Limnology (2012) 13:131–141
DOI 10.1007/s10201-011-0367-z

RESEARCH PAPER

Effect of N and P enrichment on periphytic algal community succession in a tropical oligotrophic reservoir

Carla Ferragut · Denise de Campos Bicudo

Received: 5 July 2010 / Accepted: 7 November 2011 / Published online: 7 December 2011

Abstract This enrichment experiment was conducted to evaluate how nutrient availability drives colonization and succession of a periphytic algal community in a Brazilian tropical oligotrophic reservoir. Four treatments were designed using enclosures ($n = 3$): control (no nutrient addition), P+ (isolated phosphorus addition, N-limiting condition); N+ (isolated nitrogen addition, P-limiting condition), and NP+ (phosphorus and nitrogen combined addition, no limitation). Glass microscope slides were used for periphyton growth. Samplings were carried out at short, regular intervals (3–5 days) over 31 days. Isolated P addition promoted the highest structural organization, and both NP+ and P+ promoted the highest biomass accrual. Control condition favored *Chromulina elegans* (chrysophyte) dominance, whereas enrichment favored different species descriptors belonging mainly to cyanobacteria (N+) and green algae (P+, NP+). Phosphorus was the main environmental driver in the community structural changes. All periphyton attributes were significantly affected by enrichments in the advanced successional stages, when species were strongly associated to different amendments. Periphytic algal community was quite sensitive to enrichments, allowing identification of successional sequences in each treatment; however, colonization time is relevant when monitoring strategies are considered.

Handling Editor: Ho-Dong Park.

C. Ferragut (✉) · D. de C. Bicudo
Núcleo de Ecologia, Instituto de Botânica, Caixa Postal 68041, São Paulo, SP 04045-972, Brazil
e-mail: carlaferragut@yahoo.com.br

D. de C. Bicudo
e-mail: dbicudo@terra.com.br

Keywords Periphyton · Succession · Tropical ecosystem · Enrichment

Introduction

Ecological succession is fundamentally a process of species replacement and a change in species performance (Pickett et al. 1987). The successional process in periphytic algal communities depends on a complex suite of interactions between physical habitat characteristics, allogenic factors, autogenic changes in the community, and species composition (McCormick and Stevenson 1991). Therefore, changes in species composition follow different successional trajectories depending on the local environmental conditions (Hoagland et al. 1982; McCormick and Stevenson 1991).

Nutrient availability is one of the main environmental factors responsible for periphyton structural variability in freshwater ecosystems (Borchardt 1996). Experimental studies have reported changes on biomass accumulation, species composition (Vymazal and Richardson 1995; Carrick and Steinman 2001; Rodrigues and Bicudo 2004), and adaptive strategies (Ferragut and Bicudo 2010) depending on water nutrient availability and/or nitrogen to phosphorus (N:P) ratio (Luttenton and Lowe 2006). Considering that periphyton promptly responds to shifting nutritional conditions, different attributes of this community have been used to access water ecological quality and to establish restoration targets (e.g., McCormick and Stevenson 1998; Pan et al. 2000; Gaiser et al. 2006). However, studies representing the complete microalgal assemblages are limited (Sekar et al. 2004). Besides, in lentic tropical ecosystems, very few studies describe the successional process of periphytic algae (e.g., Sekar et al. 2002, 2004; Vercellino and Bicudo 2006), and even fewer considered

the influence of experimental amendments (Ferragut and Bicudo 2009, 2010). In this study, we evaluated how nutrient enrichment drives colonization and succession processes of periphytic algae in a tropical oligotrophic reservoir. We first characterized the changes in algal community (biomass, species composition, species diversity) to identify the successional phases depending on P and/or N availability. We then identified the most sensitive successional stage to the amendments. This study contributes to a better understanding of the periphyton successional trajectories in response to nutrient enrichment in tropical reservoirs.

Methods

Study area

IAG Reservoir is located in the biological reserve of Parque Estadual das Fontes do Ipiranga (São Paulo, southeastern Brazil). This shallow reservoir is oligotrophic, has a surface area of 11,270 m^2, a volume of 76,653 m^3, a mean depth of 1.5 m, a maximum depth of 4.7 m, and a mean theoretical residence time of 9.5 days (Bicudo et al. 2002). Ammonium, nitrate and soluble reactive phosphorus (SRP) concentrations on an annual average base are 28.3, 6.2 and <9.3 μg L^{-1}, respectively (Bicudo et al. 2002).

Experimental design

Mesocosms (12 polyethylene bags, 80 × 50 cm, filled with185 L of reservoir water) were installed in the littoral region of the reservoir in July 1996 (winter). Two wooden supports containing 50 glass slides each were placed inside each mesocosm as substrate for periphyton growth. Triplicate treatments were established as follows: control (no nutrient addition); P+ treatment (isolate phosphorus addition, N-limiting condition); N+ treatment (isolate nitrogen addition, P-limiting condition); NP+ treatment (nitrogen and phosphorus combined addition, good availability of nutrients). Nutrients were added as NH_4NO_3 and potassium dihydrogen phosphate (KH_2PO_4) (Merck, North Wales, PA, USA), according to the Redfield N:P molar ratio, to establish nutrient availability (Redfield 1958). Based on previous analysis of reservoir water, dissolved inorganic nitrogen (DIN) concentration was 12 μmol L^{-1}, and SRP was below the detection limit of the method (<0.11 μmol P L^{-1}). Therefore, to reach good availability of nutrients (N:P ratio 10–16) in the NP+ treatment, 20 μmol N L^{-1} and 2 μmol P L^{-1} were added on the first day of the experiment; to the N+ treatment, 20 μmol N L^{-1} was added to establish the P-limiting condition (N:P ratio >16); to the P+ treatment, 2 μmol P L^{-1} was added to establish the N-limiting condition (N:P ratio <10). After this first enrichment, the preestablished conditions were maintained throughout the experiment by daily water monitoring and additional enrichment to adjust N:P ratios. Enclosure volume was checked daily. Monitoring data are available in Ferragut and Bicudo (2010).

Sampling and limnological variables

Regular samplings of abiotic and biological variables were carried out every 3 days up to the 15th day of periphyton succession, and then at 5-days intervals until totaling a 31-day-period. The following variables were measured on the sampling days: temperature, electric conductivity (Digimed, São Paulo, Brazil), pH (pHmeter, Jenway, Staffordshire, UK), water transparency (Secchi disc), alkalinity (Golterman and Clymo 1971), dissolved oxygen (Golterman et al. 1978), dissolved inorganic carbon (DIC), nitrite (NO_2) and nitrate (NO_3) (Mackeret et al. 1978), ammonium (Solorzano 1969), SRP, and total dissolved phosphorus (TDP) (Strickland and Parsons 1965). Samples were kept under refrigeration until arriving at the laboratory. On the sampling day, water samples were filtered under low pressure (<0.3 atm) through Whatman GF/F membrane filters for analyses of dissolved nutrients. Unfiltered water samples were used for total nitrogen (TN) and total phosphorus (TP) determinations (Valderrama 1981).

