning and end of the incubation period and analysed for nutrients (TDN, total dissolved nitrogen; DIN, dissolved inorganic nitrogen [as $NO_3 + NH_4$]; DON, dissolved organic nitrogen [calculated as the difference between TDN and DIN]; SRP, soluble reactive phosphorus), carbon (DOC, dissolved organic carbon; DIC, dissolved inorganic carbon; $SUVA_{254}$, specific ultraviolet absorbance at 254 nm), and stable isotopes of water (δ^2H, $\delta^{18}O$) and dissolved inorganic carbon ($\delta^{13}C_{DIC}$). Water samples for nutrient and carbon analysis were filtered through a sterile syringe filter (Sartorius minisart 0.45-μm). $\delta^{13}C_{DIC}$, δ^2H and $\delta^{18}O$ isotope samples were filtered through a sterile syringe filter (PALL 0.2-μm Supor) into a glass vial ensuring that all headspace was removed. Samples were refrigerated immediately (4°C) in the field for transport back to the laboratory for analysis. DOC and TDN were measured simultaneously on a Shimadzu TOC-V analyser coupled with a total nitrogen module (Shimadzu TNM-1). Ultraviolet absorbance at 254 nm was measured on a UV–visible spectrophotometer (Cary 50; Varian Medical Systems, Inc.). Specific ultraviolet absorbance ($SUVA_{254}$) was calculated using absorbance at 254 nm and DOC concentration as an estimation of dissolved aromatic carbon content (Weishaar et al., 2003). Dissolved nitrate (NO_3) and ammonia (NH_4) were determined by spectrophotometric colorimetric detection method on a Technicon Autoanalyser (Technicon). Soluble reactive phosphorus (SRP) was measured spectrophotometrically by the modified ascorbic acid method (Kuo, 1996; Murphy & Riley, 1962).

Water isotope samples were measured on a cavity ring-down spectrometer (Piccaro) following the analytical method outlined in Skrzypek and Ford (2014). All δ^2H and $\delta^{18}O$ values are given in per mil [‰ VSMOW] according to delta notation (Coplen, 1996). The evaporative loss fraction of the pool volume (*f*) over the duration of the experiment was calculated for each pool following Skrzypek et al. (2015), which is based on a revised Craig–Gordon model (Craig & Gordon, 1965). A non-steady-state model was selected for all pools as pool volume decreased in all pools during the experiment. The stable isotope composition of the moisture in the ambient air (δ_A) was calculated using the isotopic composition of the most recent large precipitation event proceeding the sampling period and slope of the local evaporation line (LEL). The DIC and isotopic ratios ($\delta^{13}C_{DIC}$) were measured on a Thermo Delta XL IRMS with Gasbench II (Thermo Fisher Scientific). All $\delta^{13}C_{DIC}$ values are given in per mil [‰ VPDB].

2.5 | Data analyses

Statistical procedures were conducted in R (R Core Team, 2017) and PRIMER 6 & PERMANOVA+ (Primer-E Ltd). Pigments were quantified into major algal groups following the CHEMTAX method (Mackey et al., 1996) with the limSolve package in R (Soetaert et al., 2009). Initial pigment ratios for determining algal groups were sourced from freshwater studies in the literature (Dalton et al., 2015; Sarmento & Descy, 2008; Schlüter et al., 2006; Tamm et al., 2015), and multiple starts were performed to ensure model convergence. Differences between nutrient and hydrological factors were assessed using a permutational multivariate analysis of variance (PERMANOVA) model (Anderson, 2001) for the response variables of (a) Chl-*a* biomass (μg/cm^2), (b) accessory pigment biomass (μg/cm^2) for each individual peak detected, and (c) proportional taxonomic groups derived from CHEMTAX analysis of Chl-*a*:pigment ratios.

TABLE 1 Characteristics of study pools along Coondiner Creek at initial and final samplings of periphyton incubation.

