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SEASONAL INFLUENCE OF NITROGEN AND PHOSPHORUS ENRICHMENT ON THE FLORISTIC COMPOSITION OF THE ALGAL PERIPHYTIC COMMUNITY IN A SHALLOW TROPICAL, MESOTROPHIC RESERVOIR (SÃO PAULO, BRAZIL)

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ABSTRACT

Effects of N and/or P experimental addition on species richness and floristic composition of periphytic algal community in a shallow, mesotrophic reservoir was evaluated four times during the year (spring, summer, fall and winter). Four treatments were designed using nutrient diffusing substrates (polystyrene vials filled up with agar solution and nutrients – control: no nutrient addition, N^+: 0.75 M, P^+: 0.05 M and NP^+: combined addition of N and P, molar N:P ratio = 15). Vial mouth was covered with a 20 µm mesh cloth for periphyton colonization. Samplings were performed on the 15th, 20th, 25th and 30th days of colonization. Two hundred and three taxa were identified; Chlorophyceae was the dominant group. Species richness per sample varied from 33 to 66 and was greater during summer and fall, mostly influenced by the time of the year than by nutrient treatment. Community similarity was mainly determined by the kind of treatment, grouping algal associations of (1) control, (2) P^+ and NP^+ treatments and (3) N^+ treatment. TWINSPAN analysis indicated that hierarchic classification of species was defined by P availability. Species and classes richness were not sensitive to changes due to nutrient enrichment, nitrogen amendments markedly contributing to the total species numbers, whereas species associations were clearly influenced by P availability. Present results indicated that the reservoir, due to its shallowness and prevalence of littoral biota, may have profound changes in its native associations with P inputs.

Keywords: Algae; enrichment; periphyton; phosphorus; similarity.

RESUMO

INFLUÊNCIA SAZONAL DO ENRIQUECIMENTO POR NITROGÊNIO E FÓSFORO SOBRE A COMPOSIÇÃO FLORÍSTICA DA COMUNIDADE PERIFÍTICA ALGAL EM UM RESERVATÓRIO TROPICAL RASO MESOTRÓFICO (SÃO PAULO, BRASIL). Avaliou-se o efeito da adição experimental de nitrogênio e/ou fósforo sobre a riqueza e composicão florística da comunidade de algas perifíticas em quatro épocas do ano (primavera, verão, outono e inverno) em represa rasa mesotrófica. Quatro tratamentos foram delineados usando substrato difusor de nutrientes (copos de poliestireno preenchidos com solução de ágar e nutrientes – controle: sem adição de nutrientes, N^+: 0,75 M, P^+: 0,05 M e NP^+: adição combinada dos dois sais, razão molar N:P = 15). A abertura dos copos foi revestida com tecido de náilon de malha de 20 µm de abertura, que foi usado como substrato para estabelecimento do perifíton. Coletas foram realizadas aos 15^o, 20^o, 25^o e 30^o dias de colonização. Foram inventariados 203 táxons, com predomínio das Chlorophyceae. A riqueza específica variou por amostra de 33 a 66 e sofreu maior influência da época do ano do que das condições experimentais, sendo mais elevada no verão e outono. A similaridade da comunidade foi, primordialmente, dirigida pelo tipo de tratamento, agrupando as associações de algas do controle, dos tratamentos P^+ e NP^+ e do tratamento N^+. A análise TWINSPAN indicou que a classificação hierárquica das espécies foi definida pela disponibilidade

de P. A riqueza de espécies e dos grandes grupos taxonômicos (classes) não foi sensível às mudanças devidas ao enriquecimento por nutrientes, sendo que os enriquecimentos por nitrogênio contribuíram marcadamente para a riqueza de espécies, enquanto que as associações de espécies foram claramente influenciadas pela disponibilidade de P. Os resultados indicaram que o Lago das Ninféias, por ser um ambiente raso com predomínio de biota litorânea, pôde apresentar profundas mudanças de suas associações algais nativas mediante o aporte de P.
Palavras-chave: Algas; enriquecimento; fósforo; perifíton; similaridade.

INTRODUCTION

Understanding factors that control the aquatic biodiversity and that are responsible for its maintenance or decline are key approaches in ecology. Loss of biodiversity due to human interference is becoming a topic of utmost concern (Hillbrand & Sommer 2000).

The high periphyton algal diversity in shallow systems is partly derived from the heterogeneity of habitats and surfaces available for colonization (plants, sediments, rocks), the different colonization strategies (Stevenson 1996, Goldsborough & Robinson 1996) and the species interaction and interchange with phytoplankton community (Margalef 1998, Tanigushi *et al.* 2005). Consequently, in shallow systems it is expected a major contribution of periphyton species compared to phytoplankton. However, very few studies address the biodiversity contribution of both communities. The only work comparing periphyton and phytoplankton communities of an oligotrophic reservoir in Brazil reported that the non-inclusion of the periphyton in a floristic survey would underestimate about 43% of the total algal biodiversity (Ferragut *et al.* 2005). Comparing data from enrichment (Ferragut & Bicudo 2009) and impoverishment experiments (Crossetti & Bicudo 2005, Barcelos 2003) carried out in reservoirs of the Parque Estadual das Fontes do Ipiranga (São Paulo) confirmed that trend.

Periphyton native species community represents an important indication of the ecosystem conditions, and the loss of biodiversity has several implications for the ecological stability, as demonstrated for the Everglades in Florida, USA (McCormick & O'Dell 1996). Therefore, decline of biodiversity due to eutrophication is calling the attention of scientists during the last decade (McCormick *et al.* 1996, Pan *et al.* 2000, Hillbrand & Sommer 2000, Stelzer & Lamberti 2001). Studies addressing the effect of artificial enrichment on the periphyton community in Brazilian ecosystems were recently carried out by Vercellino (2001) and Ferragut & Bicudo (2009, 2010).

Present study aimed at evaluating the effect of phosphorus and nitrogen experimental addition over a seasonal scale on the richness and the floristic composition of the algal periphyton community in a shallow mesotrophic reservoir, and in doing so to contribute to a better understanding of the biodiversity changes in response to nutrient enrichment in tropical ecosystems.

