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Original article

Do phytoplankton and epiphyton communities differ between organic and conventional rice fields?

Ana Paula Vestena Cassol[1*], Renato Zanella[2] and Lezilda Carvalho Torgan[1]

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ABSTRACT

This study aimed to determine if there are any differences in the attributes and composition of the phytoplankton and epiphyton communities between organic (OF) and conventional (CF) rice fields. We also strove to identify if there were any variations in these communities by comparing samples taken from two different periods (12 and 35 days) after the application of the herbicide clomazone and penoxsulam in CF. The farms are located in the Pampa Biome, Southern Brazil. Phytoplankton samples from the subsurface water and epiphyton samples from the rice stems were analyzed using the Utermöhl method. The CF and OF had distinct environmental conditions (pH, conductivity, and turbidity values), and the residual concentration of the herbicides decreased over time. There were no significant differences in epiphyton and phytoplankton density, or in phytoplankton richness, between the rice fields; only the epiphyton richness and taxonomic composition showed differences between the rice fields. Cyanobacteria and Chlorophyceae comprised a large proportion of the epiphytic density in CF and OF, respectively. However, Bacillariophyceae and Chlorophyceae had greater phytoplankton densities in CF and OF, respectively. The taxonomic composition of communities should be considered an effective tool to show the differences between the two cultivation systems.

Keywords: agroecosystem, artificial wetlands, community structure, herbicide, microalgae

Introduction

Wetlands are internationally recognized ecosystems that are subject to environmental protection and regulation (Millennium Ecosystem Assessment 2005). The recent classification proposed by Junk *et al.* (2013) defines three categories of Brazilian wetlands: coastal, continental, and artificial. According to this classification, artificial wetlands, such as rice farming, are the result of human activities.

It is possible to distinguish between both the conventional and organic rice cultivation systems. In Brazil, the current system in use is the conventional system that uses a large

1 Departamento de Botânica, Universidade Federal do Rio Grande do Sul, 9150-9900, Porto Alegre, RS, Brazil
2 Departamento de Química, Universidade Federal de Santa Maria, 9710-5900, Santa Maria, RS, Brazil

* Corresponding author: anapvcassol@gmail.com

amount of pesticides, which threatens the biota and ecosystem functioning. Organic management contributes to improving the sustainability of agroecosystems by avoiding the use of chemical fertilizers and pesticides, and is based on integrative ecosystem services. According to Maltchik *et al*. (2017), encouraging sustainable practices in these areas may transfer some of the responsibilities in biodiversity conservation to production systems and, thus, contribute positively to regional biodiversity.

Rice paddy fields are temporary environments occupied by a rich composition of fauna and flora (Bambaradeniya *et al*. 2004). The biota, especially microalgae, plays an important role in soil stabilization and fertility (Roger *et al*. 1991; Bellinger & Sigee 2010). However, little attention has been paid to phytoplankton and epiphyton in the literature. Phytoplankton diversity was revealed by Shivakurama & Patar (2015) in a rice field in India. Additionally, the rice itself provides the substrate for periphyton colonization and growth and is used as a food source in rice–fish farming (Das *et al*. 2007; Saikia & Das 2011; 2014).

The microalgae composition in paddy fields has already been registered on previous studies. Diatoms have mainly been investigated in Japan (Negoro & Higashino 1986; Ohtsuka & Fujita 2001; Fujita & Ohtsuka 2005). Furthermore, the pigmented members of Euglenophyceae were investigated by Alves da Silva & Tamanaha (2008) in Southern Brazil, Cyanobacteria were studied by Irisarri *et al.* (2001) in Uruguayan rice fields and by Prasana & Nayak (2007) in India, and members of Chlorophyceae were investigated by Kumar & Sahu (2012) in India.

However, there is no field comparative information about algal communities in organic and conventional rice farming with herbicide application. Furthermore, the effects of herbicides on epiphytic and planktonic algae in paddy rice fields have been described mainly in mesocosms (Sartori *et al.* 2011; Cassol *et al*. 2013; Reck *et al.* 2018; Liu *et al.* 2020). This knowledge gap motivated us to investigate the phytoplankton and epiphyton in the two rice cultivation systems. Quantifying the responses of these algal groups to agricultural systems will allow us to understand the limitations and benefits of the local biodiversity, and the results are relevant for implementing sustainable management practices with low environmental impacts (Katayama *et al.* 2019).

Our aim was to determine whether the attributes (richness, density, diversity, and equitability) and composition of phytoplankton and epiphyton communities differed between organic and conventional rice fields. The attribute variations after the application of clomazone and penoxsulam herbicide in the conventional field were also evaluated. Considering the differences in the environmental conditions of the rice fields, we expected to find distinctions among the attributes of the phytoplankton and epiphyton communities.

Materials and methods

Study area

The organic and conventional farms are located in Rio Grande do Sul, Southern Brazil at W 29°69’ and S 55°85’, and W 29°50’ and S 55°66’, respectively.

The conventional farm (CF) is located 12 km from the urban center of Alegrete City (Fig. 1). A no-tillage cultivation system was used with chemical fertilization and was carried out according to the recommendations of the Irrigated Rice Brazilian Society (SOSBAI, *Sociedade Brasileira de Arroz Irrigado*, in Portuguese). The rice farm is located in the lowland of the Ibirapuitã River, which have traditionally been used for irrigated rice farming since 1986. The region is characterized by the predominant occurrence of basaltic volcanic rocks (with relief in the form of plateaus and escarpments) that are a part of the rocky and sandy riverbed (Hasenack *et al*. 2010). The Ibirapuitã River has an extension of 260 km in a sinuous course and an almost flat relief that floods in rainy seasons. These floods are supplied with organic load from livestock and agriculture, which are the predominant activities in the region.

The organic farm (OF) is located in Manoel Viana City in the Santa Maria do Ibicuí settlement, 22 km from the urban center and 45 km away from the CF (Fig. 1). Currently, 400 ha of rice are cultivated by 224 families belonging to the Association of Organic Producers of Santa Maria do Ibicuí Settlement (Ramos 2012). The rice paddy water comes from the Ibicuí River, which extends 385 km into the lowlands and sandy riverbed in the Paleozoic lands of the Paraná sedimentary basin (Central Depression). This river has several tributaries, one of which is the Ibirapuitã River. The cultivation system used in the organic farm involved pre-germinated seedlings and does not use chemical fertilization or pesticides.

Sampling and data

Phytoplankton and epiphyton samples were taken after the flooding of the rice field in two periods (12 and 35 days after the spraying of herbicides). From both fields, three sample units were collected 600 m apart from each other. Three unit samples from the CF were taken on December 2, 2018 and on December 25, 2018: CF1 (29’69”09 55’84”95), CF2 (29’69”18 55’85”11), CF3 (29’69”74 55’84”53). Three unit samples from the OF were also taken at OF1 (29’50”26 55’66”07), OF2 (29’50”10 55’66”35), OF3 (29’50”21 55’65”75).