Hydrological status	Site	Sampling	TDN (mg/L)	DIN (mg/L)	DON (mg/L)	SRP (μg/L)	DOC (mg/L)	$SUVA_{254}$ (L mg C^{-1} m^{-1})	DIC (mg/L)	$\delta^{13}C_{DIC}$ (‰)	$\delta^{2}H$ (‰)	$\delta^{18}O$ (‰)	*f*	
Persistent	Pool 1	Initial	0.11 ± 0.02	0.03 ± 0.03	0.08 ± 0.03	1.92 ± 0.23	1.81 ± 0.21	2.23	66.17	−12.19	−48.8	−6.93		
		Final	0.09 ± 0.01	0.01 ± 0.01	0.08 ± 0.01	1.57 ± 0.98	2.09 ± 0.62	1.06	69.90	−12.11	−48.6	−6.74	0.02	
	Pool 2	Initial	0.16 ± 0.09	0.08 ± 0.11	0.08 ± 0.03	2.15 ± 0.73	2.01 ± 0.56	2.87	69.98	−12.05	−46.8	−6.85		
		Final	0.11 ± 0.01	0.03 ± 0.04	0.08 ± 0.05	1.69 ± 0.48	2.54 ± 0.45	1.54	66.02	−10.39	−44.3	−6.58	0.03	
	Pool 6	Initial	0.07 ± 0.00	0.01 ± 0.01	0.06 ± 0.01	1.66 ± 1.32	1.30 ± 0.11	1.85	56.46	−12.19	−41.1	−6.27		
		Final	0.07 ± 0.01	0.02 ± 0.02	0.05 ± 0.02	2.21 ± 0.28	1.41 ± 0.26	1.23	56.20	−12.15	−44.0	−5.99	0.04	
Ephemeral	Pool 3	Initial	0.16 ± 0.01	0.01 ± 0.02	0.14 ± 0.01	2.09 ± 0.97	3.39 ± 0.11	2.48	54.85	−11.25	−35.2	−3.82		
		Final	0.15 ± 0.01	0.02 ± 0.02	0.13 ± 0.01	2.53 ± 0.49	3.59 ± 0.23	1.99	56.20	−10.65	−30	−3.15	0.24	
	Pool 7	Initial	0.18 ± 0.11	0.04 ± 0.02	0.15 ± 0.09	1.05 ± 0.63	2.13 ± 0.27	2.07	43.63	−11.39	−26	−3.18		
		Final	0.11 ± 0.01	0.03 ± 0.01	0.08 ± 0.00	2.04 ± 0.10	2.48 ± 0.39	1.63	45.57	−10.74	−26.9	−2.78	0.21	

Note: Dissolved inorganic nitrogen (DIN) as NO_3 + NH_4; stable isotopes of filtered water samples ($\delta^{13}C_{DIC}$, $\delta^{2}H$ and $\delta^{18}O$); and pool evaporative loss (*f*). Values reported are mean ± standard deviation ($n = 3$).
Abbreviations: DOC, dissolved organic carbon; DON, dissolved organic nitrogen; SRP, soluble reactive phosphorus; $SUVA_{254}$, specific absorbance at 254 nm; TDN, total dissolved nitrogen.

Univariate PERMANOVA of Chl-*a* biomass was performed on a euclidean distance matrix produced from log(x + 1) transformed values. The three-factor model had a crossed design with pool hydrological status (random: persistent vs. ephemeral), grazing (fixed: grazed vs. ungrazed) and nutrient addition (fixed: C, N, P, NP) as factors. Multivariate PERMANOVAs of accessory pigment biomass and proportional taxonomic groups were performed on a Bray–Curtis similarity matrix of log(x + 1) transformed values. The two-factor mixed effects model was designed with pool hydrological status (random) and nutrient treatment (fixed) as the factors. Each PERMANOVA model was run for 999 permutations with Type I (sequential) sum of squares. We report permutation *p*-values at a significance level of $\alpha = 0.05$. Multivariate data were visualised using the distance based linear model DistLM procedure to produce dbRDA plots. Two-way ANOVA were performed to compare pool water nutrient and C concentrations between pool hydrological status (persistent vs. ephemeral pools) and time of sampling (initial and final).

3 | RESULTS

3.1 | Stream pool nutrient status and hydrological characteristics

The proportion of pool volume evaporative loss (*f*) ranged from 0.02 to 0.04 for persistent pools, whereas *f* ranged from 0.21 to 0.24 for "ephemeral" pools, showing that overall volumes of persistent pools over the 28 day experimental period remained relatively constant, whereas ephemeral pools lost up to one quarter of their volume over the same period (Table 1). TDN ranged between 0.07 and 0.18 mg/L and the concentration was significantly higher in ephemeral pools than persistent pools (ANOVA: $F_{1,26} = 8.01$, $p = 0.009$), whereas there was no significant change in TDN concentration over the course of the experiment. The bulk of dissolved nitrogen was in the form of DON (median = 83% of TDN), whereas DIN concentrations ranged from 0.01 to 0.08 mg/L and were similar across pools. SRP ranged between 1 and 2 μg/L with similar concentrations across both persistent and ephemeral pools and did not change significantly over the experimental period (Table 1). DOC ranged between 1.30 and 3.59 mg/L and the concentration was significantly higher in ephemeral pools than persistent pools (ANOVA: $F_{1,26} = 20.91$, $p < 0.001$), whereas there was no significant change in DOC concentration over the course of the experiment.