Periphyton was collected by random sampling of glass slides and removed from the substrate by scraping and rinsing with distilled or ultrapure water. All biological analyses were carried within a maximum of 8 months from the collecting date. Samples for quantitative analyses were adjusted to a constant volume with distilled water and preserved with acetic Lugol's solution. Algal quantifications were performed under a Zeiss Axiovert microscope (400×) according to the Utermöhl (1958) and sedimentation time in chamber, following Lund et al. (1958). Counting limit was established according to the species rarefying curve and until reaching 100 individuals of the most common species. Biomass (μm^3 cm^{-2}) was estimated using the biovolume obtained by multiplying each species' density by the mean volume of its cells considering—whenever possible—mean dimension of 30 individuals, following Hillebrand et al. (1999). Taxonomic samplings were preserved with 4% formaldehyde water solution, and permanent diatom slides were prepared according to Hasle and Fryxell (1970). Shannon–Wiener diversity index (bits ind^{-1}), dominance, and evenness were used as measures of the community structure (Krebs 1999). Chlorophyll-*a* analyses corrected for pheophytin were carried out using acetone extraction (Golterman et al. 1978). Periphyton dry mass and ash-free dry mass (AFDM) were determined according to the American Public Health Association (APHA) (1995).

Univariate analysis was performed using the software MINITAB for Windows 14.1. One-way analysis of

Table 1 Minimum and maximum value and *between parentheses* mean and standard errors ($n = 36$) of water variables in the four treatments

Variable	Control	Treatment N+	Treatment P+	Treatment NP+
Temperature (°C)	14.2–17.5 (15.6 ± 0.3)	14.2–17.5 (15.6 ± 0.3)	14.2–17.5 (15.6 ± 0.3)	14.2–17.5 (15.6 ± 0.3)
Electric conductivity ($\mu S\ cm^{-1}$)	36.0–46.6 (39.6 ± 1.2)	37.5–58.9 (44.8 ± 2.4)	37.1–45.6 (39.6 ± 0.8)	38.4–58.3 (43.8 ± 2.3)
Dissolved oxygen ($mg\ L^{-1}$)	7.5–8.9 (8.2 ± 0.2)	6.8–9.0 (8.1 ± 0.2)	6.5–10.1 (8.5 ± 0.5)	7.0–10.2 (8.9 ± 0.4)
HCO_3 ($mg\ L^{-1}$)	3.6–6.5 (4.5 ± 0.8)	3.5–4.4 (4.0 ± 0.3)	4.2–6.6 (5.0 ± 0.7)	3.0–4.3 (3.7 ± 0.6)
Free CO_2 ($mg\ L^{-1}$)	4.1–10.1 (7.2 ± 1.9)	6.9–13.9 (2.1 ± 4.4)	2.1–12.5 (3.7 ± 1.7)	2.2–12.9 (2.8 ± −2.8)
pH	5.9–6.5 (6.1 ± 0.2)	5.8–6.1 (5.9 ± 0.1)	5.8–6.7 (6.3 ± 0.4)	5.7–6.6 (6.0 ± 0.3)
SRP ($\mu g\ L^{-1}$)	<10	<10	58–144 (87 ± 10)	33.0–106 (71.0 ± 8.0)
TDP ($\mu g\ L^{-1}$)	<10	<10	61.0–141.0 (92.0 ± 9.0)	40.0–111.0 (78.0 ± 9.0)
TP ($\mu g\ L^{-1}$)	2.0–13.0 (8 ± 1.0)	2–10 (7.0 ± 1.0)	85.0–164.0 (109.0 ± 10.0)	75.0–148.0 (101.0 ± 9.0)
$N\text{-}NO_2^-$ ($\mu g\ L^{-1}$)	1.0–4.0 (2.0 ± 0.3)	1.0–9.0 (3.0 ± 1.0)	1.0–2.0 (1.0 ± 0.1)	1.0–10.0 (4.0 ± 1.0)
$N\text{-}NO_3^-$ ($\mu g\ L^{-1}$)	0.00–91.0 (34 ± 11)	10.0–221.0 (69 ± 22)	0.00–68.0 (20 ± 9)	7.0–168.0 (62.0 ± 19.0)
$N\text{-}NH_4^+$ ($\mu g\ L^{-1}$)	62–178 (95 ± 15)	176–876 (417 ± 88)	17–87 (33 ± 8)	109–624 (231 ± 58)
DIN ($\mu g\ L^{-1}$)	73–181 (111 ± 25)	225–893 (488 ± 129)	14.3–156 (58 ± 25)	157–639 (296 ± 90)
TN ($\mu g\ L^{-1}$)	179–416 (287 ± 27.0)	392–1,499 (719 ± 114)	133–249 (208 ± 14.0)	330–1,109 (535 ± 77.0)
Soluble reactive silica ($mg\ L^{-1}$)	0.83–1.09 (0.95 ± 0.03)	0.81–1.00 (0.90 ± 0.02)	0.74–0.98 (0.88 ± 0.02)	0.81–0.99 (0.88 ± 0.02)
DIN:SRP molar ratio	55.9–121.6 (88.8 ± 24.0)	152.6–607.2 (332.0 ± 150.4)	0.4–4.0 (1.8 ± 1.1)	6.1–15.0 (9.7 ± 3.0)

N+ nitrogen, *P+* phosphorus, *NP+* nitrogen plus phosphorus, *HCO_3* bicarbonate, *CO_2* carbon dioxide, *SRP* soluble reactive phosphorus, *TDP* total dissolved phosphorus, *TP* total phosphorus, *$N\text{-}NO_2$* nitrite, *$N\text{-}NO_3$* nitrate, *$N\text{-}NH_4$* ammonium, *DIN* dissolved inorganic nitrogen, *TN* total nitrogen

variance (ANOVA) ($\alpha = 0.05$) was applied to test significant differences among treatment means. For periphyton attributes (total density, total biovolume, AFDM, and chlorophyll-*a*), ANOVA was performed for the last successional stage (31st day). Specific means were compared using Tukey's multiple-comparison test ($\alpha = 0.05$). Multivariate analysis was processed by applying principal component (PCA) and canonical correspondence (CCA) analyses to the abiotic and biotic data using a covariance matrix with data-transformed log ($x + 1$). Software PC-ORD version 3.0 for windows (McCune and Mefford 1999) was used for the analysis.

Results

Limnological variables

Phosphorus concentrations were the highest in P+ and NP+ treatments (Table 1) and below or near the method-detection limit in the control and N+ treatment groups. Soluble reactive phosphorus concentration did not differ between P+ and NP+ treatments (ANOVA $F = 1.63$, $p = 0.2223$). DIN concentration was higher in N+ and NP+ treatments and significantly different in both treatments (ANOVA $F = 15.19$, $p = 0.0002$). According to the Redfield N:P ratio, mean values for N:P molar ratio (DIN:SRP) indicated P-limiting condition in N+ treatment, N-limiting condition in P+ treatment, and good availability of nutrients in NP+ treatment.