STUDY SITE

We conducted our study in Ninféias Reservoir, which is located in the Parque Estadual das Fontes do Ipiranga (23^{o}38'08" S to 23^{o}40'18" S; 46^{o}36'48" W to 46^{o}38'00" W), a Conservation Unit circumscribed by heavily urbanized area in the megalopolis of São Paulo, southeast Brazil. It is a reservoir formed in 1930 by the damming of Pirarungaua creek. The reservoir is small and shallow, having a surface area of 5433 m^2, a volume of 7170 m^3, maximum and mean depths of 3.6 m and 1.3 m, and a mean theoretical residence time of 7.2 days (Bicudo *et al.* 2002a). It is a polymictic mesotrophic ecosystem (Bicudo *et al.* 2002b) with extensive multispecies banks of floating and submerged macrophytes. Climate of the region is tropical of altitude (Conti & Furlan 2003).

MATERIAL AND METHODS

Experimental design – We used nutrient diffusing substrates (NDS) to evaluate the effect of nutrients (nitrogen and phosphorus) on algal community composition. The experiment was performed using one control (no nutrient addition) and three enriched treatments as follows: N$^+$ (nitrogen addition, 0.75

M of $NaNO_3$, P-limiting condition), P^+ (phosphorus addition, 0.05 M of Na_2HPO_4, N-limiting condition) and NP^+ (nitrogen and phosphorus addition, molar ratio N:P = 15). Treatments were located in four locations of the littoral region of the reservoir according to the water flux and distant from each other in order to avoid contamination among treatments. NDS were constructed using a polystyrene vial (330 ml, 110 mm height, 80 mm diameter at mouth) filled with 2% agar solution plus N and/or P according to the treatment. The vial mouth was covered with a 20 µm mesh nylon cloth (phytoplankton net cloth), that was used as substrate for periphyton growth (substrate area 47.75cm²). Details about NDS construction are found in Fermino *et al.* (2004).

Sampling covered a seasonal cycle, and was performed during the spring (23 November-08 December 2001), summer (21 February-08 March 2002), fall (03-18 May 2002) and winter (10-25 July 2002). For each period and treatment, four samplings (n = 2) were performed at random corresponding to the 15th, 20th, 25th and 30th days of succession.

Analyses of periphyton algae – Periphyton was removed from substrate using gentle brushing and distilled water jets, and was immediately fixed and preserved with formalin 3-4% (Bicudo 1990a). Taxonomic study was based on 64 samples collected in each succession period and was carried out for each treatment and season of the year. Oxidation and preparation of diatom slides for microscope observation followed Hasle & Fryxell (1970), using Hyrax as the inclusion medium. Observations were done under a Zeiss binocular microscope with camera-lucida and digital measuring ocular.

Periphyton floristic comparisons were based on the algal quantification procedure for purposes of standardizing the sampling and the analyses efforts among treatments and seasons of the year. Material removed for quantification from substrate was immediately fixed and preserved with 0.5% acetic lugol (Bicudo 1990a, Villafañe & Reid 1995). Counting followed Utermöhl (1958) using a Zeiss inverted microscope and 400 times magnification (Lund *et al.* 1958). Counting limits were based on two procedures, i.e. species rarefaction curve and counting of a minimum of 100 individual specimens of the most common species (Bicudo 1990b).

Statistical treatment – Data were analyzed using multivariate statistical analyses. For dichotomous hierarchic classificatory analyses of periphyton algae referring to floristic composition, Double Entrance Indicator Species Analysis - TWINSPAN (Hill 1979) was used. Analysis followed the standard configuration for sampling unit information *versus* species: 5 as the minimum size for the group, 4 as the maximum number of indicators, and 142 as the maximum number of species in the final matrix (McCune & Grace 2002). Cluster analysis was also performed, measured by group analysis (UPGMA) using the Sørensen binary index. Both analyses were based on a species density matrix (with relative abundance ≥ 1.0% for each sampling unit), and data were transformed into a presence/absence matrix. Analyses were carried out using PCORD version 4.1 (McCune & Mefford 1999).

RESULTS

Taxonomic composition of periphytic algae community, including treatments and seasons of the year, totaled 203 infrageneric taxa distributed in 9 classes, 13 orders and 85 genera.

Average species richness per treatment and season of the year had a 2-fold variation, i.e. from 33 (winter, treatment N^+) to 66 (fall, control) (Figure 1). Considering just treatments, year average varied from 43 species (N^+) to 49 (control), whereas during the seasons richness among treatments varied between 39 (spring and winter), 52 (summer) and 54 (fall).

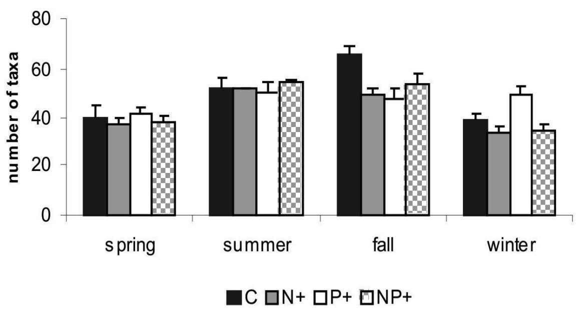

Figure 1. Species richness average number and respective Standard error (n = 4) under enrichment experimental conditions (C = control, P^+, N^+, NP^+) in each season of the year in the Ninféias Pond.

Concerning classes, N isolated addition (N^+) presented the least number (5 in the spring and 6 during all other seasons), control and P^+ treatment varied from 6 to 8 depending on the season, and the greatest number of classes (8) was detected in the NP^+ treatment across all seasons (Figure 2).