Phytoplankton samples were collected from the surface of the rice paddy water using a 100 mL glass bottle, and the epiphyton samples were collected via brushing three rice stems of 3cm lengths, totaling a composite sample of 25 cm^2. This was done in both the conventional and organic fields. The samples were preserved in Lugol’s alkaline solution (Sournia 1978) and housed at Alarich Schultz Herbarium (number 110934 – 110957), Museu de Ciências Naturais, Fundação Zoobotânica do Rio Grande do Sul in Porto Alegre, Brazil.

The densities of phytoplankton (ind.mL^{-1}) and epiphyton (ind.cm^{-2}) were estimated according to the method of Uthermöhl (1958) in 5 mL chambers, using an inverted microscope (Zeiss Axiovert) after 24h of sedimentation. This was done via counting in transect fields, with the aim of achieving an efficiency of 80 % (Pappas & Stoermer 1996). Only individuals with plastids and those that were alive at the time of collection were quantified. Identification was performed using an inverted Zeiss microscope (640× magnification). Richness was estimated based on the number of taxa at generic and infra-specific levels. The abundance was determined according

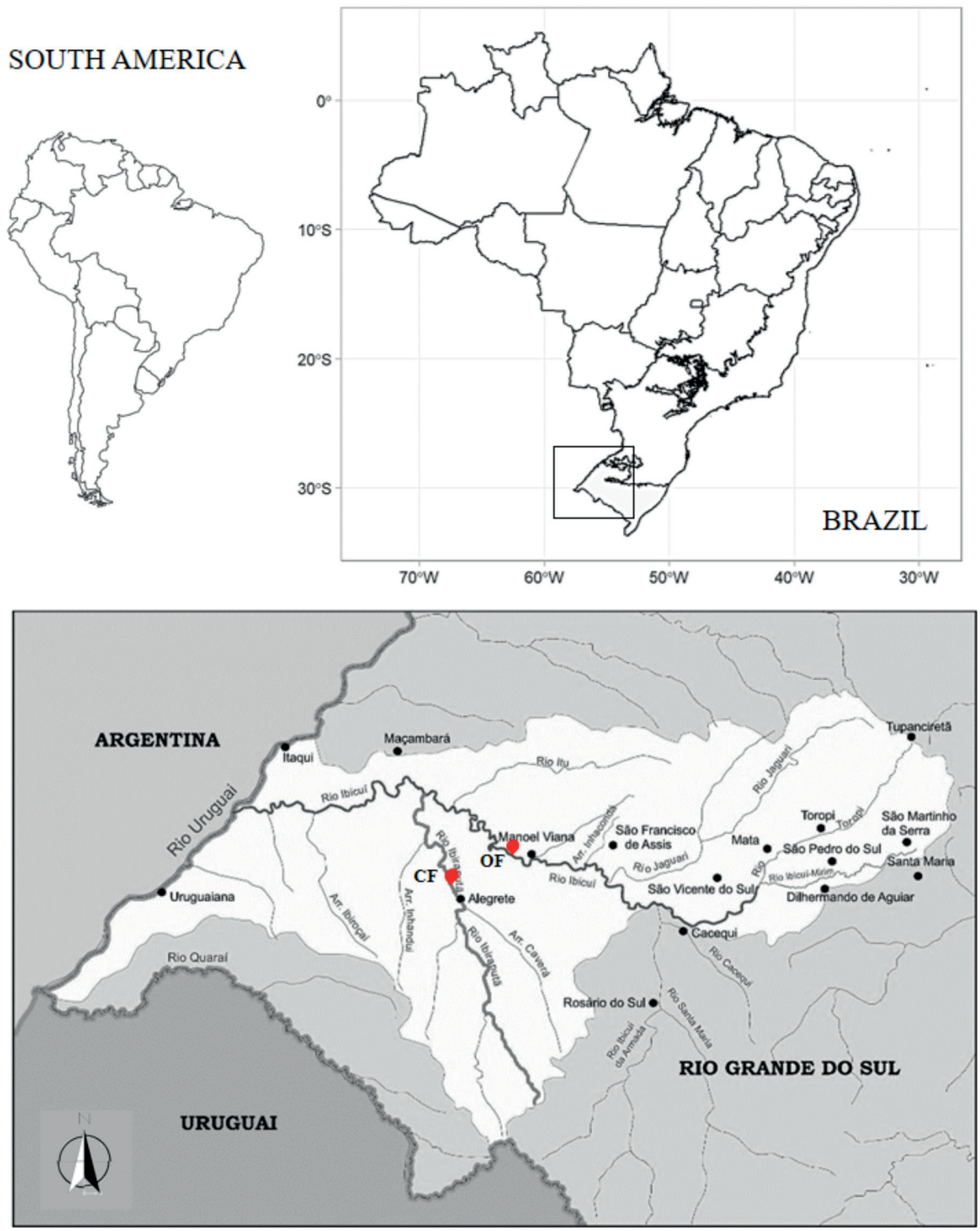

Figure 1. Location of the study sites of conventional farm (CF) and organic farm (OF) in the Ibicuí River watershed, Southern Brazil.

to the method of Lobo & Leighton (1986). Shannon's diversity (H´) and equitability indices (E) were used as measures of community structure.

The presence and concentration of residual herbicides (penoxsulam and clomazone) in the water were detected using the solid phase extraction method. These were then analyzed via liquid chromatography with mass spectrometry (LC-MS/MS) in the Pesticide Residue Laboratory (LARP) at the Federal University of Santa Maria, according to the method of Donato *et al.* (2012).

The water temperature (temp), hydrogen potential (pH), electrical conductivity (Cond), dissolved oxygen (DO), turbidity (Turb), and total dissolved solids (TDS) were measured in situ using a Horiba U-50 – Multiparameter probe. The data from the conventionally farmed field were obtained in the morning, but with the organically farmed field the measurements were made in the afternoon on the same sampling day.

After logarithmic transformation of the data to achieve normality, we used the two-sample T test to determine if there are differences between the periods and the cultivation systems in both communities. Permutational analysis of variance (PERMANOVA) was performed to verify differences in the composition of species in the phytoplankton and epiphyton communities of the rice fields. Nonmetric multidimensional scaling (NMDS) was applied to the abundant species matrix to investigate the species distribution at the sites of the organically and conventionally farmed rice fields. The community matrices were transformed using Wisconsin double standardization and a Bray–Curtis dissimilarity matrix was calculated using the vegan "metaMDS" function in R. The IndVal method (Dufrêne & Legendre 1997) was used to identify indicator species in the cultivation systems. An overall analysis was performed using the R software (R Development Core Team 2021) and the Vegan package (Oksanen *et al.* 2020).