3.2 | Periphyton biomass response to nutrient additions

The Chl-*a* biomass ranged from 0.4 to 38.5 μg/cm^2 across all treatments at the end of the 28 day NDS experiment (Figure 2). Grazing exclusion did not significantly affect Chl-*a* biomass (Pseudo-$F = 1.61$, p [perm] = 0.139). There also was no significant interaction between grazing and nutrient treatments (Pseudo-F = 0.844, p

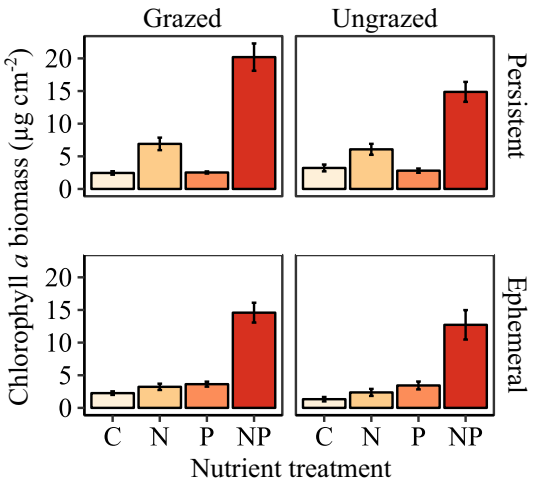

FIGURE 2 Periphyton chlorophyll-*a* response to nutrient additions in "persistent" and "ephemeral" pools. Nutrients added to substrates were nitrogen (N) as NH_4NO_3, phosphorus (P) as KH_2PO_4, and nitrogen + phosphorus (NP). The control (C) received no nutrient additions. The experiment was duplicated with "grazed" and "ungrazed" NDS treatments.

TABLE 2 Peak identification table of pigments identified in mixed standard and periphyton samples.

Peak no.	Pigment	Retention time (min)	Wavelength maxima (nm)		
1	Chlorophyllide *a*	6.36	459	590	668
2	Chlorophyllide *b*	7.64	453	598	645
3	Chlorophyll *c2*	9.58	449	584	635
4	Peridinin	11.11			476
5	19′-But-fucoxanthin	12.51		454	471
6	Fucoxanthin	15.26		445	470
7	Pheophorbide *a*	16.58	410	505	535
8	Neoxanthin	18.35	413	437	467
9	Prasinoaxanthin	19.94		454	471
10	Violaxanthin	22.46	416	442	470
11	Pheophorbide *a*-like	23.50	410	505	535
12	Diadinoxanthin	26.15	420	445	478
13	Antheraxanthin	27.29		446	471
14	Alloanthin	29.38		454	481
15	Diatoxanthin	30.51		453	481
16	Lutein	30.80	420	446	473
17	Zeaxanthin	31.55		453	479
18	Chlorophyll *b*	34.52	472	601	650
19	Chlorophyll *a*	38.93	410	433	666
20	α-Carotene	49.20		441	469
21	β,β-Carotene	50.70		447	475

[perm] = 0.572). The three-factor PERMANOVA model showed that there was a significant difference in how the periphyton biomass responded to nutrient availability between persistent and ephemeral pools (Pseudo-F = 20.338, p [perm] = 0.001), with a significant interaction between hydrological status and nutrient treatment (Pseudo-F = 9.7, p [perm] = 0.001). In both persistent and ephemeral pools, simultaneous N and P additions increased algal biomass by more than three-fold compared to the control (Figure 2). Biomass also more than doubled in persistent pools in response to N alone (Figure 2). All other treatments combinations showed no significant effect on periphyton biomass.

3.3 | Chemotaxanomic response of autotrophic periphyton

The identity of 21 chlorophyll and accessory pigments were determined in the periphyton samples collected from the different substrates (Table 2). Peak separation was achieved for all of the main pigments of interest with the exception of diatoxanthin, which eluted within the broad peak base formed when lutein was present in high concentrations (peaks 15 and 16; Figure 3a). Hence, we excluded diatoxanthin from further analysis. Typical raw chromatograms from the sample set are shown in Figure 3b.

Changes to community structure were evident in shifts in the biomass of accessory pigments such as fucoxanthin, peridinin and lutein. The periphyton community structure responded to nutrient availability differently between pools of persistent and ephemeral hydrological status (Pseudo-F = 7.55, p [perm] = 0.001) (Table 3a). The variation in response among pools and between hydrological status to different nutrients can be observed on axis-1 of the dbRDA plot, with more negative values corresponding to higher relative biomass (Figure 4a). Axis-1 explains 63.7% of the variation in the fitted model. Many of the major pigments also align with axis-1 and are highly correlated (e.g., Chl-*a*, Chl-*b*, Lutein, Fucoxanthin). Axis-2 accounts for 16.9% of the variation of the fitted model and distinguishes ephemeral pools from persistent pools by a greater response to the P treatment. This axis illustrates an increase in peridinin and diadinoxanthin pigments, which are indicative of a higher proportion of dinoflagellates in ephemeral pools, especially with P treatment.