PCA summarized 75.7% of data total variability in the two first axes (Fig. 1). On the negative side of axis 1, the sampling units of N+ treatment and control were ordered and were associated with higher NH_4 and TN concentrations ($r > -0.7$). On the positive side, sampling units of P+ and NP+ treatments were ordered and were associated with high availability of P+ ($r > 0.8$). On the positive side of axis 2, observations from NP+ and partially N+ treatments were ordered and were associated with higher values of electric conductivity, NH_4, and TN ($r > 0.6$). Therefore, preestablished limnological conditions were maintained over the experimental period.

Changes in periphyton attributes

Periphyton chlorophyll-*a* increased exponentially over succession, except for with N+ treatment (Fig. 2). The highest biomass increment was observed under P and N combined addition. Significant differences were observed in all treatments after 12-day succession ($F = 6.56$–22.07, $p = 0.001$–0.015).

As for chlorophyll-*a*, AFDM increased exponentially over succession, except for N+ isolated addition (Fig. 2). Considering colonization time, AFDM increment was

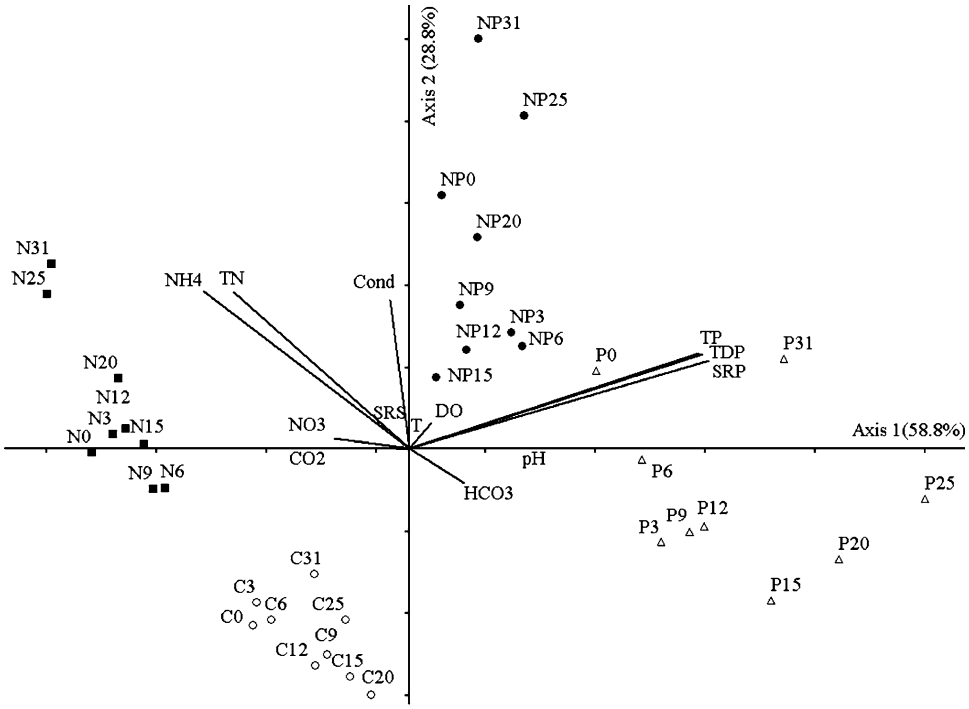

Fig. 1 Principle component analysis (PCA) biplot of abiotic variables and scores for the four treatments during the experimental period (*C* control, *N* nitrogen treatment, *P* phosphorus treatment, *NP* nitrogen plus phosphorus treatment). NH_4 ammonium, *Cond* conductivity, *DO* dissolved oxygen, *T* temperature, NO_3 nitrate, PO_4 orthophosphate, *pH* pH, *TDP* total dissolved phosphorus, *TN* total nitrogen, *TP* total phosphorus, CO_2 free carbon dioxide, HCO_3 bicarbonate. *First letters* of scores refer to treatment; *numbers* refer to experiment day

significantly different among treatments, except for the 3rd and 12th days ($F = 5.55$–47.85, $p = 0.002$–0.008). Total algal density also increased exponentially over succession under P addition (P+, NP+), whereas in N+ treatment, there was a decrease from day 15 on (Fig. 2). In relation to control, density peak was 1.2 and 1.7 times higher in the NP+ and P+ amendments, respectively. Significant differences among treatments occurred from day 25 ($F = 7.32$–12.53, $p = 0.017$–0.042). Algal biovolume increased exponentially over succession in all treatments, being significantly higher under isolate and combined P addition (Fig. 2). In relation to control, biovolume peak was 2.4 and 7.6 times higher in NP+ and P+ treatments, respectively, and only 1.4 times higher under isolated N+ addition. Biovolume was significantly different among treatments after the 15th day ($F = 7.44$–22.01, $p = 0.006$–0.027). The attributes of periphyton responded to enrichment in late successional stages (days 25 and 31) based on each attribute treatment/control ratio. Responses were markedly prominent under isolated and combined P addition, particularly for total density and biovolume (Fig. 3).

Species composition and succession

Relative abundance of algal classes markedly changed according to treatment. In the control, Chrysophyceae and Chlorophyceae prevailed (39–67%, 23–55%, respectively), whereas in N+ treatment, Cyanobacteria was dominant until the 12th day (60–67%) and, again, in the latter successional stages (56–62%). In P+ treatment, although Cyanobacteria was dominant until the 3rd day (67%), it was outnumbered by Chlorophyceae towards advanced successional stages (57–81%), whereas in NP+ treatment, this class was highly dominant over succession (79–93%). Considering species responses (Fig. 4), the control was characterized by the prevalence of *Chromulina elegans* until day 25 (50–65% of the total density), followed by *Chlamydomonas sordida* at the end of succession (34%). In the N+ treatment, *Synechococcus nidulans* was better represented up to day 12 (31–39%), being outnumbered by *Chromulina elegans* (43–53%) until the 20th day. Toward the end of succession, two Cyanobacteria, *Pseudanabaena galeata* (17–35%) and *Synechococcus nidulans* (7–23%), were the most represented species. P+ treatment was characterized by the highest number of abundant species (32), with no clear dominance except for the 3rd day, when *Synechococcus nidulans* (50%) prevailed. Toward the advanced stages, three Chlorophyceae were more highly represented: *Scenedesmus ecornis* (11–17%), *Chlamydomonas planctogloea* (7–14%), and *Monoraphidium contortum* (3–13%). In NP+ treatment, two flagellated Chlorophyceae prevailed up to day 9, *Chlamydomonas planctogloea* (21–45%) and *Chlamydomonas sordida* (39–51%), and subsequently several species contributed, chiefly *Scenedesmus ecornis* (20–27%), *Chlamydomonas planctogloea* (21–39%), and *Chlamydomonas sordida* (7–18%).