Results

Physical and chemical water conditions

The physical and chemical rice paddy water conditions are presented in Table 1. The water temperature varied between 18.4 °C and 24.5 °C in the CF and between 22.2 °C and 31.0 °C in the OF.

The pH varied from neutral to slightly acidic in the CF, and was strongly acidic in the OF. The electrical conductivity showed a slight increase in the CF, varying between 60 µs cm^{-1} and 100 µs cm^{-1}, whereas in the OF, the maximum electrical conductivity found in the study period was 20 µs cm^{-1}.

Turbidity varied between a minimum of 2.48 NTU and maximum of 31.20 NTU in the CF, and was greater in the OF (62 - 542 NTU). Meanwhile, total dissolved solids varied from 0.03 mg L^{-1} to 0.06 mg L^{-1} in the CF and between 0.01 mg L^{-1} and 0.02 mg L^{-1} in the OF. Dissolved oxygen showed higher values in the CF (6.5 - 14.8 mg L^{-1}) than those in the OF (4.0 - 7.8 mg L^{-1}).

Herbicide concentrations in the conventionally farmed rice field

The residual concentrations of herbicides in the conventionally farmed fields and in the channel that takes water from the Ibirapuitã River to the crops are presented in Table 2. Average values of 1.29 µg L^{-1} and 0.28 µg L^{-1} were detected for clomazone and penoxsulam, respectively. The residual concentrations of the two herbicides decreased over time, and penoxsulam, which was not detected in some samples after 35 days of spraying, dissipated faster than clomazone.

Phytoplankton community

The phytoplankton species richness in the CF ranged from 24 to 26 species in period 1 and from 25 to 36 species in period 2. In the OF, the richness ranged from 17 to 25

Table 1. Physical and chemical variables of sample units in the conventional farm in Alegrete City, RS, and in the organic farm in Manoel Viana City, RS, 12 and 35 days after the spraying of herbicides in three sample units in periods 1 (12/02/2018) and period 2 (12/25/2018). These sample units were taken at the same day.

Farming	Period	Sample Unit	Temp (°C)	pH	Cond (µs cm^{-1})	Turb (NTU)	DO (mg L^{-1})	TDS (g L^{-1})
Conventional Farming	Period 1	CF 01	18.4	6.4	90	2.7	9.2	0.06
		CF 02	22	6.9	90	2.5	12.0	0.05
		CF 03	21.9	7.4	100	4.5	14.8	0.06
	Period 2	CF 01	22.8	6.1	60	31.2	7.7	0.04
		CF 02	22.9	6.2	80	24.7	6.5	0.05
		CF 03	24.5	6.4	60	20.8	7.3	0.03
Organic Farming	Period 1	OF 01	31	5.3	10	243	7.8	0.01
		OF 02	28.4	5.8	20	174	7.3	0.01
		OF 03	28.8	5.0	20	542	4.0	0.01
	Period 2	OF 01	22.8	5.4	20	100	4.4	0.01
		OF 02	22.2	5.3	20	172	5.4	0.02
		OF 03	23.3	4.6	10	62	4.9	0.01

species in period 1 and from 14 to 40 species in period 2 (Fig. 2). There was no significant variation in richness between periods in either CF or OF ($p > 0.05$). There was also no significant difference in richness between the two cultivation systems ($p > 0.05$).

Table 2. Residual concentrations of herbicides from conventional farm after 12 days (period 1) and 35 days of spraying (period 2). Nd=non detected.

Period	Sample Unit	Clomazone (µg L^{-1})	Penoxsulam (µg L^{-1})
Period 1	CF 01	1.402	0.225
	CF 02	1.336	0.322
	CF 03	1.140	0.304
Period 2	CF 01	0.297	n.d.
	CF 02	0.433	0.091
	CF 03	0.184	n.d.

Specific diversity showed higher values in period 2 (2.43 – 3.05 bits. ind^{-1}) than in period 1 (1.38 - 2.23 bits. ind^{-1}) in the CF. The specific diversity seen in OF was greater in period 2 (1.81 - 2.79 bits. ind^{-1}) than in period 1 (0.45 - 2.15 bits. ind^{-1}) (Fig. 2). The equitability in the CF showed higher values in period 2 (0.72 - 0.84) than in period 1 (0.43 - 0.69) and the equitability in the OF showed higher values in period 2 (0.68 - 0.79) than in period 1 (0.15 - 0.66).

The total density of phytoplankton in CF obtained higher values in period 1 (699 - 8244 ind. mL^{-1}) than in period 2 (175 - 1094 ind. mL^{-1}). In the OF, the density obtained higher values in period 1 (4368 - 62103 ind. mL^{-1}) than in period 2 (374 - 1313 ind. mL^{-1}) (Fig. 2). There was no significant difference in density between the periods in CF, but there was a significant difference in density between the periods in