Periphyton pigment compositions are considered representative of changes in major taxonomic groups. Based on the CHEMTAX approach, communities colonising the control NDS (no nutrient addition) were estimated to consist of 60% diatoms, 13%–20% chlorophytes, 7%–12% euglenophytes, 9% cyanobacteria and 7% dinoflagellates in both ephemeral and persistent pools (Figure 5). However, at the end of the experiment the nutrient treatments differed in their periphyton community structure between pool hydrological status (Pseudo-F = 2.55, p [perm] = 0.030). Relative to the control, P treatments in persistent pools had a decrease in the proportion of diatoms (P: 37%, NP: 45%; F = 10.86, p = 0.002) and euglenophytes (P: 3%, NP: 1%; F = 39.09, p < 0.001), and an increase in the proportion of chlorophytes (P: 37%, NP: 38%; F = 28.98, p < 0.001). There was no change in the proportion of dinoflagellates. In contrast, P treatments in ephemeral pools showed decreased proportions of diatoms (P: 18%, NP: 22%; F = 32.95, p < 0.001), and an increase in dinoflagellates (P: 44%, NP: 36%;

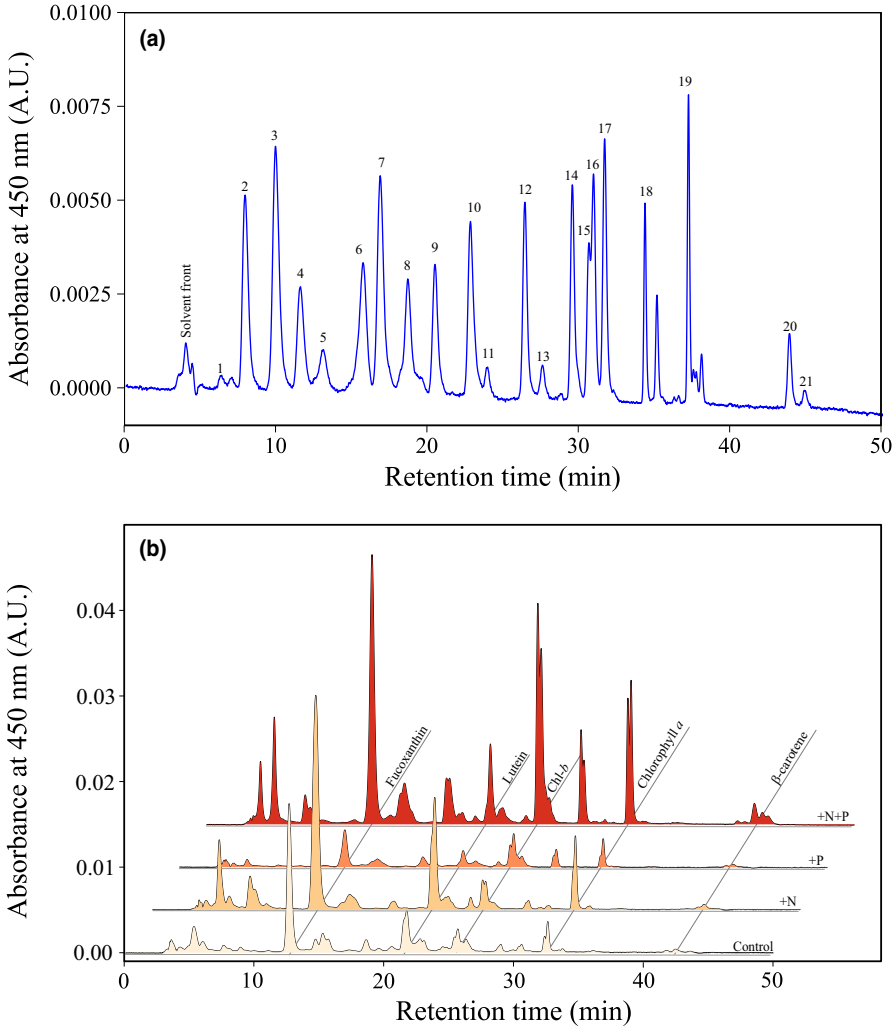

FIGURE 3 HPLC chromatograms showing (a) standard pigment mix, peak numbers correspond with those in Table 2, and (b) a typical HPLC chromatogram from a persistent pool showing control (C), nitrogen (N), phosphorus (P), and nitrogen + phosphorus (NP) treatments. Absorbance was measured at 450 nm.

$F = 13.92$, $p = 0.001$) relative to the control, but no change in the proportion of chlorophytes (Figure 5). The community structure of N treatments in persistent pools was similar to the control, although the proportion of cyanobacteria was reduced ($F = 12.54$, $p = 0.001$). By contrast, in ephemeral pools, the proportion of dinoflagellates (N: 44%, NP: 36%) and euglenophytes (N: 14%) all increased when N was added either alone or with P.