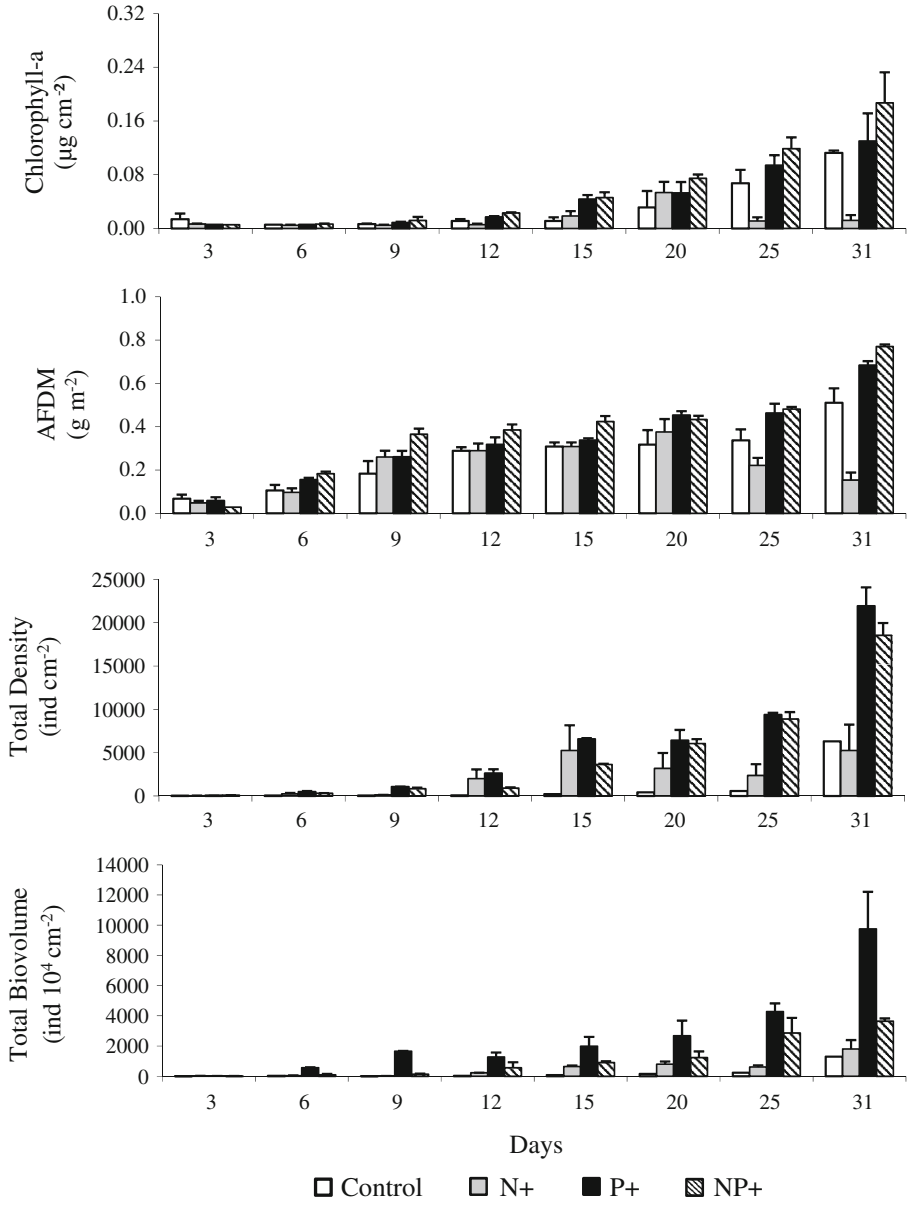

Fig. 2 Mean chlorophyll-*a* concentration ($n = 3$), ash-free dry mass (AFDM, $n = 3$), periphytic total density ($n = 2$), and total biovolume ($n = 2$) over succession in nutrient addition treatments [*C* control: phosphorus (P+), nitrogen (N+), nitrogen plus phosphorus (NP+)]. *Vertical bars* denote 1 standard error or 1 standard deviation (density and biovolume)

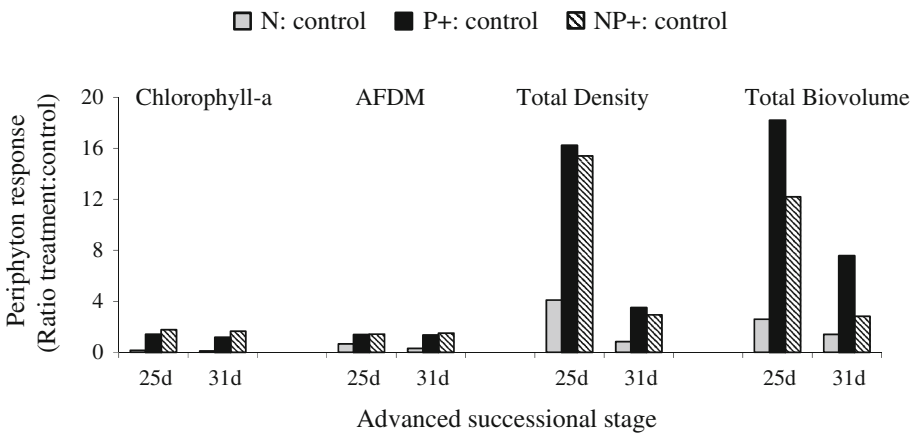

Fig. 3 Ratio between periphyton attribute [chlorophyll-*a*, ash-free dry mass (AFDM), total density or total biovolume] in control and nutrient addition treatments [phosphorus (P+), nitrogen (N+), nitrogen plus phosphorus (NP+)] for the advanced successional stages