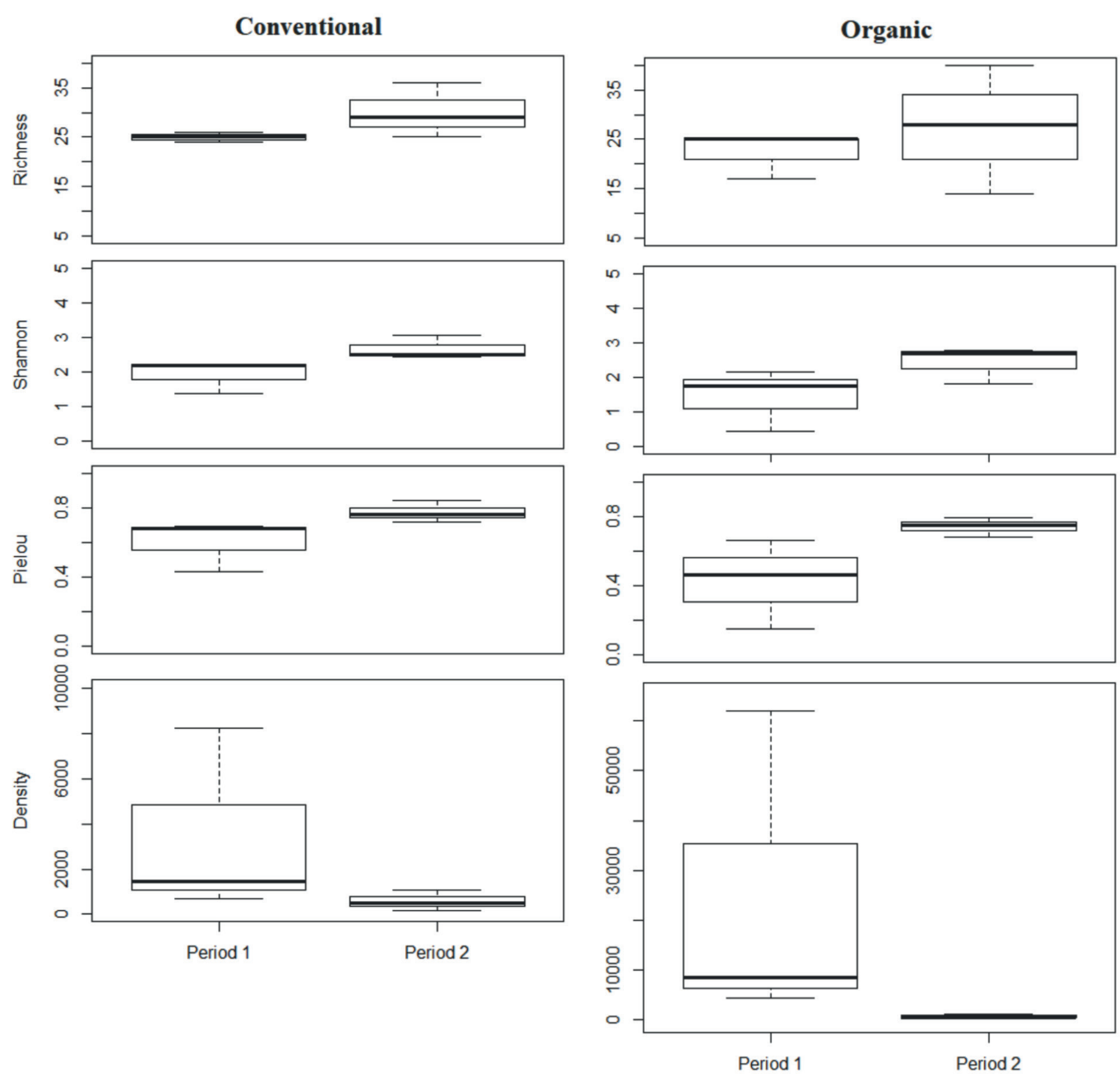

Figure 2. Phytoplankton density (ind.mL^{-1}), richness, diversity, and equitability in conventional (CF) and organic (OF) farms in three samples from the two experimental periods.

OF ($p < 0.05$). Notably, there were no significant differences between the crops in these fields ($p < 0.05$).

The NMDS model showed species distribution according to the organic and conventional fields (Fig. 3). Moreover, the difference in species composition was statistically significant according to PERMANOVA analyses ($p = 0.0003$, $R^2 = 0.22$). The results of the indicator species analysis between the cultivation systems showed that *Nitzschia* cf. *vixnegligenda* Lange-Bertalot & Hofmann and *Stauroneis reichardtii* Lange-Bertalot, Cavacini, Tagliaventi & Alfinito were associated with CF ($p = 0.01$), and *Radiococcus planctonicus* J.W.G.Lund was associated with OF ($p = 0.01$). We identified 86 species belonging to seven classes in CF. The most representative class in terms of density was Bacillariophyceae, followed by Chlorophyceae and Cyanophyceae in period 1; this was followed by Euglenophyceae and Cryptophyceae in period 2. In period 1, within Bacillariophyceae there were abundant *Nitzschia palea* (Kützing) W. Smith and *Nitszchia gracilis* Hantzsch. Within Chlorophyceae, the abundance of *Dictyosphaerium* sp., *Monoraphidium caribeum* Hindák, and *Schoederia* sp. were observed. Among the other classes, we also noted the presence of *Aphanocapsa* sp., *Pseudanabaena* sp. (Cynaophyceae), *Trachelomonas curta* A. M. Cunha (Euglenophyceae), *Cryptomonas ovata* Ehrenberg (Cryptophyceae), *Actinotaenium perminutum* (G. S. West) Teiling, and *Zygnema* sp. (Zygnematophyceae). In period 2, within Bacillariophyceae, *Encyonema silesiacum* (Bleisch) D. G. Mann 1990, *Navicula incarum* U. Rumrich & Lange-Bertalot, *N. palea*, *N. gracilis*, *Pinnularia subcapitata* Gregory, and *S. reichardtii* were abundant. Within Euglenophyceae, the presence of *Trachelomonas verrucosa* var. *granulosa* (Playfair) Conrad & Meel was highlighted, as well as that of *Cryptomonas ovata* Ehrenberg within Cryptophyceae.

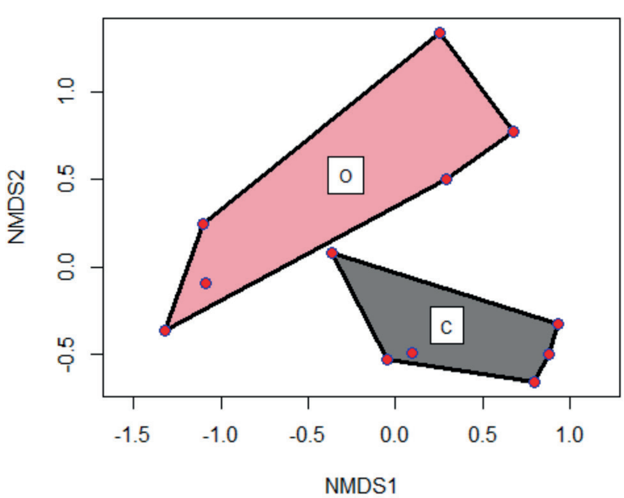

Figure 3. Nonmetric multidimensional scaling (NMDS) ordination of abundant phytoplankton species (stress value = 0.05) in the sample units (●) taken in two time periods from organic and conventional rice paddy fields.

In the OF, we identified 79 species belonging to seven classes. Chlorophyceae followed by Zygnematophyceae were dominant in period 1, with abundance of *Dyctiosphaerium* sp., *Kirchneriella irregularis* (G. M. Smith) Korshikov, *Monoraphidium. caribeum Hindák, M. flexuosum Komárek, M. griffthii (Berkeley) Komárková-Legnerová, Radiococus planktonicus, Actinotaenium perminutum (G.S.West) Teiling, Cosmarium* sp3, *N. palea*, and *C. ovata*. In period 2, the composition of the community was changed to having a dominance of Bacillariophyceae (*Nitzchia* spp.) in sample units 1 and 2, and Euglenophyceae (*Monomorphina* sp., *T. verrucosa* var. *granulosa*, *T. curta*, and *Cryptomonas* sp3) in sample unit 3 (Fig. 4).