The periphyton community responded significantly differently between pools of persistent and ephemeral hydrological status based on CHEMTAX analysis (Pseudo-$F = 14.767$, p [perm] = 0.001; Table 3b). For both persistent and ephemeral pools we observed a shift away from a diatom-dominated periphyton community when nutrients were added. For persistent pools we observed a shift from diatoms to chlorophyta, whereas in ephemeral pools there was a shift towards a dinoflagellate-dominated periphyton community. These results are graphically illustrated on the dbRDA plot with diatoms, chlorophytes and dinoflagellates separated out strongly (Figure 4b). Axis-1 aligns with communities being chlorophyte- or diatom-dominated and explained 58.4% of the variation of the fitted model. Axis-2 of dbRDA plot aligns with an increase in the proportion of dinoflagellates and explained 35% of the variation of the fitted model.

4 | DISCUSSION

Our results demonstrate that autotrophic periphyton productivity in Coondiner Creek was primarily N and P co-limited at the time the experiment was conducted. This outcome was regardless of pool hydrological status, with both persistent and ephemeral pools displaying N and P co-limitation, persistent pools also displayed secondary N limitation. Nonetheless we observed subtle differences in Chl-*a* biomass response and clear shifts in periphyton community structure among pools of differing hydrological status. The taxonomic response of periphyton to nutrient additions varied considerably with hydrological status: in general, "persistent pools shifted towards a chlorophyta-dominated community, whereas ephemeral pools shifted towards a dinoflagellate-dominated community. Control treatments which did not receive N or P additions generally were diatom-dominated. This study builds on our understanding of how individual taxonomic groups comprising freshwater periphyton communities respond to changes in nutrient availability and provides new information of the biogeochemical processes shaping stream communities in northwestern Australia and elsewhere. Few freshwater algae studies have been conducted in the Pilbara (Masini, 1988,

TABLE 3 Factorial two-way mixed effects PERMANOVA of (a) periphyton pigment biomass (μg/cm^2), and (b) estimates of algal group contributions from CHEMTAX analysis of Chl-*a*:pigment ratios.

Source	*df*	SS	MS	Pseudo-*F*	*p* (perm)	Unique perms	*p* (M.C.)
(a) Pigment biomass							
Hydrological status	1	1,556.6	1,556.6	7.5501	**0.001**	999	**0.001**
Nutrient	3	10,122	3,373.9	4.2535	0.065	738	**0.016**
Hyd. × Nut.	3	2,207.8	735.93	3.5694	**0.001**	998	**0.001**
Residuals	52	10,721	206.17				
Total	59	24,607					
(b) Comparison of estimates of algal group contributions from CHEMTAX analysis							
Hydrological status	1	7,112.2	7,112.2	14.767	**0.001**	999	**0.001**
Nutrient	3	12,332	4,110.7	3.1159	0.082	769	0.116
Hyd. × Nut.	3	3,689	1,229.7	2.5532	**0.030**	998	**0.037**
Residuals	52	25,045	481.63				
Total	59	48,178					

Note: Pool hydrological status and nutrient treatment are included as factors. Significant *p*-values are indicated in bold.