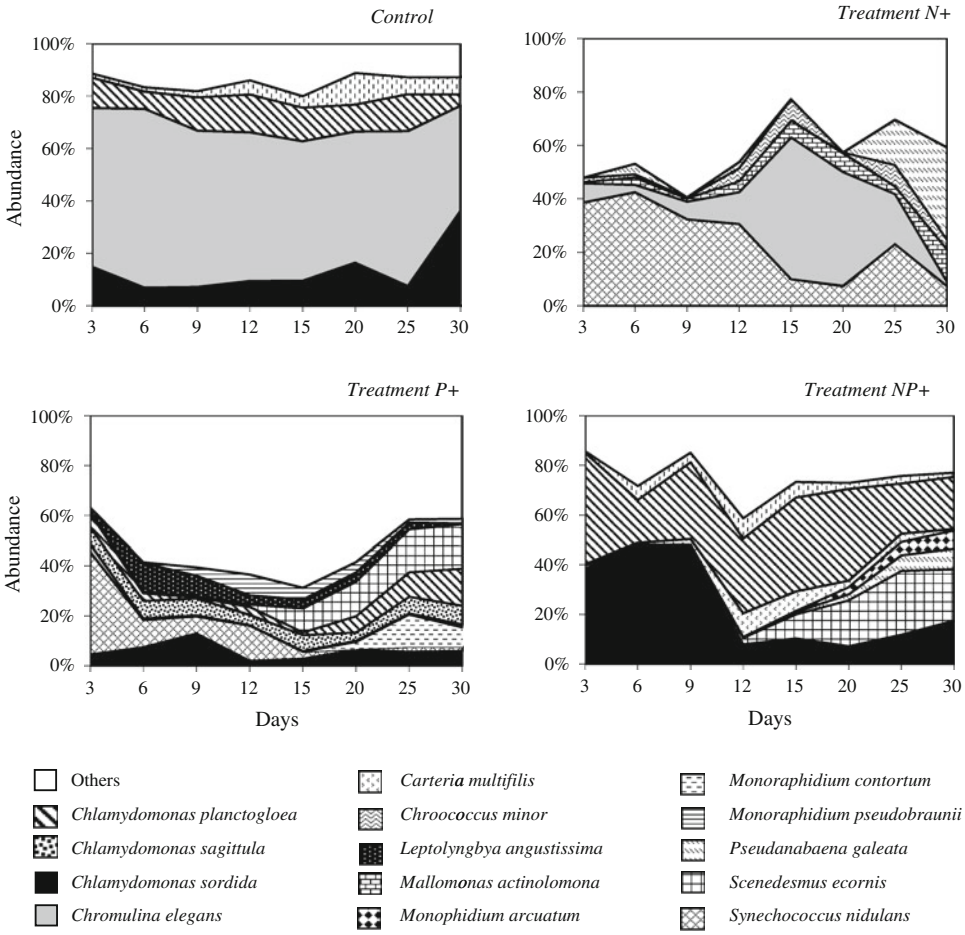

Fig. 4 Relative abundance (%) of descriptor species (>3% of total density) over succession in nutrient addition treatments [*C* control, phosphorus (P+), nitrogen (N+), nitrogen and phosphorus combined (NP+)]

CCA accomplished with 22 species (≥5% of total density in each treatment) extracted 42.1% of data total variability in the first two axes ($\lambda 1 = 0.499$, $\lambda 2 = 0.258$) (Fig. 5; Table 2). The high Pearson correlation between species and environmental data for both axes ($r = 0.96$, $r = 0.95$) indicated a strong relationship between species distribution and amendments, and Monte Carlo permutation test was significant for both axes 1 and 2 ($p = 0.01$). The canonical coefficients indicated SRP as the main environmental variable in the ordination of axis 1 (SRP $= -1.341$), whereas DIN availability was the most important in axis 2 (DIN $= -1.180$). Intermediate and advanced successional stages (15–31 day) in NP+ and P+ treatments were ordered on the negative side of axis 1 and were associated with higher values of SRP, DO, and pH ($r = -0.901$, $r = -0.610$, $r = -0.518$, respectively). On the positive side, all successional stages of the control and N+ treatment, besides the initial stages (3–9 days) of the P+ and NP+ treatments, were ordered, being mostly correlated with free CO_2 ($r = 0.531$). On the negative side of axis 2, the intermediate and advanced successional stages in N+ and NP+ treatments were ordered and were highly associated with DIN availability($r = -0.79$).

Several species presented high negative scores, mainly *Monoraphidium arcuatum*, *Scenedesmus ecornis*, *Desmodemus spinosus*, and *Kirchneriella pinguis*, and were closely associated with the advanced successional stages in the NP+ treatment ($r = >0.6$). Four species (*Nitzschia palea*, *Chlamydomonas sagittula, Monoraphidium contortum, M. pseudobraunii*) presented greater affinity to the most advanced stages in the P+ treatment ($r = >0.5$). Considering the species with positive score in axis 1 and high negative score in axis 2, two species (*Cyanosarcina* sp., *Lemmermaniella pallida*) were more closely associated to N+ treatment.

Biological indexes

Periphytic algal community increased both in diversity and richness over succession, regardless of the amendments (Fig. 6). In general, all biological indexes fluctuated over succession, mainly with N amendments (N+, NP+). Under P+ addition, fluctuation over succession was less pronounced and the community was characterized by higher diversity, species richness, and uniformity.