Epiphytic community

The epiphyton species richness between the two systems and in the CF between the periods in the experiment was significantly different ($p < 0.05$). Higher values were observed in period 1 (18-23 species) than in period 2 (7-17 species). In the OF, the richness varied between 30 species and 38 species in period 1 and between 10 species and 39 species in period 2; these values were not significantly different ($p < 0.05$) (Fig. 5).

Epiphyton density did not differ significantly between systems ($p > 0.05$). However, the density did vary between 20,315 ind. cm^2 and 36,216 ind. cm^2 in period 1, and between 15.355 ind. cm^2 and 158.058 ind. cm^2 in period 2 in the CF. In the OF, higher values were observed in period 2 (115.542 - 1.986782 ind. cm^2) than in period 1 (50.873 - 248.288 ind. cm^2). Significant differences in density between the collection periods were observed only in the OF ($p < 0.05$) (Fig. 5).

The epiphyton diversity index showed higher values in period 1 (1.24 - 2.19 bits. ind^{-1}) than in period 2 (0.71 - 1.96 bits. ind^{-1}) in the CF. In the OF, the attribute showed higher values in period 1 (1.93 - 2.63 bits. ind^{-1}) than in period 2 (0.11 - 1.57 bits. ind^{-1}). In the CF, the equitability ranged from 0.42 to 0.70 in period 1 and from 0.36 to 0.69 in period 2. In the OF, the values ranged from 0.54 to 0.72 in period 1 and ranged from 0.05 to 0.55 in period 2 (Fig. 5).

The NMDS model showed distribution of abundant epiphytic species corresponding to OF and CF (Fig. 6). However, according to PERMANOVA analyses, there was no significant difference in composition between the two cultivation systems ($p > 0.05$). Moreover, there were no epiphytic indicator species in either of the cultivation systems.

We identified 54 species belonging to six classes in the CF. In period 1, the most representative classes in density were Cyanophyceae and Bacillariophyceae, with an abundance of *Anagnostidinema amphibium* (C. Agardh ex Gomont) Strunecký, Bohunická, J. R. Johansen & J. Komárek ; *N. palea*; and *N.* cf. *vixnegligenda*. In period 2, Cyanophyceae (*A. amphibium*), Cryptophyceae (*Cryptomonas erosa* Ehrenberg, *Chroomonas* cf. *coerulea* (Geitler) Skuja), and Bacillariophyceae (*Nitzschia* spp.) were highlighted in sample unit 3 (Fig. 7).

We identified 91 epiphytic species in the OF. In period 1, Chlorophyceae was predominant, followed by Zygnematophyceae, with abundance of *Dictyosphaerium* sp., *K. lunaris* var. *irregularis*, *Monoraphidium circinale* Nygaard, *M. griffithii* (Berkeley) Komárková-Legnerová, *Oedogonium* sp., *R. planktonicus*, *Actinotanenium perminutum*, and *N. palea*. Cyanophyceae and Cryptophyceae dominated in period 2, with *A. amphibium*, *C. erosa*, and *C.* cf. *coerula* as the main species (Fig. 7).

Discussion

Organic versus conventional farming

Organic and conventional farming have different planting systems. Pre-germinated planting is used in the OF, where flooding occurs during soil preparation and there is the turning over of residual plants from the previous crop; whereas, in the CF, flooding occurs after the spraying of herbicides and no-tillage is used (SOSBAI 2018). These planting systems result in different environmental conditions. In the rice paddy water in OF, higher turbidity values were recorded (> 62 NTU) because of the suspension of solids that is promoted by the practice of turning over residual plants (Furtado & Luca 2003). The lower values of pH observed (4.6 - 5.8) is because the anaerobic conditions in flooded soil as a result of decomposition process that produces organic acids (Bohnen *et al.* 2005). Lower pH values were obtained in rice fields in India that were organically fertilized (Sihi *et al.* 2020). The lower water stream flow in the OF due to cultivation in the plots may explain the high temperature and low oxygen concentration in the water during the experimental period. Lower values of dissolved oxygen (4 - 7.8 mg L^{-1}) and pH were also observed in the OF when compared to those in the CF (Suárez-Serrano *et al.* 2010).