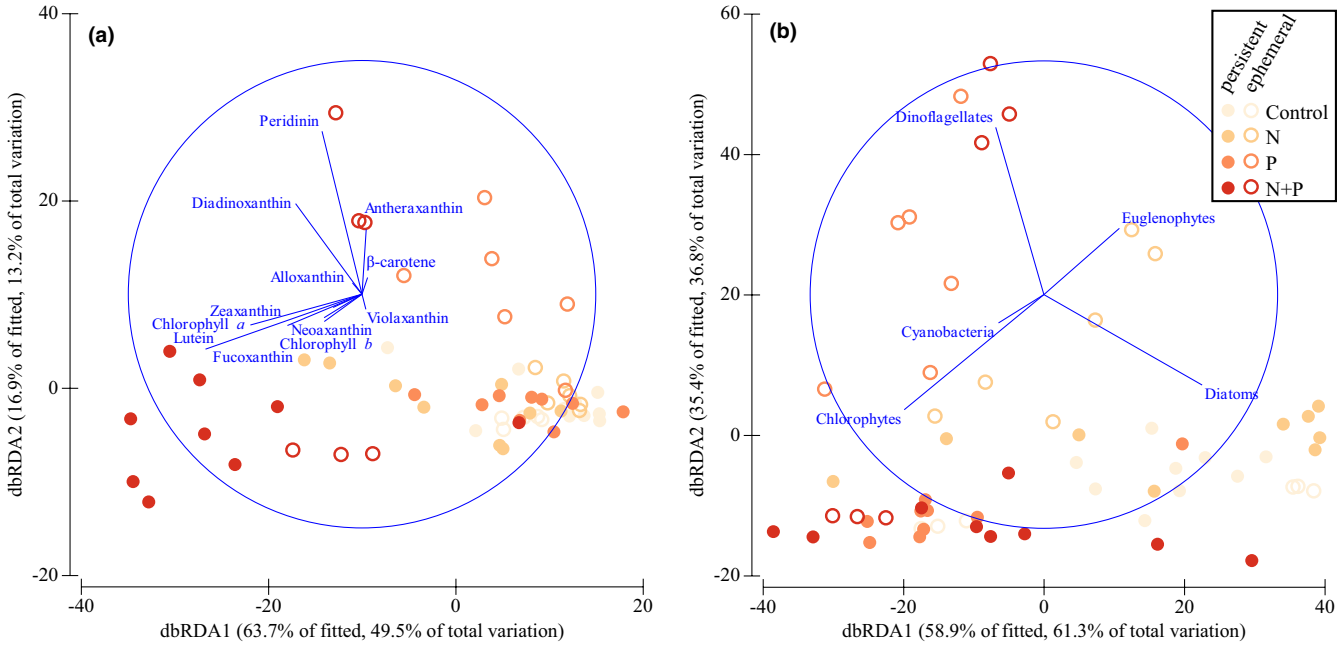

FIGURE 4 Multidimensional dbRDA plots of pigments extracts from the periphyton NDS experiment: (a) pigment biomass (μg/cm^2) and (b) estimates of algal group proportions by CHEMTAX analysis. Results are based on a Bray–Curtis similarity matrix of log(x + 1) transformed samples (n = 60).

1989; McIntyre, 2009), with this study being the first to directly test nutrient limitation on stream ecosystems in this remote region.

Periphyton production (Chl-*a* biomass) was co-limited by N and P. The combination effect of adding both N and P causes a synergistic response in autotrophic production. The responses observed in the pools investigated in the present study are consistent with widespread co-limitation by N and P that has been observed across a broad range of aquatic systems (Elser et al., 2007; Francoeur, 2001). In Coondiner Creek, a relatively pristine environment, periphyton communities are most probably highly adapted to scavenging nutrients that may only be episodically available via increased uptake efficiencies and nutrient recycling between autotrophic and heterotrophic components (Scinto & Reddy, 2003).

Much of the pioneering research on biogeochemical processes in intermittent streams focused on nitrogen limited arid systems, such as Sycamore Creek in Arizona (Grimm et al., 1981; Grimm & Fisher, 1986). These classical streams are fed by seasonal snowmelt, are frequently flow-regulated by lock or flood controls, and therefore have a comparatively predictable hydrological status. Less is understood of the relative importance of N versus P

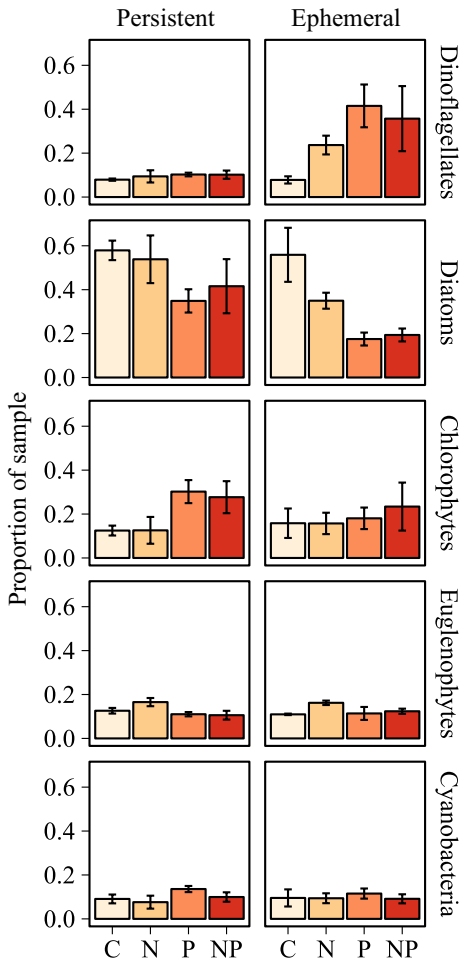

FIGURE 5 Estimates of algal group contributions to periphyton community structure calculated from Monte Carlo perturbations of CHEMTAX analysis. Nutrients added to substrates were nitrogen (N) as NH_4NO_3, phosphorus (P) as KH_2PO_4, and nitrogen + phosphorus (NP). The control (C) received no nutrient additions. Mean proportion of each group per nutrient and hydrological treatment is shown with standard error ($n = 3$).

availability in systems broadly described as "intermittent rivers and ephemeral streams (IRES)" that occur in largely unmodified catchments, receive very episodic flows, and where the surrounding catchments are dominated by nutrient-poor soils. Factors controlling stream productivity in intermittent streams remain largely undescribed. However, expanding our understanding of metabolic processes in IRES is considered fundamentally important for the future management of freshwater systems given that there is a global trend of increasing stream intermittency (Acuña et al., 2017). The Pilbara region where this study was located in many ways exemplifies these global processes, with changing spatial and temporal patterns in rainfall, and changed land use (Cullen & Grierson, 2007; O'Donnell et al., 2015; Rouillard et al., 2015, 2016).