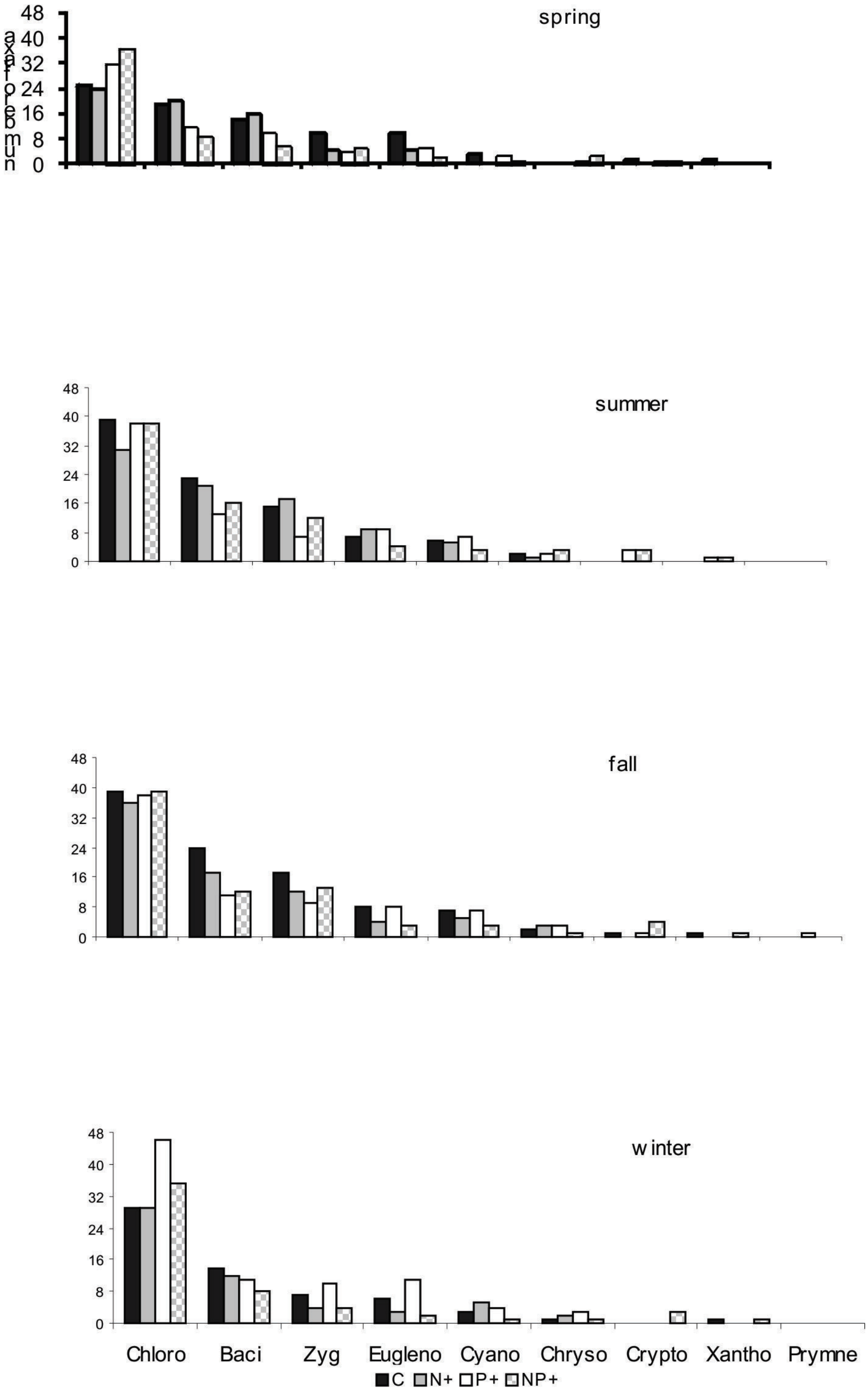

Figure 2. Periphytic algal classes average numbers (n = 8) under experimental enrichment conditions (C = control, N^+, P^+, NP^+) in each season of the year in the Ninféias Pond. Abbreviations: Chloro: Chlorophyceae, Baci: Bacillariophyceae, Zyg: Zygnemaphyceae, Eugleno: Euglenophyceae, Cyano: Cyanophyceae, Chryso: Chrysophyceae, Crypto: Cryptophyceae, Xantho: Xanthophyceae, Prymne: Prymnesiophyceae.

In general, the greatest number of taxa per class was observed in all treatments during fall and summer, whereas the opposite was observed during the spring and winter. Chlorophyceae was the class with the highest species number (24 to 46) in all treatments and seasons of the year, followed by Bacillariophyceae, Zygnemaphyceae and Euglenophyceae (Figure 2). The genera with the highest number of species were *Scenedesmus* (17 species), *Cosmarium* (10 species) and *Monoraphidium* (8 species).

Cluster analysis performed with the presence/ absence of 140 species formed two main groups (Figure 3). The first one included two subgroups, characterized by the subgroup control (SIMI 32%) and the P isolated (P^+) and combined (NP^+) addition subgroup (SIMI 26%), except for the combined addition during the winter that formed a separated group (SIMI 90%). In more detail, subgroup control was separated according to the seasons of the year (spring + summer and fall + winter) and, later on, by the succession days whose similarities were above 90%. The same way, in the P^+ and NP^+ subgroup subsequent cutting levels separated the treatments by the season of the year (SIMI > 90%). There was not, however, a complete grouping of treatments P^+ or NP^+, because spring always remained somewhat more separated from the other subgroups.

The second main group brought together the isolated N addition treatment (37.5% SIMI), whose subsequent cutting levels grouped the seasons of the year, with similarities varying between 70 and 75%, i.e. lesser than in all other treatments.