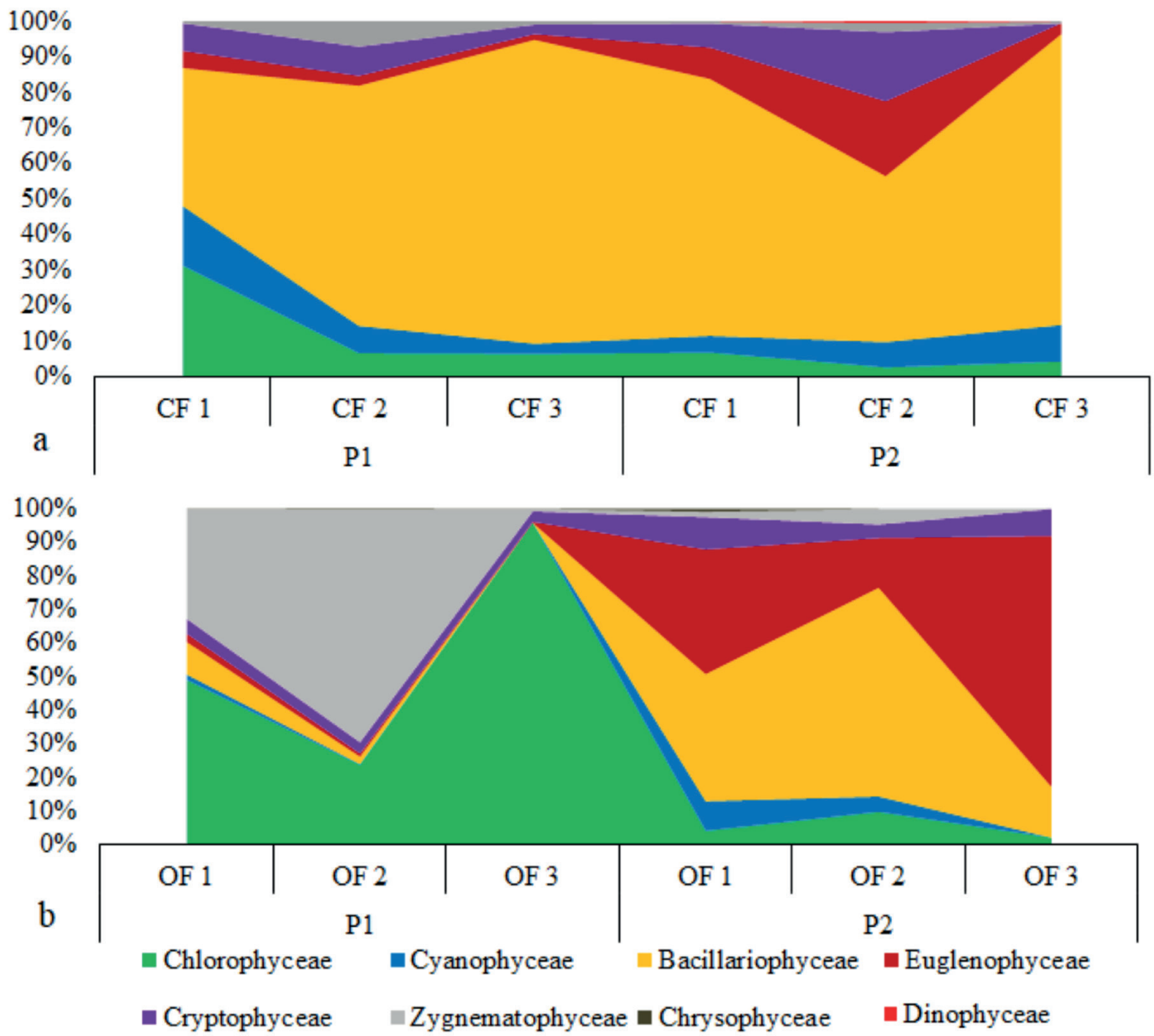

Figure 4. Relative contribution (total number of individuals) of seven classes of phytoplankton in the conventional farm (a) and organic farm (b) in the three sample units from the two experimental periods (P1, P2).

In the CF, the values of pH and dissolved oxygen were close to the ones registered in the Ibirapuitã River (7.1 - 9.1 and 7.02 - 9.06 mg L^{-1}, respectively) in 2018, according to the ANA (2018). The water pH ranging from slightly acidic to neutral from the CF is similar to that of mesocosms that underwent herbicide spraying and the control treatment (Reck *et al*. 2018; Reimche *et al*. 2015).

The values of dissolved solids and electrical conductivity were higher in CF than in OF. This result was also observed under the same conditions of rice cultivation in India due to the chemical fertilization utilized in conventional fields (Sihi *et al*. 2020). In contrast, the values of electrical conductivity in CF and OF were higher than those in the Ibirapuitã (37 - 55.8 µs cm^{-1}) and Ibicuí rivers (46.2 – 60.4 µs cm^{-1}), respectively, in 2018 (ANA 2018). This was most likely due to the higher organic matter concentration.

Effects of penoxsulam and clomazone in the environment

The herbicide penoxsulam inhibits acetolactate synthase, an enzyme responsible for building protein with a branched amino acid chain, whose inactivation results in a protein deficit that affects cell multiplication (Senseman *et al*. 2007). The results of herbicide residue analysis from rice paddy water showed that penoxsulam degraded faster than clomazone in the environment and, after 35 days the application of the herbicide, was not detected in most of the sample units. Contact with water promotes the solubilization of chemicals and occurs in a different way for each herbicide according to its own characteristics. For example, the solubility of clomazone is higher than that of penoxsulam (Senseman *et al*. 2007). It is important

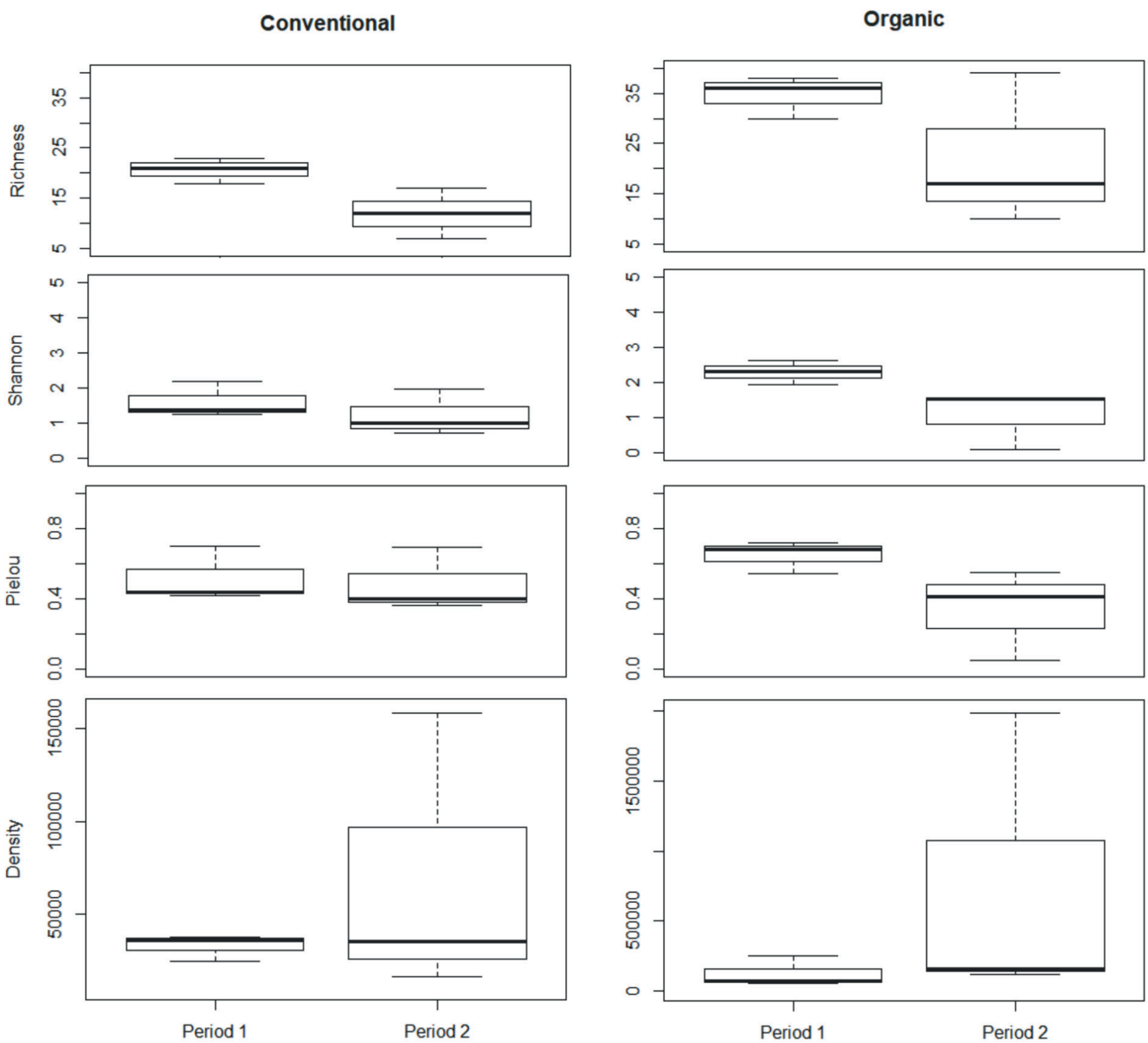

Figure 5. Epiphyton density (ind.cm^2), richness, diversity and equitability in conventional (CF) and organic (OF) farms in three samples from the two experimental periods.

to note that the aqueous photodegradation of herbicides occurs quickly in the environment; however, their products can remain for long periods and can be difficult to detect (Jabusch & Tjeerdema 2006). In addition, penoxsulam concentrations were detected during the period of rice cultivation in the superficial waters of rivers in Rio Grande do Sul (Silva *et al.* 2009), where it possibly affects other organisms.