An interesting finding of this study was that P was the primary nutrient responsible for shifts in periphyton community structure. Other periphyton limitation studies have indicated that although P addition may promote algal biomass, it does not generally result in a change in periphyton community structure in freshwater streams (Dalton et al., 2015; DeNicola & Lellock, 2015; Vizza et al., 2018). Within Australia, Townsend et al. (2012) found no change in autotrophic periphyton community structure in a tropical stream between control and nutrient additions. However, nutrient concentrations in the Townsend et al. (2012) study were only marginally above ambient conditions. In an alpine stream, pulses of P were found to result in a reduction in periphyton species diversity (Davies & Bothwell, 2012). Hence, in some instances the source and duration of phosphorus enrichment to periphyton, rather than the P concentration *per se* may be more controlling of community structure. Another explanation as to why P more strongly altered periphyton community structure lies in the strongly P-limited system that we are investigating. Streams in the Pilbara region are highly P-limited as a result of (a) the underlying geology which is depauperate in P and highly weathered (Arndt et al., 2007; Kranendonk et al., 2002), and (b) geochemical constraints where stream sediments are extremely Fe-rich (up to ~70% Fe in the form of goethite and haematite). As P is rapidly adsorbed to surface sites on Fe minerals, it becomes less available for algal uptake (Iles et al., 2022). Both introducing excess P via the NDS, and presenting a novel growth surface free of Fe (agar and glass fibre paper), may be indirectly altering the P response. This is a known limitation of NDS studies, where emulating natural growth surfaces are not fully achievable. A recent review on P limitation experiments highlighted that the choice of cation or phosphate form may cause growth inhibition (Beck & Hall, 2018). In that study the Chl-*a* concentration of P and N + P treatments was always higher than the control. Hence, while we did not encounter gross P inhabitation, there may have been some unequal effect on individual algal groups which potentially explains why we observed a change in algal community structure in response to excess P. How individual algal groups or species respond to a researcher's choice in cation and P form used in NDS studies would be worthy of further investigation.

Not all algal taxa responded evenly to nutrient additions in our study, a finding which would be overlooked by classical gross biomass studies. Overall response to N additions was comparatively lower in ephemeral compared to persistent pools, with Chl-*a* biomass in the N treatment for ephemeral pools being similar to the control treatment. Interestingly, the surface water in ephemeral pools had higher TDN concentrations—and presumably N availability—but the response to N additions by some algal taxa was stronger in these pools. We found that adding inorganic N and/or P to ephemeral pools favoured the relative growth of dinoflagellates, whereas adding P to persistent pools favoured relative growth of chlorophytes. In both cases the change in periphyton community composition resulting from nutrient additions was at the expense of diatoms. The majority of pool water TDN consisted of organic N, with very low DIN concentrations across all pools at the start and end of the NDS experiment. Only very low concentrations of bioavailable inorganic N generally are present within this oligotrophic creek system (Siebers et al., 2016). The higher TDN measured in ephemeral pools probably was due to higher evapoconcentration of pool water

(Datry et al., 2018) and accumulation of recalcitrant organic matter, rather than an increase in DIN. This is also demonstrated by increasing DOC concentrations and decreasing $SUVA_{254}$ values measured in the pools over the duration of the experiment. We suspect that bioavailable inorganic N (i.e., DIN)—and inorganic P—are rapidly utilised by autotrophs when they do become available in the system.

This *in situ* study showed little impact of grazing on periphyton biomass over 28 days, which was a surprising finding. It was assumed before deploying the NDS experiment that grazing by fish and macroinvertebrates would have a negative effect on the periphyton biomass (Hill et al., 2010; Hillebrand & Kahlert, 2001). We observed fish schools within all pools when selecting sites for this study and as isolated pools act as refuge for native fish populations between flow events (Beesley & Prince, 2010; Lostrom et al., 2015; Morgan & Gill, 2004), predation pressure by fish such as Rainbowfish (*Melanotaenia australis*) presumably would increase as the pools contract, thus reducing grazing effects of macroinvertebrates on periphyton. Alternatively, the mesh may have protected small (<5 mm) grazers from fish predation, and excluded fish from directly grazing the periphyton themselves. These conclusions around grazing are tentative as we did not directly measure grazing pressure via invertebrate and fish counts or observational studies. Nonetheless, it would be of interest for future studies to address this more formally. Shading is well known to be a limiting factor in periphyton production (Guo et al., 2016; Hill et al., 2009; Von Schiller et al., 2007). The mesh used in this study blocked ~5% light onto the GF/F. Hence, we assume that the shading effect would be minimal and possibly insignificant.