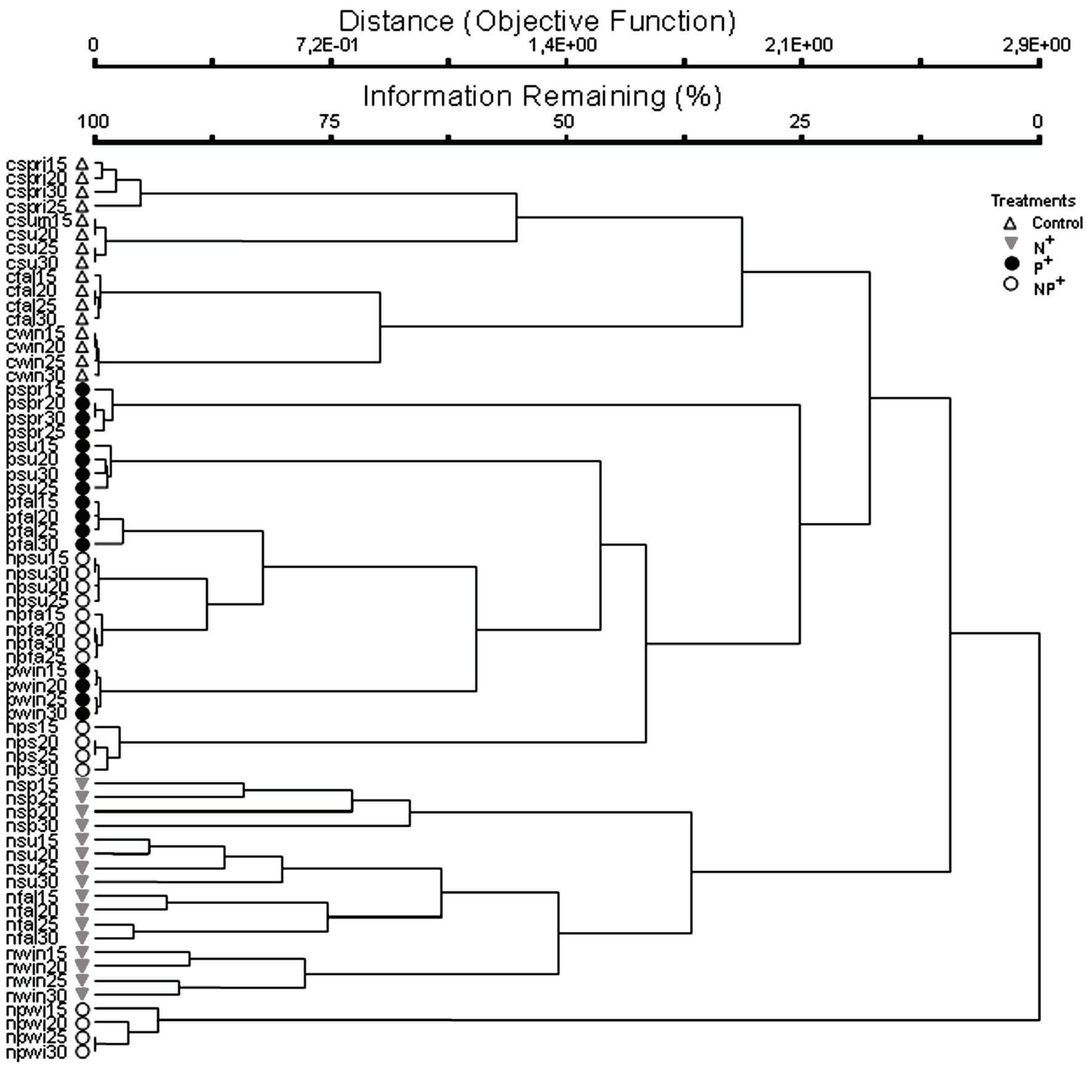

Figure 3. Cluster similarity analysis (Sørensen Binary Index) between periphytic algal classes in the control and enriched treatments. Abbreviations: first letter = treatment (c = control, n = treatment N^+, p = treatment P^+, np = treatment NP^+), the two or three following letters: season of the year (spr = spring, fal = fall, sum = summer, win = winter); numbers: days of succession

TWINSPAN classification analysis differentiated, after the third division level, 13 sampling groups – A to K (Figure 4, Table 1).