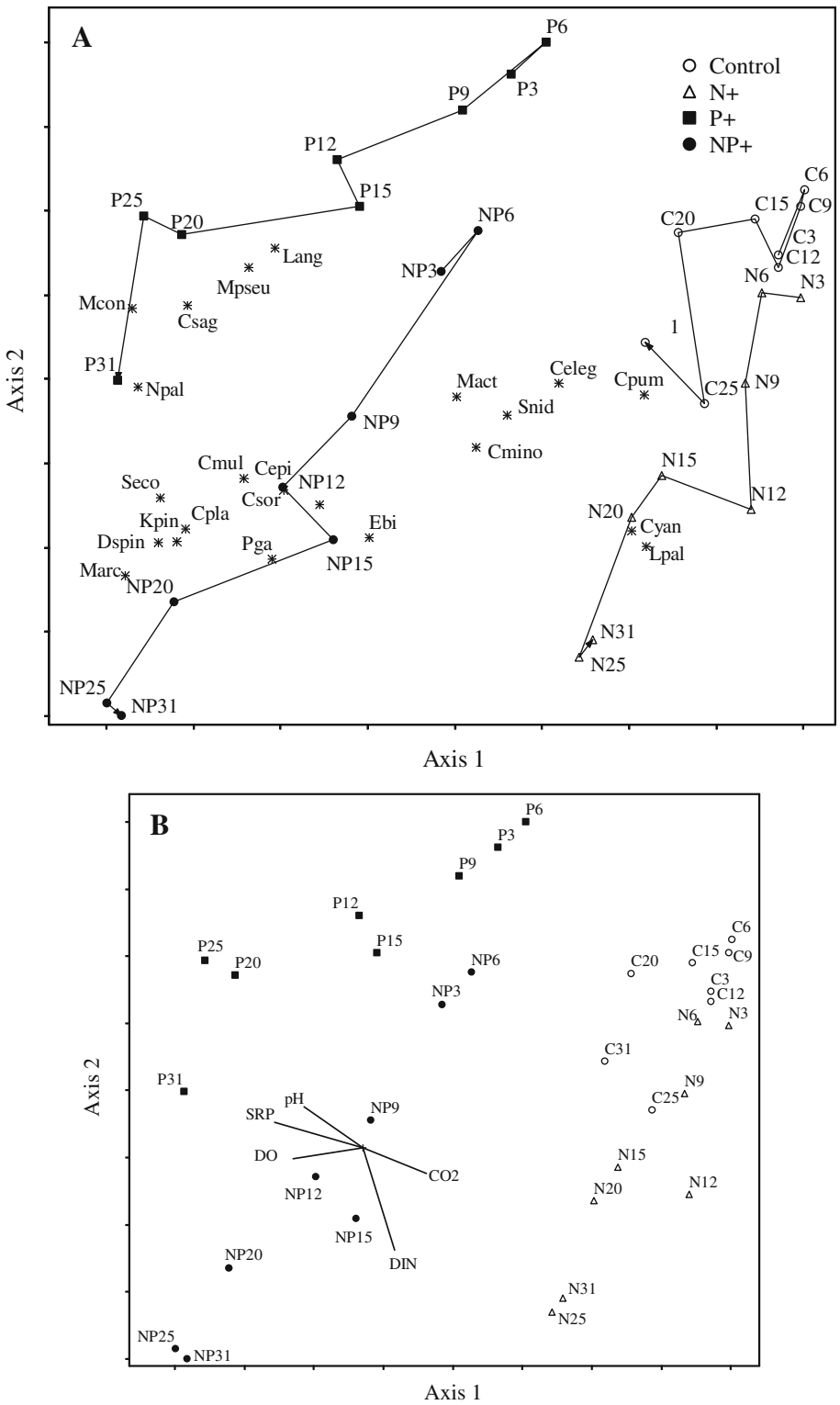

Fig. 5 Canonical correspondence (CCA) biplot of periphytic algal density and scores for the treatments during the experimental period [*C* control, phosphorus (P+), nitrogen (N+), and nitrogen plus phosphorus (NP+)]. **a** Successional trajectories with species and sampling-unit scores; **b** biplot with the sampling units. *First letters* of scores refer to treatment; *numbers* refer to experiment day. For correlation of species with axes 1 and 2, and respective codes, see Table 2

Discussion

Dynamic and successional sequence of periphyton community markedly changed with the amendments, particularly under phosphorus addition. Isolated and combined P+ addition promoted the highest biomass increment over succession considering all analyzed attributes. Moreover, under isolated N+ addition (high P+ limitation), periphyton growth decreased and biomass had a sharp reduction from day 25 on. Therefore, phosphorus availability was

Table 2 Species' Pearson correlations (*r*) with axes 1 and 2 of canonical correspondence (CCA) and their respective codes

Species	Code	Axis 1	Axis 2
Carteria multifilis (Fresenius) Dill.	Cmul	−0.695	−0.416
Chlamydomonas epibiotica G.M. Smith	Cepi	−0.449	−0.453
Chlamydomonas planctogloea Skuja	Cpla	−0.706	−0.490
Chlamydomonas sagittula Skuja	Csag	−0.472	0.087
Chlamydomonas sordida Etll	Csor	−0.499	−0.386
Chloromonas pumilio Ettl	Cpum	0.141	−0.059
Chromulina elegans Doflein	Celeg	0.099	−0.086
Chroococcus minor (Kutz.) Nag.	Cmin	−0.077	−0.253
Cyanosarcina sp.	Cyan	0.230	−0.445
Eunotia bilunaris (Ehr.) var. *bilunaris* Mills	Ebi	−0.423	−0.698
Kirchneriella pinguis Hindák	Kpi	−0.636	−0.459
Lemmermanniella pallida (Lemm.) Geitl.	Lpal	0.214	−0.400
Leptolyngbya angustissima (West & West) Anagnostidis & Komárek	Lang	−0.534	0.300
Mallomonas actinolomonas Takahashi	Mact	−0.120	−0.122
Monoraphidium arcuatum (Korš.) Hind.	Marc	−0.564	−0.416
Monoraphidium contortum (Thur) Kom.-Leg.	Mcon	−0.439	0.065
Monoraphidium pseudobraunii (Belcher & Swale) Heyning	Mpseu	−0.504	0.208
Nitzschia palea (Kütz.) W. Smith	Npal	−0.406	−0.048
Pseudanabaena galeata Boch.	Pgal	−0.419	−0.461
Desmodesmus spinosus Chod.	Dspin	−0.632	−0.434
Scenedesmus ecornis (Ehr. ex Ralfs) Chod.	Seco	−0.707	−0.374
Synechococcus nidulans (Pringsh.) Kom.	Snid	−0.015	−0.225

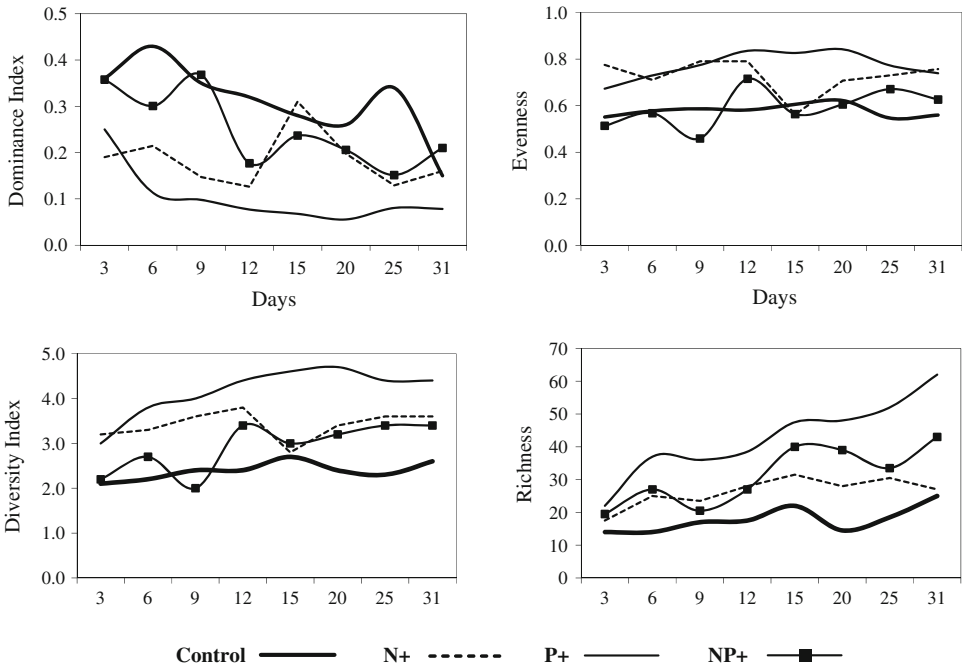

Fig. 6 Periphytic algal diversity (bits ind^{-1}), species richness, evenness, and dominance index over succession in nutrient addition treatments [*C* control, phosphorus (P+), nitrogen (N+), nitrogen plus phosphorus (NP+)]

considered the primary limiting factor for periphyton growth. Accordingly, Huszar et al. (2005) stated that P+ limitation is more commonly reported in tropical ecosystems than previous expected. Other findings, based on experimental and observational periphyton studies in tropical reservoirs, also reported P+ as the limiting nutrient (Vercellino and Bicudo 2006; França et al. 2009; Ferragut and Bicudo 2010). Studies in the subtropical region, mainly

in the Everglades, also pointed out P+ as the main controlling factor for the periphytic community organization (e.g., McCormick and Stevenson 1998; Pan et al. 2000). So far, only one study carried out in Brazil in the Amazon floodplain lake reported N+ as the main periphyton limiting factor (Engle and Melack 1993).

Successional stages of periphytic algal communities have been analyzed by immigration and growth rates (Stevenson et al. 1991), competitor/stress tolerator/ruderal (CRS) strategies (Biggs et al. 1998; Carrick and Steinman 2001; Ferragut and Bicudo 2010) or were simply based on colonization time (Peterson and Grimm 1992). In the study presented here, successional stage was first identified based on classes and/or algal species replacement and secondarily considered the set of periphyton attributes (density, total biovolume, chlorophyll-*a*, AFDM).