This study demonstrates that chemotaxonomic analysis is an effective method for assessing changes in periphyton community structure. Chemotaxonomic analysis may be a relatively inexpensive and straightforward approach for monitoring responses to environmental change, such as altered flows or nitrate inputs from mining discharge (Degnan et al., 2016; Dogramaci et al., 2015), increased concentrations owing to reduced flows (Bestland et al., 2017; Siebers et al., 2016), increased inputs from dust deposition from fertiliser applications to surrounding catchments, and N and P from cattle (McDowell & Stewart, 2005; Pettit et al., 2012). Overall, these results demonstrate that periphyton biomass in Pilbara streams is sensitive to both N and P inputs. Surface and groundwater runoff of N, along with atmospheric deposition are increasing in northwestern Australia due to industrial activities (fertiliser production, disturbance and airborne dust from resource extraction) and agricultural (nutrient-supplemented irrigation schemes, rangeland grazing) sources. Hence, these new N sources have the potential to increase rates of periphyton production in Pilbara streams, which in turn may affect higher trophic levels.

Productivity and biogeochemical processes in these streams also may vary over the wet–dry hydrological cycle (time-scales of ~seasons to years). It is likely also that the system fluctuates between primarily N or P limitation through the seasons and the streams' natural hydrological phases (Francoeur et al., 1999; Reisinger et al., 2016). Whilst characterisation and identification of biogeochemical processes during this "dry" part of the hydrological phase is important, we are missing the most energetic and dynamic period within these systems. How nutrients and carbon are transported and processed during flood-flow events has not been characterised in this study. However, these first flows are important releases of remineralised nutrients and carbon upon rewetting of sediments (Baldwin & Mitchell, 2000). Consequently, further investigations which capture the distinct hydrological phases that distinguish IRES from perennial systems may provide further insights into stream nutrient processes.

Although it is a region of extreme climate variability, the Pilbara region has showed noticeable wettening in recent decades compared to previous centuries (Cullen & Grierson, 2007; O'Donnell et al., 2015; Rouillard et al., 2015, 2016). Future climate modelling scenarios predict increased air temperature, with the delivery of rainfall also projected to change, with a reduction in frequency, but increase in intensity, of tropical cyclones (Charles et al., 2015; Sudmeyer, 2016). Importantly, potential evaporation also is projected to increase (Charles et al., 2015), which will increase the rainfall deficit, and directly affect surface waters. Hence, the extent of stream surface water throughout the region may be expected to contract and become fragmented more rapidly after flow cessation—a key process in IRES. Future climates may alter both the extent and duration of surface water, and the evapo-concentration of nutrients, increasing nutrient retention in isolated stream pools (McLaughlin, 2008). For streams where evaporation already plays a critical role in shaping the differences between persistent and ephemeral pools along these streams will increase. Spring-fed persistent and the arguably more vulnerable ephemeral pools across the region both have unique character and will require complementary approaches to enable each to be managed sustainably. Understanding and managing impacts against a background of extreme variability remains a challenge.

AUTHOR CONTRIBUTIONS

Conceptualisation: JAI, NEP, PFG. Developing methods: JAI, GC. Data analysis: JAI, GC. Preparation of figures and tables: JAI. Conducting the research, data interpretation, writing the manuscript: JAI, NEP, GC, PFG.

ACKNOWLEDGMENTS

We acknowledge the Traditional Owners, the Nyiyaparli, of the lands which this study was conducted, and would also like to pay our respects to Elders past and present. Andre Siebers and Doug Ford (UWA) assisted in the field. Michael Donn (CSIRO), Grzegroz Skrzypek, Kate Bowler, Caroline Mather, Jen Middleton and Ela Skrzypek (UWA) assisted with analytical procedures. Open access publishing facilitated by James Cook University, as part of the Wiley - James Cook University agreement via the Council of Australian University Librarians.

FUNDING INFORMATION

This research was funded by ARC linkage grant LP120200002 (RTIO) and Pilbara Corridors Biodiversity Scholarship (RangelandsNRM).

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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How to cite this article: Iles, J. A., Pettit, N. E., Cawthray, G., & Grierson, P. F. (2022). Chemotaxonomic responses of autotrophic periphyton communities to nutrient additions in pools of an intermittent stream. *Freshwater Biology*, *67*, 2148–2160. https://doi.org/10.1111/fwb.14002