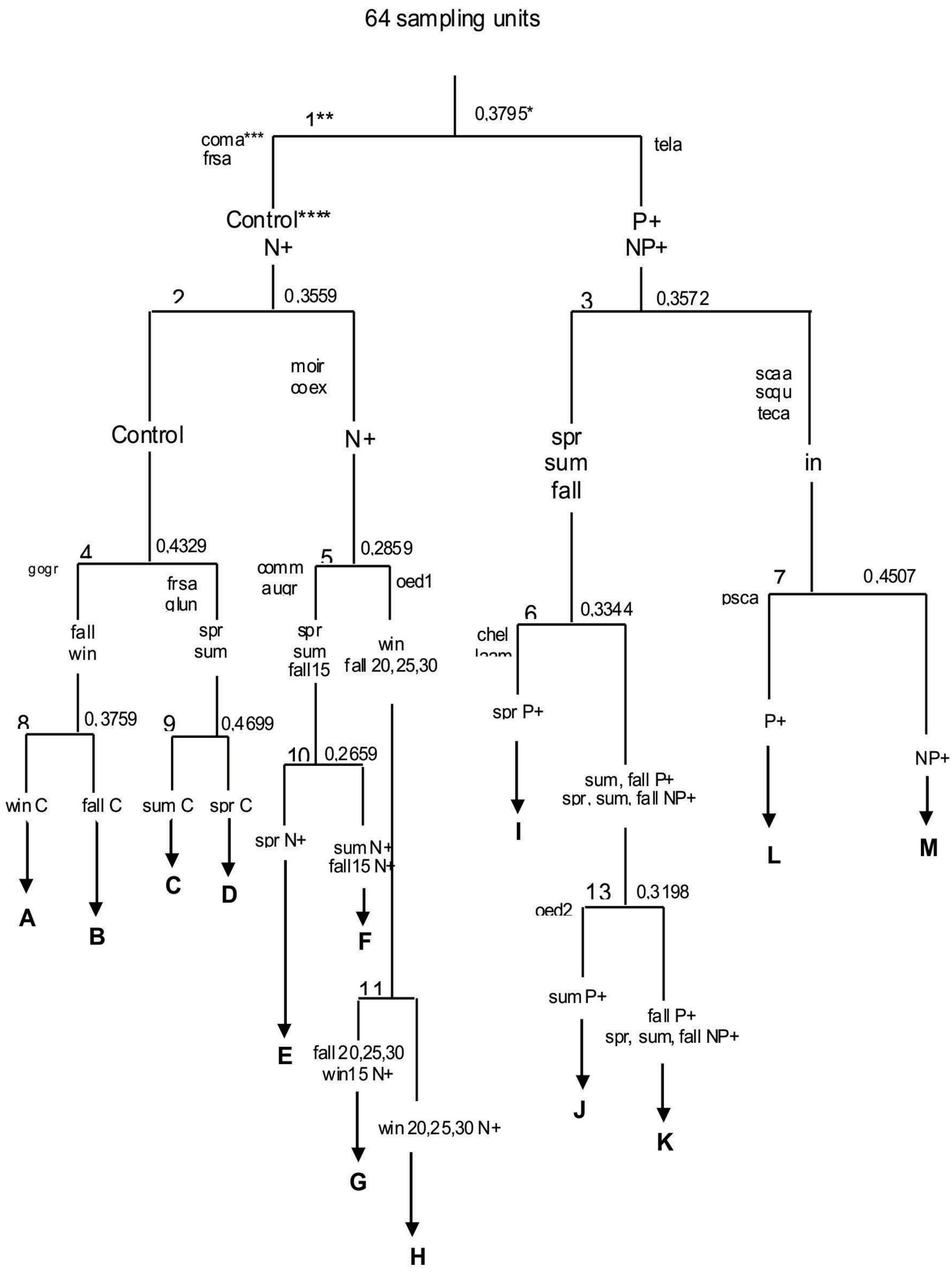

Figure 4. Hierarchic divisor classification (TWINSPAN) of periphytic algal species under enrichment experimental conditions (control, N^+, P^+, NP^+) and the four seasons of the year (spr: spring; sum: summer; fall: fall; win: winter) in the Ninféias Pond. * (eigenvalues), ** (division), *** (associated species) and **** (seasons of the year and treatments), number: succession stages. Abbreviations: augr = *Aulacoseira granulata*, coex = *Cosmarium exiguum,* chel = *Chromulina elegans*, coma = *Cosmarium margaritatum*, comm = *Cosmarium contractum* var. *minutum*, frsa = *Frustulia rhomboides* var. *saxonica*, glun = *Geitlerinema unigranulatum*, gogr = *Gomphonema gracile*, laam = *Lagynion ampullaceum*, moir = *Monoraphidium irregulare*, oed1 = *Oedogonium* sp. 1, oed2 = *Oedogonium* sp. 2, scaa = *Scenedesmus acuminatus*, scqu = *S. quadricauda*, psca = *Pseudanabaena catenata*, teca = *Tetraëdron caudatum* and tela = *Tetralanthos lagerheimii*.

Division 1 separated control and N addition treatment sampling units from those with P addition (P^+ and NP^+), with an eigenvalue (λ) of 0.3795.

Division 2 (λ = 0.3559), including 32 sampling units, separated control from the N^+ treatment. Subsequent divisions resulted in 8 sampling groups designated A, B, C, D, E, F, G and H.

The first and second division levels separated sampling units by treatments. From that level on, separation was mainly due to the season of the year, and groups were the following:

Group A: 4 sampling units referring to control in the winter.

Group B: 4 sampling units referring to control in the fall.

Group C: 4 sampling units referring to control in the summer.

Group D: 4 sampling units referring to control in the spring.

Group E: 4 sampling units referring to N^+ treatment in the spring.

Group F: 4 sampling units referring to N^+ treatment in the summer and one in the fall.

Group G: 3 sampling units referring to N^+ treatment in the fall and one in the winter.

Group H: 3 sampling units referring to N^+ treatment in the winter.

Division 3 (λ = 0.3572) included all sampling units from P^+ enriched treatments (P^+, NP^+) and separated seasons of the year: spring, summer and fall were placed in the negative group and winter in the positive one. Subsequent divisions resulted in 5 sampling groups (I to M). In the second division level, seasons of the year weighted more than treatments. From the 3rd level on, sometimes either season of the year or kind of treatment most contributed to the division (Figure 4, Table 1). Groups were the following:

Group I: 4 sampling units referring to P^+ treatment in the spring.

Group J: 4 sampling units referring to P^+ treatment in the summer.

Group K: 16 sampling units referring to P^+ treatment in the fall and to NP^+ in the spring, summer and fall.

Group L: 4 sampling units referring to P^+ treatment in the winter.

Group M: 4 sampling units referring to NP^+ treatment in the winter.

Three species most contributed for division 1: *Cosmarium margaritatum* (Lundell) Roy & Bisset (coma: 91% frequency) and *Frustulia rhomboides* (Ehrenberg) De Toni var. *saxonica* (Rabenhorst) De Toni (frsa: 69% frequency), which were exclusively present in the control and N^+ treatment, and *Tetrallantos lagerheimii* Teiling (tela: 72% frequency) due to its unique presence in treatments with P^+ (P^+, NP^+).

Indicating species for division 2 were: *Monoraphidium irregulare* (G.M. Smith) Komárková-Legnerová (moir) and *Cosmarium exiguum* Archer (coex), both with 81% frequency in the nitrogen enriched treatment and absent in the control. In all other divisions of the latter two treatments (divisions 4, 5, 8, 9, 10 and 11), the frequency of indicating species varied from 50 to 100% in one of the groups, being absent in the remaining ones.

Indicating species of division 3 (sampling units of isolated and combined P additions) were *Scenedesmus acuminatus* (Lagerheim) Chodat (scaa), *Scenedesmus quadricauda* (Turpin) Brébisson *sensu* Chodat (scqu) and *Tetraëdron caudatum* (Corda) Hansgirg (teca), all of them with a 100% frequency during winter and absent in all other treatments and seasons of the year.

Table 1 summarizes the variation in the species composition in all treatments. Two initial groups are easily distinguished, which include the species exclusively present in the isolated N addition treatment and in the control, respectively. In the middle of table are the species found in all treatments and all seasons of the year, such as *Chlamydomonas sordida* Ettl, *Scenedesmus ecornis* (Ehrenberg) Chodat and *Gomphonema parvulum* Kützing, with 94, 88 and 84% distribution in the sampling units, respectively. Some species were more frequently distributed in treatments with P addition (P^+, NP^+), although not exclusive of such treatments. The most prominent among them is the diatom *Nitzschia palea* (Kützing) W. Smith, occurring with 81% frequency in treatments with isolated or combined phosphorus addition, contrasting to 15% in the remaining treatments.

Table 1. Species *versus* sampling units (64) classified by TWINSPAN. Letters (A-M) indicate the sampling groups formed from the experimental conditions (control, N^+, P^+ and NP^+) in the four seasons of the year. Underlined species: indicators of divisions 1, 2 or 3. Presence (1) and absence (--).