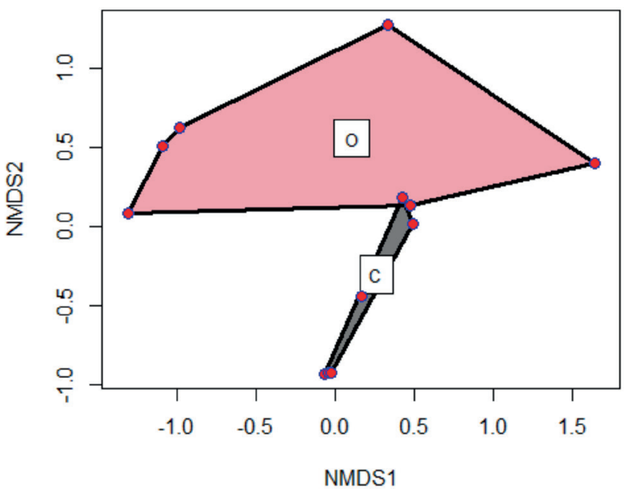

Figure 6. Nonmetric multidimensional scaling (NMDS) ordination of abundant epiphyton species (stress value = 0.06) in sample units (●) taken in two time periods from organic and conventional rice paddy fields.

The other herbicide evaluated was clomazone, which belongs to the isoxazolidinone chemical group and acts as an inhibitor of carotenoid biosynthesis (Senseman *et al.* 2007). Clomazone was the most frequently herbicide found in the Vacacaí and Vacacai-Mirim rivers, during rice cropping between 2000 and 2003, in the central region of Rio Grande do Sul (Marchesan *et al.* 2007). In this study, clomazone showed a slower degradation rate than penoxsulam, which was detected in the second sampling period, 35 days after herbicide spraying. The persistence of clomazone was detected up to 130 days in rice paddy water in the central region of Rio Grande do Sul (Zanella *et al.* 2002). Notably, the half-life of clomazone varies in the literature, ranging from 11 to 126 days (Du *et al.* 2018) and from 8 to 32 days (Noldin *et al.* 2001).

Phytoplankton and epiphyton associated with cultivation systems

Research on the algae community in organic and conventional fields is scarce in the literature. Comparatively, the total phytoplankton richness was similar between the two cultivation systems (79 spp. in OF and 86 spp. in CF) and was also close in value to that of natural wetlands within the territorial limits of the Pampa biome (74 taxa) (Matsubara *et al.* 2008).

In mesocosms, however, greater values of phytoplankton community richness were found in rice cultivated without the spraying of pesticides but with residual imidazolinone (151 species), such as in rice cultivated with imazapyr and imazapic herbicides (60 genera, with 44 genera observed by us), according to Cassol *et al.* (2013) and Reck *et al.* (2018), respectively. Baert *et al.* (2016) exposed the plankton diatom community at different levels of richness to different concentrations of atrazine herbicide and observed that more diverse communities were less affected by herbicide stress.

The total epiphyton richness (42 genera) was similar to rice-fish farm in India (38 genera) (Das *et al.* 2007); this was, however, less than that of another rice-fish farm, which had 97 genera (Saikia & Das 2011). The number of epiphytic taxa (about 90 species) was similar to that found in a mesocosm under residual imidazolinone close to the region of this study (Cassol *et al.* 2013). The results of different studies do not seem to illustrate a unique pattern of phytoplankton and epiphyton richness between the cultivation systems.

In this study, we expected to find differences in community attributes owing to the differences in environmental conditions. However, only the difference in epiphyton richness was statistically significant. The phytoplankton community had a taxonomic homogenization after 12th and 35th days of herbicide application. The effects of herbicides on algal growth possibly occurred in the first days after spraying, followed by recovery events in the community during this time. Faster recovery events observed in other studies, associated with the phytoplankton being subjected to the water flow, can hinder the detection of punctual impacts because the evaluation was not done 24 h after herbicide spraying. Imazapyr and imazapic herbicides, whose mechanism of action is the same as that of penoxsulam, inhibited the growth of the phytoplankton community 24 h after the spraying in mesocosms (Reck *et al.* 2018). Residual water from CF after spraying with herbicides, such as clomazone, inhibited the growth of *Chlorella vulgaris, Pseudokirchneriella subcapitata*, and *Desmodesmus subcapitatus* strains 2 days after spraying (Suárez-Serrano *et al.* 2010).

The decrease in epiphyton richness after herbicide spraying may be the result of the longer contact time with the chemical compound. At the same time, the species has strategies, such as mucous tubules and peduncles, for resisting environmental pressure (Rimet & Bouchez 2011). According to the sensitivity assessment, the periphyton remains exposed for longer to the pollutant that promotes community growth (Lobo *et al.* 2002).

Differences between the algal communities subjected to conventional and organic farming were observed in the phytoplankton species composition, particularly in period 1 of the experiment. In the phytoplankton of CF, Bacillariophyceae was the most representative class for the total number of individuals, with a predominance of *Nitzschia* spp. This genus is common in wetlands and tolerates high