Without enrichment (P+ limitation), structural changes with time were little, with no clear differentiation of phases due to the prevalence of Chrysophyceae represented by *Chromulina elegans*, characterizing a low biofilm organization over succession. Nutrient amendments promoted changes even in higher taxonomic levels, indicating a strong structural modification. Under extreme P+-limitation (N+), three successional phases were identified, with clear replacement of classes and species (Cyanobacteria → Chrysophyceae → Cyanobacteria). The initial phase was characterized by the highest participation of *Synechococcus nidulans* (3–12 days) in all treatments, followed by *Chromulina elegans* in the intermediate phase (15–20 days), and the later colonizer *Pseudanabaena galeata*, besides maintenance of *Synechococcus nidulans* (25–31 days). Chrysophyceae and Cyanobacteria have effective adaptive strategies to maintain growth under P+ limitation. In general, Chrysophyceae are nutritional opportunists (Sandgreen 1988), and Cyanobacteria are able to accumulate P+ internally (Oliver and Ganf 2000). The prevalence of *Synechococcus nidulans* under high N+ supply was also reported by Ahlgren and Hyenstrand (2003) and can be associated with this species' high P+ assimilation efficiency in depleted conditions (Moutin et al. 2002).

With isolated P+ amendment, three successional phases were also identified, and periphyton structural organization was the highest. The initial colonizers were mostly represented by *Synechococcus nidulans* (Cyanobacteria), followed by several co-occurring species, with more stable abundance in the intermediate phase with no species/class prevalence. In the late phase, community was mostly composed of Chlorophyceae represented by *Scenedesmus ecornis*, *Monoraphidium contortum*, *Chlamydomonas planctogloea*, and *Chlamydomonas sordida*. Great affinity of *Monoraphidium contortum* and other species (e.g., *Chlamydomonas sagittula*, *Nitzschia palea*) to the advanced successional stage was corroborated by CCA. Other studies reported the replacement of Cyanobacteria by Chlorophyceae with P+ availability increase (e.g., Vymazal et al. 1994), and that this class has a preference for high nutrient levels, particularly P+ (Havens et al. 1999). Considering that pioneer species have great influence on resource availability for species in subsequent stages (Sommer 1999), under N+-limiting condition (P+), *Synechococcus nidulans* may improve community nutrient status in the initial successional stage due to its ability to fix N+ (Tsygankov 2007). Several studies associated *Nitzschia palea* to high availability of nutrients, particularly P+ (e.g., van Dam et al. 1994; Fore and Grafe 2002). Therefore, phosphorus enrichment favored the arrival and permanence of several colonists over succession, leading to the highest diversity, richness, and evenness among treatments. The increased periphytic algae diversity and non-occurrence of competitive exclusion were reported in experiments with P+ enrichment (Ferragut and Bicudo 2009). Considering that the periphytic community is primarily limited by P+ (Vercellino and Bicudo 2006; Ferragut and Bicudo 2009), increased nutrient availability probably favored limiting the increase in complexity.

Under good nutrient (NP+) availability, only two successional phases were distinguished, as Chlorophyceae dominated and species replacement was less pronounced over succession. Two pioneer species were highly represented (*Chlamydomonas sordida*, *Chlamydomonas planctogloea*), followed by a second phase with maintenance of the two pioneers and the sharp increase of *Scenedesmus ecornis* toward the later stages. Great affinity of *Scenedesmus ecornis* and other Chlorophyceae species (*Desmodesmus spinosus*, *Kirchneriella pinguis*, *Monoraphidium arcuatum*, *Chlamydomonas planctogloea*) to the advanced successional stage in NP+ treatment was corroborated by CCA. As mentioned previously, it the preference of Chlorophyceae for high nutrient availability has been frequently reported (e.g., Havens et al. 1999). Yet, flagellate Chlorophyceae success (in this case, *Chlamydomonas*) is favored in both oligotrophic and eutrophic environments, as small cells are better competitors for nutrients and faster reproducers, and mobility allows the efficient exploitation of substrate resources (Happey-Wood 1988). In general, community diversity, richness, and mainly evenness were lower than the periphyton under isolated P+ amendment, and dominance index was similar to that of control. Although community presented structurally simplified successional phases under both nutrient additions (NP+), isolated or combined P+ enrichment promoted high biomass accumulation over succession.

In summary, this study demonstrated that periphyton structural changes over succession were strongly driven by nutrient amendments, and the algal community allowed

identification of successional sequences in each treatment. All biomass attributes, particularly total density and biovolume, demonstrated phosphorus as the main environmental driver for periphyton growth. However, all periphyton structural attributes were significantly sensitive to amendments only in the advanced successional phase (25–31th days), when species were also much more strongly associated with different kinds of enrichments by CCA. Our results showed that periphyton is a good sensor of water enrichment; however, colonization time is relevant when monitoring or recovery strategies are considered.

Acknowledgments Authors are indebted to FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo) for the first author's Doctoral Fellowship, and to CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) for DCB's grant (Grant no. 305072/2009-9).

References

Ahlgren G, Hyenstrand P (2003) Nitrogen limitation effects of different nitrogen sources on nutritional quality of two freshwater organisms *Scenedesmus quadricauda* (Chlorophyceae) and *Synechococcus* sp. (Cyanophyceae). J Phycol 39:906–917

American Public Health Association(APHA) (1995) Standard methods for the examination of water and wastewater. American Public Health Association, Washington, DC

Bicudo DC, Forti MC, Bicudo CEM (2002) Parque Estadual das Fontes do Ipiranga (PEFI): unidade de conservação que resiste à urbanização de São Paulo. Secretaria do Meio Ambiente do Estado de São Paulo, São Paulo

Biggs BJF, Stevenson RJ, Lowe RL (1998) A habitat matrix conceptual modes for stream periphyton. Arch Hydrobiol 143: 21–56

Borchardt MA (1996) Nutrients. In: Stevenson RJ, Bothwell ML, Lowe RL (eds) Algal ecology: freshwater benthic ecosystems. Academic Press, San Diego, pp 184–227

Carrick HJ, Steinman AD (2001) Variation in periphyton biomass and species composition in Lake Ockeechobee, Florida (USA): distribution of algal guilds along environmental gradients. Arch Hydrobiol 152:411–438

Engle DL, Melack JM (1993) Consequences of riverine flooding for seston and the periphyton of floating meadows in a Amazon floodplain lake. Limnol Oceanogr 38:1500–1520

Ferragut C, Bicudo DC (2009) Efeito de diferentes níveis de enriquecimento por fósforo sobre a estrutura da comunidade perifítica em represa oligotrófica tropical (São Paulo, Brasil). Rev Bras Bot 32:569–583

Ferragut C, Bicudo DC (2010) Periphytic algal community adaptive strategies in N and P enriched experiments in tropical oligotrophic reservoir. Hydrobiologia 646:295–309

Fore LS, Grafe C (2002) Using diatoms to assess the biological condition of large rivers in Idaho (U.S.A.). Freshw Biol 47: 2015–2037