Periphytic algal taxa	Treatments and sampling groups		
	Control	Treatment N^+	Treatment with addition of P (P^+, NP^+)
	A B C D	E F G H	I J K L M
	AAAABBBBCCCCDDDD	EEEEFFFFFGGGGHHH	IIIIJJJJKKKKKKKKKKKKKKKKLLLLMMMM
Nephrocytium schilleri	----------11----	----111-11------	--------------------------------
Aphanothece smithii	----------------	----1--11-------	--------------------------------
Merismopedia elegans	----------------	-1-1------------	--------------------------------
Oscillatoria sancta	----------------	-------1--------	--------------------------------
Ankistrodesmus bibraianus	----------------	1-1--1--1--1----	--------------------------------
Closteriopsis acicularis	----------------	111111----------	--------------------------------
Dictyosphaerium chlorelloides	----------------	-----1----------	--------------------------------
Monoraphidium contortum	----------------	-1---1--11------	--------------------------------
Nephrocytium lunatum	----------------	----111---1-----	--------------------------------
N. limneticum	----------------	-------11-------	--------------------------------
Protoderma viride	----------------	-1--------------	--------------------------------
Raphidocelis contorta	----------------	-------1--------	--------------------------------
Scenedesmus acutus	----------------	-1--------------	--------------------------------
Desmodesmus armatus	----------------	1---------------	--------------------------------
Willea irregularis	----------------	----11-1--------	--------------------------------
Closterium dianae	----------------	---111-1--------	--------------------------------
C. setaceum	----------------	-1-11-----1-----	--------------------------------
Cosmarium abbreviatum	----------------	111---1---------	--------------------------------
C. contractum var. *minutum*	----------------	111111111-1-----	--------------------------------
C. bioculatum	----------------	11--------------	--------------------------------

Continuation of Table 1

Periphytic algal taxa	Treatments and sampling groups		
	Control	Treatment N^+	Treatment with addition of P (P^+, NP^+)
	A B C D	E F G H	I J K L M
	AAAABBBBCCCCDDDD	EEEEFFFFFGGGGHHH	IIIIJJJJKKKKKKKKKKKKKKKKLLLLMMMM
C. pygmaeum	----------------	----1-----------	--------------------------------
Euastrum sp.	----------------	11--11-11-1-----	--------------------------------
Staurastrum iversenii var. *americanum*	----------------	----1-----------	--------------------------------
S. dickiei	----------------	-1--------------	--------------------------------
S. rotula	----------------	11----1---------	--------------------------------
S. volans	----------------	1---------------	--------------------------------
S. mamillatus	----------------	-----111--------	--------------------------------
Staurodesmus convergens	----------------	1---11--11------	--------------------------------
Xanthidium armatum	----------------	--11------------	--------------------------------
Dinobryon divergens var. *schauinslandii*	----------------	----11----------	--------------------------------
Euglena acus	----------------	-1111111--1-1---	--------------------------------
Lepocinclis ovum	----------------	-------1--------	--------------------------------
Phacus orbicularis	----------------	-----1----------	--------------------------------
P. platalea	----------------	-----1----------	--------------------------------
P. pleuronectes	----------------	--1--1-1--------	--------------------------------
Trachelomonas armata	----------------	---1--1---------	--------------------------------
T. superba	----------------	-----1----------	--------------------------------
Anomoeoneis vitrea	----------------	----11-11--1----	--------------------------------
Amphipleura lindheimerii var. *lindheimerii*	----------------	-11111----------	--------------------------------
Aulacoseira granulata	----------------	111-1111--------	--------------------------------

Continuation of Table 1

Periphytic algal taxa	Treatments and sampling groups		
	Control	Treatment N+	Treatment with addition of P (P+, NP+)
	A B C D	E F G H	I J K L M
	AAAABBBBCCCCDDDD	EEEEFFFFFGGGGHHH	IIIIJJJJKKKKKKKKKKKKKKKKLLLLMMMM
A. granulata var. *angustissima*	----------------	11--1-----------	--------------------------------
Cymbella ventricosa	----------------	1-1-1-----------	--------------------------------
Eunotia lunaris	----------------	----1-----------	--------------------------------
E. flexuosa	----------------	----111---------	--------------------------------
E. monodon	----------------	--1-------------	--------------------------------
Gomphonema acuminatum	----------------	1---11-1--------	--------------------------------
G. angustatum	----------------	--11------------	--------------------------------
Pinnularia divergens	----------------	11--------------	--------------------------------
P. gibba	----------------	----111---------	--------------------------------
P. viridis	----------------	--1--111-1------	--------------------------------
Rhizosolenia longiseta	----------------	--------1-------	--------------------------------
Sellaphora pupula var. *pupula*	----------------	1-11--1-1-1-1---	--------------------------------
Synedra ulna	----------------	-------1--------	--------------------------------
Aphanocapsa delicatissima	----------------	--------11111---	--------------------------------
Merismopedia tenuissima	----------------	-1-----1------1-	--------------------------------
Phormidium tenue	----------------	--------1111----	--------------------------------
Snowella atomus	----------------	--------111-----	--------------------------------
Botryococcus braunii	----------------	----------11----	--------------------------------
Bulbochaete sp.	----------------	--------------1-	--------------------------------
Chlorela vulgaris	--------------1-	----1-1--1--1111	--------------------------------

Continuation of Table 1

Periphytic algal taxa	Treatments and sampling groups		
	Control	Treatment N⁺	Treatment with addition of P (P^+, NP^+)
	A B C D	E F G H	I J K L M
	AAAABBBBCCCCDDDD	EEEEFFFFFGGGGHHH	IIIIJJJJKKKKKKKKKKKKKKKKLLLLMMMM
Coelastrum astroideum	----------------	---1---1--11----	--------------------------------
C. microporum	----------------	111111-11-1111--	--------------------------------
Kirchneriella irregularis	----------------	----------1-----	--------------------------------
K. irregularis var. *spiralis*	----------------	---------1------	--------------------------------
K. lunaris	----------------	---1--------1---	--------------------------------
Monoraphidium irregulare	----------------	11111111--1111-1	--------------------------------
M. minutum	----------------	--------1111----	--------------------------------
M. nanum	----------------	----------11-1-1	--------------------------------
Pediastrum tetras	----------------	-1----1----11---	--------------------------------
Tetrastrum triangulare	----------------	1---------11---1	--------------------------------
Cosmarium contractum	----------------	----------11----	--------------------------------
C. margaritatum f. *minor*	----------------	------1---11----	--------------------------------
C. subtumidum	----------------	-----1111111----	--------------------------------
Staurodesmus cuspidatus	----------------	------1--111----	--------------------------------
Phacus oblongus	----------------	------------1---	--------------------------------
Cymbella silesiana	----------------	---------111--11	--------------------------------
Eunotia tenella	----------------	1-------11--1---	--------------------------------
Gomphonema augur var. *augur*	----------------	----------1-----	--------------------------------
G. augur var. *turris*	----------------	-------11-----11	--------------------------------