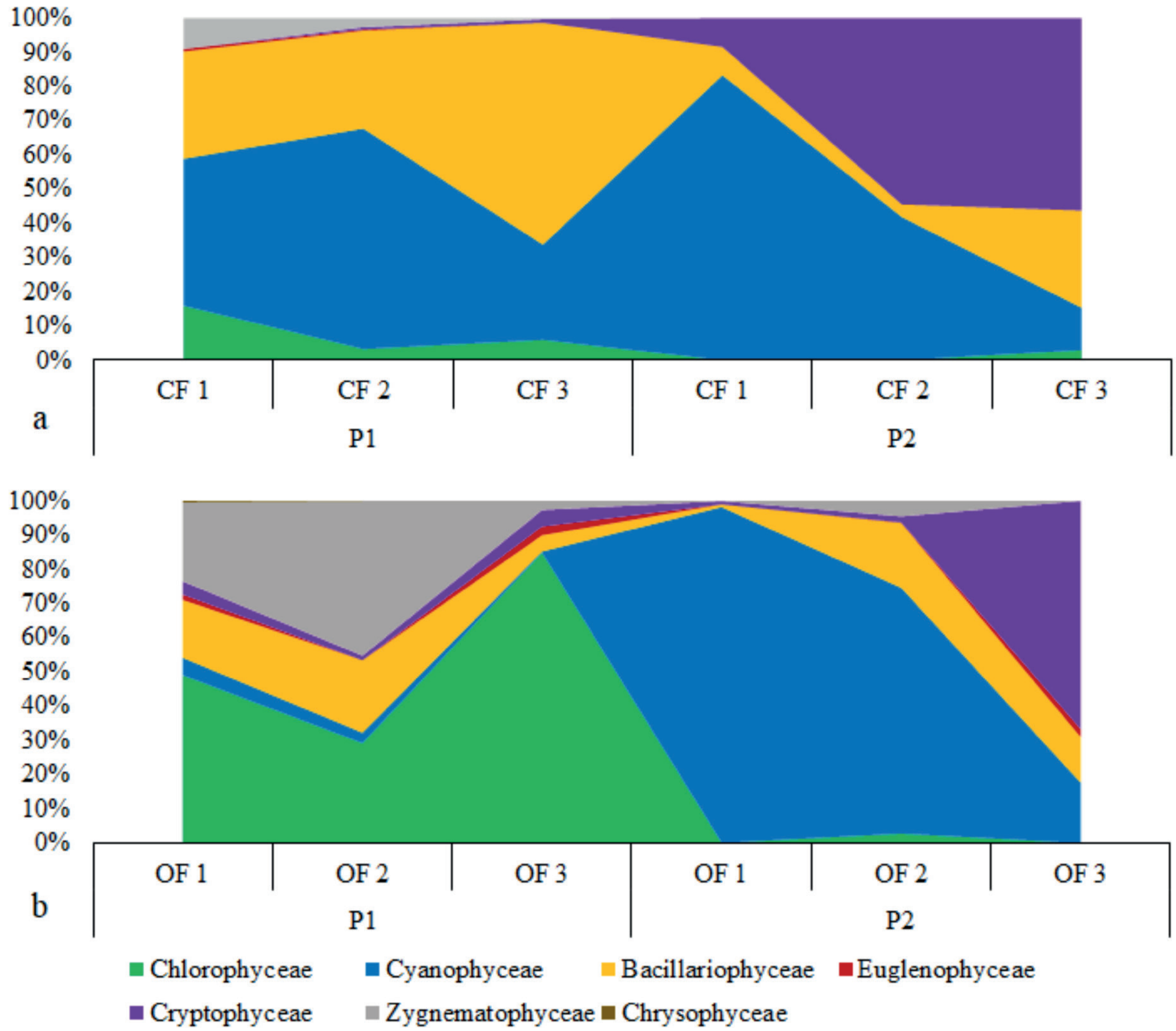

Figure 7. Relative contribution (total number of individuals) of different classes of epiphyton from the conventional farm (**a**) and organic farm (**b**) in three sample units from the two experimental periods (P1, P2).

nutrient and organic matter concentrations (Sheat & Wehr 2003; Trobajo & Sullivan 2010). *Nitzschia* cf. *vixnegligenda* is an indicator species in conventional rice paddy fields. This is a little-known taxon that has been recorded in high-electrolyte streams in Ecuador and Central Europe (Rumrich *et al.* 2000). *Nitzschia palea* was also abundant; this species is distributed in different lotic and lentic environments, and is associated with environments under stress. Trobajo *et al.* (2009) recognized this species as being tolerant to atrazine, a photosystem II inhibitor herbicide (Debenest *et al.* 2010). *Stauroneis reichardtii* has also been reported to be an indicator species in conventional rice paddy fields. This cosmopolite species is found in circumneutral waters with low specific conductance (Bahls 2010).

The relationships between species composition and periods after flooding were verified. Euglenophyceae and Cryptophyceae increased densities in the phytoplankton community in period 2. Cyanophyta and Euglenophyta showed a positive relationship with these factors in mesocosms, whereas Chlorophyta, Bacillariophyta, Cryptophyta, and Dinophyta showed a decrease in density (Furtado & Luca 2003). Cryptophyceae was the Y functional group; it is tolerant to low luminosity and, therefore, uses the phagotrophic mode to obtain energy (Reynolds *et al.* 2002). The incidence of solar radiation in rice paddy water decreased over the time(Cassol *et al.* 2013), which may favor the growth of heterotrophic species in period 2. In experiments, the photosystem II inhibitor herbicide may favor facultative heterotrophic species, thus preventing their deleterious effects (Debenest *et al.* 2009; Larras *et al.* 2012).

In OF, Chlorophyceae was abundant among phytoplankton during period 1 of the experiment. The association between higher turbidity recorded in this study and the presence of *R. planktonicus* has been previously described (Reynolds *et al.* 2002). The density of green algae decreased consistently during period 2. The same result was observed for higher herbicide concentrations in rice farming, showing the sensitivity of species to photosystem inhibitor chemicals (Cochard *et al.* 2014); the effect was also noted ecotoxicologically (Suárez-Serrano *et al.* 2010). *Radiococcus* *planktonicus*, an indicator species of organic farming, has cells that are enveloped by a mucilaginous sheath making it easy to float. It has been suggested that colonial genera such as *Aphanocapsa*, *Eudorina*, *Pandorina*, and *Sphaerocystis* are sensitive to the presence of imazapyr and imazapic herbicides belonging to the penoxsulam chemical group in rice farming mesocosms (Reck *et al*. 2018). The low abundance of green algae in CF and their predominance in OF suggest the effects that herbicide sprays have on the environment. Heterotrophic groups were also abundant in OF in period 2.

In the epiphyton, Bacillariophyceae and Cyanophyceae made notable contributions to the composition of the communities in both rice farms. However, Chlorophyceae had a higher contribution in the OF, mainly in period 1. The preference of Chlorophyceae and Cyanophyceae in colonizing the rice stem was also observed in rice–fish farming without pesticides in India, where a natural substrate with a rough surface promoted the development of even more weakly attached forms of algal flora when compared to a glass slide that was preferentially colonized by Bacillariophyceae (Saikia & Das 2011). The authors suggested that there might be a relationship between the life cycle of the substrate and periphyton diversity even though they had not been able to describe such a relationship. In bioassays with periphytic communities, the herbicide metribuzin, a photosystem II inhibitor, negatively affected Chlorophyta, whereas diatoms and Cyanobacteria showed an increase in biomass under its exposure (Gustavson *et al.* 2003).

The results of this study showed that organic and conventional farming had distinct environmental conditions and expressed differences in community attributes; only epiphyton richness showed differences between conventional and organic fields. Therefore, phytoplankton and epiphyton taxonomic compositions should be considered as an effective tool to illustrate the differences between the two systems.

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