França RCS, Lopes MRM, Ferragut C (2009) Temporal variation of biomass and status nutrient of periphyton in shallow Amazonian Lake (Rio Branco, Brazil). Acta Limnol Bras 21:175–183

Gaiser EE, Childers DL, Jones RD, Richards JH, Scinto LJ, Trexler JC (2006) Periphyton responses to eutrophication in the Florida Everglades: cross-system patterns of structural and compositional change. Limnol Oceanogr 51:617–630

Golterman HL, Clymo RS (1971) Methods for chemical analysis of freshwaters. Blackwell Scientific, International Biological Program, Oxford

Golterman HL, Clymo RS, Ohmstad MAM (1978) Methods for physical and chemical analysis of freshwaters. Blackwell Scientific, International Biological Program, Oxford

Happey-Wood VM (1988) Ecology of freshwater planktonic green algae. In: Sandgreen CD (ed) Growth and reproductive strategies of freshwater phytoplankton. Cambridge University Press, Cambridge, pp 175–226

Hasle GR, Fryxell GA (1970) Diatoms: cleaning and mouting for light and electron microscopy. Trans Am Microsc Soc 89: 469–474

Havens KE, East TL, Rodusky AJ, Sharfstein B (1999) Littoral periphyton responses to nitrogen and phosphorus: an experimental study in a subtropical lake. Aquat Bot 63:267–290

Hillebrand H, Durselen C-D, Kirschtel D, Pollingher U, Zohary T (1999) Biovolume calculation for pelagic and benthic microalgae. J Phycol 35:403–424

Hoagland KD, Roemer SC, Rosowski JR (1982) Colonization and community structure of periphyton assemblages, with emphasis on the diatoms (Bacillariophyceae). Am J Bot 69:188–213

Huszar VLM, Bicudo DC, Giani A, Ferragut C, Martinelli LA, Henry R (2005) Subsídios para compreensão sobre a limitação de nutrientes ao crescimento do fitoplâncton e perifíton em ecossistemas continentais lênticos no Brasil. In: Roland F, César D, Marinho M (eds) Lições em Limnologia: Fronteiras Conceituais. RiMa Editora, São Carlos, pp 243–260

Krebs CJ (1999) Ecological methodology. Addisson Wesley Longman, Menlo Park

Lund JWG, Kipling C, Le Cren ED (1958) The inverted microscope method of estimating algal numbers and statistical basis of estimation by counting. Hydrobiologia 11:143–170

Luttenton MR, Lowe RL (2006) Response of a lentic periphyton community to nutrient enrichment at low N:P ratios. J Phycol 42:1007–1015

Mackeret FJH, Heron J, Talling JF (1978) Water analysis: some revised methods for limnologists. Titus Wilson & Son Ltd., Kendall (Freshwater Biological Association Scientific Publication no. 36)

McCormick PV, Stevenson RJ (1991) Mechanisms of benthic algal succession in lotic environments. Ecology 72:1835–1848

McCormick PV, Stevenson RJ (1998) Periphyton as a tool for ecological assessment and management in the Florida Everglades. J Phycol 34:726–733

McCune B, Mefford MJ (1999) PC-ORD for Windows. Multivariate analysis of ecological data, version 4.10. MjM Software Design, Oregon, p 47

Moutin T, Thingstad TF, Wambeke FV, Marie D, Slamyk G, Raimbault P, Claustre H (2002) Does competition for nanomolar phosphate supply explain the predominance of the cyanobacterium *Synechococcus*. Limnol Oceanogr 47(5):1562–1567

Oliver RL, Ganf GG (2000) Freshwater blooms. In: Whitton BA, Potts M (eds) The ecology of Cyanobacteria their diversity in time and space. Kluwer, London, pp 149–194

Pan Y, Stevenson RJ, Vaithiyanathan P, Slate J, Richardson CJ (2000) Changes in algal assemblages along observed and experimental phosphorus gradients in a subtropical wetland, USA. Freshw Biol 44:339–353

Peterson CG, Grimm NB (1992) Temporal variation in enrichment effects during periphyton succession in a nitrogen-limited desert stream ecosystem. J N Am Benthol Soc 11:20–36

Pickett STA, Collins SL, Armesto JJ (1987) Models, mechanisms and pathways of succession. Bot Rev 53:335–371

Redfield AC (1958) The biological control of chemical factors in the environment. Am Sci 46:205–221

Rodrigues L, Bicudo DC (2004) Periphytic algae. In: Thomaz SM, Agostinho AA, Hahan NS (eds) Upper Paraná River and its floodplain: physical, aspects, ecology and conservation. Backhuys Publishers, Leiden, pp 125–143

Sandgreen CD (1988) The ecology of chrysophyte flagellates: their growth and perennation strategies as freshwater phytoplankton. In: Sandgreen CD (ed) Growth and reproductive strategies of freshwater phytoplankton. Cambridge University Press, Cambridge, pp 9–104

Sekar R, Nair KVK, Rao VNR, Venugopalan VP (2002) Nutrient dynamics and successional changes in a lentic freshwater biofilm. Freshw Biol 47:1893–1907

Sekar R, Venugopalan VP, Nandakumar K, Nair KVK, Rao VNR (2004) Early stages of biofilm succession in a lentic freshwater environment. Hydrobiologia 512:97–108

Solorzano L (1969) Determination of ammonia in natural waters by the phenolhypochlorite method. Limnol Oceanogr 14:799–801

Sommer U (1999) A comment on the proper use of nutrient ratios in microalgal ecology. Arch Hydrobiol 146:55–64

Stevenson RJ, Peterson CG, Kirschtel DB, King CC, Tuchman NC (1991) Density dependent growth ecological strategies and effects of nutrients and shading on benthic diatom succession in streams. J Phycol 27:59–69

Strickland JDH, Parsons TR (1965) A manual of sea water analysis. Bull Fish Res Board Can 125:1–185

Tsygankov AA (2007) Nitrogen-fixing cyanobacteria: a review. Appl Biochem Microbiol 43:250–259

Utermöhl H (1958) Zur vervollkommnung der quantitativen phytoplankton-methodik. Mitt Int Ver Theor Angew Limnol 9:1–38

Valderrama GC (1981) The simultaneous analysis of total nitrogen and total phosphorus in natural waters. Mar Chem 10:109–112

Van Dam H, Mertens A, Sinkeldam J (1994) A coded checklist and ecological indicator values of freshwater diatoms from the Netherlands. Neth J Aquat Ecol 28:117–133

Vercellino IS, Bicudo DC (2006) Sucessão da comunidade de algas perifíticas em reservatório oligotrófico tropical (São Paulo, Brasil): comparação entre período seco e chuvoso. Rev Brasil Bot 29:363–377

Vymazal J, Richardson CJ (1995) Species composition, biomass, and nutrient content of periphyton in the Florida Everglades. J Phycol 31:343–354

Vymazal J, Craft CB, Richardson CJ (1994) Periphyton response to nitrogen and phosphorus additions in Florida Everglades. Algol Stud 73:75–97