Continuation of Table 1

Periphytic algal taxa	Treatments and sampling groups		
	Control	**Treatment N⁺**	**Treatment with addition of P (P⁺, NP⁺)**
	A B C D	E F G H	I J K L M
	AAAABBBBCCCCDDDD	EEEEFFFFFGGGGHHH	IIIIJJJJKKKKKKKKKKKKKKKKLLLLMMMM
Ankistrodesmus falcatus	------------11-1	11-11111111111--	--------------------------------
Frustulia rhomboides	------------1111	111-1111111111--	--------------------------------
Gomphonema intricatum	--1-------------	1---1111-1111---	11-1----------------------------
Synedra acus	1111------------	11111-1-11111111	--------------------------------
Ankistrodesmus spiralis	------11--------	--------1-111---	--------------------------------
Frustulia rhomboides var. *saxonica*	11111111--------	111-1111111-1111	--------------------------------
Ankistrodesmus bernardii	----1111--------	-1-----11-11----	--------------------------------
Scenedesmus obtusus	--------1111----	1---111----1----	--------------------------------
Cosmarium margaritatum	1111111111111111	1111111111111---	--------------------------------
Staurastrum quadrangulare	----111111111111	111-111111111-11	--------------------------------
Geitlerinema unigranulatum	11111111--------	----------------	--------------------------------
Chlamydomonas epibiotica	1111----------1-	----------------	11------------------------------
C. gloeopara	11111111--11----	--1--------111--	--------------------------------
Dictyosphaerium pulchellum var. *minutum*	----111-11111111	------1---------	--------------------------------
Peridinium umbonatum	----------------	----1111---1-111	------------------------1111----
Cymbella mesiana	----------------	----1111111111--	----1111------------------------
Dictyosphaerium pulchellum	111--111--------	1111111111111111	----------------111111111111----
Scenedesmus acuminatus var.	------------1111	1111111111111111	------------------------11111-11
Cosmarium exiguum	----------------	-1--111111111111	------------1111--------1111----

Continuation of Table 1

Periphytic algal taxa	Treatments and sampling groups		
	Control	Treatment N^+	Treatment with addition of P (P^+, NP^+)
	A B C D	E F G H	I J K L M
	AAAABBBBCCCCDDDD	EEEEFFFFFGGGGHHH	IIIIJJJJKKKKKKKKKKKKKKKKLLLLMMMM
Scenedesmus disciformis	--------1111----	----111111111--1	-1111111------------------------
Staurastrum tetracerum	----111111111111	1111111111-1----	1111----------------1111--------
Chlamydomonas sordida	11111111111111-1	1111111111111111	1111111111111111111111111111----
Scenedesmus ecornis	1111111111111111	1111111111111111	--------111111111111111111111111
Gomphonema parvulum	1111111111111-11	1111111111111111	1111111111111111111-1111--------
Monoraphidium arcuatum	----1111----111-	1111--1111-11111	1111------------1111----1111----
Gomphonema gracile	--------11111111	1111111111111111	----1111----1111----1111----1111
Aphanocapsa elachista	----1111--------	1--1--1111111111	-1-1------------11111111111-----
Leptolyngbya perelegans	--------1111----	1111111111111111	----1111----11111-111111--------
Scenedesmus quadricauda	----------------	1-1-1-111--1-111	------------------------11111111
Lagynion ampullaceum	----------------	-------11-----11	1111----------------------------
Scenedesmus acutus	----------------	------------111-	----------------------------1111
S. acuminatus	---------11-----	------------1-1-	------------------------11111111
Tetraëdron caudatum	----------------	----------11---1	------------------------11111111
Nephroclamys willeana	----------------	----11--11-1----	------------1111-111----11111111
Oedogonium sp. 2	----------------	----1111111-----	----1111----------------11111111
Chlamydomonas sagitulla	----------------	----------11--1-	----------------111------1------
Tetraëdron minimum	----------------	----------11-1-1	--------------------1111--------
Achnanthidium microcephalum	----------------	---1111-111111-1	------------1111111-1111--------
Monoraphidium circinale	----------------	-----1----111---	---------111--------11111111----

Continuation of Table 1

Periphytic algal taxa	Treatments and sampling groups		
	Control	Treatment N^+	Treatment with addition of P (P^+, NP^+)
	A B C D	E F G H	I J K L M
	AAAABBBBCCCCDDDD	EEEEFFFFFGGGGHHH	IIIIJJJJKKKKKKKKKKKKKKKKLLLLMMMM
M. griffithii	----11111111----	1-1-1-1111111111	1111111111111111111111111111----
Oedogonium sp. 1	--------1111----	---------111111-	----1111----1111111111111-111111
Desmodesmus dispar	----------------	------------11--	------------11-1--------------11
Cryptomonas marssonii	----1111--------	----------------	----11-111111111-------------1--
Trachelomonas volvocinopsis	--------1111----	-11-111111111111	11111111----11111111111111111111
Chromulina verrucosa	111111111111----	----------111111	11111111111111111111111111111-11
Heterothrix stichococcoides	1111--------111-	----------------	1111111-111----1-------------111
Pseudoanabaena catenata	----------------	----------------	-----111--------1111----1111----
Chlamydomonas planctogloea	----------------	------------11-1	------11--------111111111111----
Scenedesmus spinosus	----------------	----------------	----------------------------11--
Ulothrix subtilissima	----------------	----------------	------------------------------11
Cryptomonas erosa	----------------	----------------	-----------------------------111
Chromulina elegans	----------------	----------------	1111--------------------11------
Tetrallantos lagerheimii	----------------	----------------	--------11111111111111111111-111
Chloromonas grovei	----------------	----------------	----------------111-------------
Oocystis lacustris	----------------	---------------1	----1111------------------------
O. parva	----------------	----------------	----------------11--------------
Stigeoclonium sp.	----------------	----------------	-------------111-----11---1-----
Heimansia pusilla	----------------	------11--------	----1111------------1111--------
Cryptomonas curvata	----------------	----------------	---------1111111----1111-----111

Continuation of Table 1

Periphytic algal taxa	Treatments and sampling groups		
	Control	Treatment N+	Treatment with addition of P (P+, NP+)
	A B C D	E F G H	I J K L M
	AAAABBBBCCCCDDDD	EEEEFFFFFGGGGHHH	IIIIJJJJKKKKKKKKKKKKKKKKLLLLMMMM
C. ovata	----------------	----------------	----11-1-11-----------1---------
C. obovata	----------------	----------------	----1-11----1-------------------
Nitzschia palea	------------1-11	1-1-------------	-1111111111111111111111-1111----
Spirogyra sp.	----------------	--1-11----111---	-----111----111111111-11--------

DISCUSSION

Ninféias Pond periphyton species richness was more affected by the season of the year than by the experimental conditions, since it was greater in summer and fall, independently of the kind of treatment. These results confirm previous studies carried out in the PEFI area, that involved comparison of the periphyton succession in two seasons of the year, one of them in an oligotrophic and the other one in a eutrophic reservoir (Vercellino 2001), and the experimental oligotrophication study carried out in one eutrophic system (Barcelos 2003). In the latter two systems, species richness did not vary in relation to the system trophy. Enrichment works carried out during the winter in an oligotrophic reservoir located in the PEFI area (IAG Pond) demonstrated the increase of species richness with isolated P addition, although little richness change occurred with the increasing P addition levels (Ferragut & Bicudo 2009). Particularly for the Ninféias Pond, only during the winter increase in species richness of periphyton was observed after isolated P addition.

In terms of classes, Chlorophyceae were represented by the greatest number of taxa in all treatments and seasons of the year (Figure 2). According to Stevenson (1996), in general, Chlorophyceae present great species richness in the periphyton. Qualitative dominance of Chlorophyceae and mostly of Chlorococcales is common in various tropical and subtropical lacustrine environments both in the periphyton (e.g. Ferragut *et al.* 2005, Vercellino & Bicudo 2006, Ferragut & Bicudo 2009) and the phytoplankton (e.g. Figueiredo & Giani 2001, Ferragut *et al.* 2005, Fonseca & Bicudo 2011), regardless of the system's nutritional conditions.

Regarding the species present in different experimental conditions, response of periphyton community was markedly distinct. Similarity was mainly influenced by the kind of treatment, since associations of control, isolated or combined P addition and isolated N addition treatments were grouped. Mainly when P was not added (control, N^+), seasonal variation was important, followed by succession days that grouped together the respective treatments and seasons of the year with similarities greater than 80%. It is also observed that during winter, periphyton community formed a separate group (90% SIMI) under NP combined addition and a separated subgroup with isolated P addition. Periphyton community composition response to different N and/or P addition was also verified in an oligotrophic reservoir within the PEFI area (IAG Pond), in which similarity responded mostly to P availability (Ferragut & Bicudo 2009). Periphyton species associations were also good indications of environmental conditions in the floodplain of the high Paraná river, since it separated the kind of environment (lentic, semilotic and lotic), followed by the season of the year (high waters, low waters) and, finally, the kind of substrate (Rodrigues & Bicudo 2001).

Indicative species double entrance analysis indicated that the hierarchic divisor classification of periphyton species was guided by P availability. Under low P availability (control and N^+), type of treatment and season of the year were important factors in determining the indicative species grouping. Under P addition (P^+ and NP^+), the most important variable was first the season of the year, followed by the kind of treatment. Thus, with P availability increase, seasons of the year became more importance in the species classification.

Species associated to low P availability (C, N^+) are *Cosmarium margaritatum* and *Frustulia rhomboides* var. *saxonica* (TWINSPAN division 1). Other *Cosmarium* species (*C. exiguum*, *C. contractum* Kirchner) were also associated with that (TWINSPAN divisions 2 and 5) depending on the treatment (C, N^+) and the season of the year. Desmids have a preference for acid waters with pH between 4.5 and 7.0, several species being commonly found in oligotrophic environments (Ruts 1983) and, mostly, in the periphyton or metaphyton communities (Coesel 1996). Luxurious aquatic vegetation at the Ninféias Pond, slightly acid (pH 5.9-6.8), but mainly nutritional conditions (total phosphorus 7.3-22.7 mg L^{-1}, present study) most probably favored dominance of desmids. Regarding diatoms, either *Frustulia rhomboides* var. *saxonica* (TWINSPAN division 1) or *Gomphonema gracile* Ehrenberg (TWINSPAN division 4, control, fall and winter) were reported associated to oligotrophic systems (Moro & Fürstenberger 1997).

Four Chlorococcales species (*Tetrallantos lagerheimii*, *Scenedesmus acuminatus*, *S. quadricauda* and *Tetraëdron caudatum*, (TWINSPAN division 1 and 3) indicated isolated or combined P enrichment conditions. In the phytoplankton, especially genus *Scenedesmus* is favored by high P concentrations (Reynolds 1984, Happey-Wood 1988, Gonzalez & Ortaz 1998), *S. ecornis* and *S. quadricauda* being frequently related to mesotrophic and eutrophic environments (Rosen 1981, Patrick & Palavage 1994). In the periphyton, *Scenedesmus* species were also present in N and/or P enriched systems (Fairchild *et al.* 1989, Ferragut & Bicudo 2009) and in the eutrophic reservoir in the PEFI area (Barcelos 2003).

Therefore, periphyton algal species and class richness were not sensitive to artificial enrichment at the Ninféias Pond. Regarding exclusive species, nitrogen amendments markedly contributed to the total species numbers. However, species associations were clearly influenced by P availability that was considered the limiting or primary limiting nutrient of periphyton in the Ninféias Pond, as well as in other reservoirs in the PEFI area (Huszar *et al.* 2005).

Descriptive and experimental studies carried out in the Florida Everglades also recognized P as the main limiting factor for determining taxonomic composition and the loss of oligotrophic species associations, and leading to consequences for the ecosystem stability even with P levels a little above the basal one (McCormick *et al.* 1996, McCormick & O'Dell 1996, Pan *et al.* 2000).

Present results suggested that Ninféias Pond, a shallow system with almost total light penetration throughout the entire year (Fonseca & Bicudo 2011) and luxurious aquatic macrophytes (i.e. with prevalence of littoral biota), may have profound changes in its native associations with P inputs.

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