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Original Article

Soil diatoms in rice fields: comparison between organic and conventional systems and alterations after herbicide application

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ABSTRACT

This study is the first effort to provide comparative information about soil diatom assemblages in organic (OF) and conventional (CF) rice fields. We aimed to investigate whether soil diatom assemblages (richness, relative abundance and species composition) differ between organic and conventional systems and detect alterations after the application of herbicides (clomazone and penoxsulam). Surface sediment was collected using a core (10 cm diameter, 2 cm depth) at three sites in each system in Rio Grande do Sul State, South Brazil. Live motile diatoms were isolated from the sediment using the trapping method. The presence and concentration of residual herbicides in the soil and the pH and temperature of interstitial water were measured. OF had higher temperatures and lower pH than CF. The diatom assemblages differed significantly in richness, composition, and relative abundance; OF had greater total richness (123 species) than CF (79 species). *Pinnularia* and *Nitzschia* were the richest genera for CF, while *Pinnularia* and *Eunotia* were the richest for OF. Exclusive taxa were detected in OF and the relative abundance of species was related to the sampling period in CF.

Keywords: agroecosystem, organic, pesticides, rice field, terrestrial diatoms.

Introduction

Diatoms are the most widespread and diverse group of algae. They are well-known in aquatic ecosystems but are infrequently studied in agricultural soils. Recent efforts described the structure of soil diatom communities in response to anthropic disturbance (Vacht *et al.*, 2014; Antonelli *et al.*, 2017) and different land uses such as forests, agricultural fields and preserved or disturbed grasslands (Foets *et al.*, 2020a).

Diatoms of soil rice fields have rarely been investigated, although they are a component of tropical and subtropical landscapes, especially in Asia and South America. These agroecosystems are complex temporary environments occupied by a rich composition of fauna and flora (Bambaradeniya *et al.*, 2004). Two rice cultivation systems can be distinguished — conventional and organic. The conventional system is the most frequently used and employs high amounts of pesticides to control weeds, fungi and insects. The organic system, on the other hand, is being used by some producers and employs no pesticides

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or inorganic fertilizers, and usually has a more diverse crop rotation (SOSBAI, 2018).

Worldwide, studies on rice fields have focused on phytoplanktonic and epiphytic flora (*e.g.*, Irisarri *et al*., 2001; Prasanna & Nayaki, 2007; Alves-da-Silva & Tamanaha, 2008; Kumar & Sahu, 2012). Investigations have also addressed the variability of microalgae communities under agricultural practices (*e.g.*, Sartori *et al*., 2011; Cassol *et al*., 2013; Reck *et al*., 2018; Liu *et al*., 2020) and organic versus conventional systems (*e.g.*, Cassol *et al*., 2022). However, the soil diatom flora of rice fields has only been described by Negoro & Higashino (1986), Ohtsuka & Fujita (2001) and Fujita & Nakahara (2006) in Japan. Diatoms are important contributors to organic compounds and play a crucial role in the formation and stability of soil aggregates (Meetting, 1981). It remains unclear if soil diatoms can be used to monitor agricultural soil quality, however, there is evidence that diatom community composition is sensitive to land use (Heger *et al*., 2012; Foets *et al*., 2020a).

Diatom diversity in conventionally cultivated soils has been documented under maize crops (Stanek-Tarkowska & Noga, 2012), under wheat and oat crops (Stanek-Tarkowska e*t al*., 2017; 2018), in fallow soil (Stanek-Tarkowska *et al*., 2015) and in pasture soil (Poradowska, 2020) in Poland. Terrestrial diatoms of rice fields have been mainly investigated in Asia (Negoro & Higashino, 1986; Ohtsuka & Fujita, 2001; Fujita & Nakahara, 2006; Vijayan & Ray, 2016; Nashima & Palanisamy, 2016).

Organic farming often has positive effects on species richness and abundance, but these effects differ among groups of organisms in landscapes (Almeida *et al*., 2023; Bengtsson *et al*., 2005; Komatsuzaki & Syuaib, 2010; Katayama *et al*., 2019). A single study of soil diatoms in organic systems was carried out in fields in Laos (Fujita & Ohtsuka, 2005).

In Brazil, the most common form of rice farming is the conventional system. Studies on the association between diatom species and pesticides improved in the first years of the 2000s (Falasco *et al*., 2009). Recent field studies highlighted pesticides as an important driver of structuring periphyton biomass in subtropical streams (Bartozek *et al*., 2022). However, most studies of diatoms and pesticides have used artificial conditions and usually a single species (Debenest *et al*., 2009; Larras *et al*., 2014; Wood *et al*., 2016). Thus, there is a need to analyze the ecological preferences of diatom taxa in areas exposed to constant anthropic pressure such as agricultural activity (Stanek-Tarkowska *et al*., 2021). Describing how herbicides can directly influence soil diatom species composition and distribution is fundamental to understanding their ecology (Foets *et al*., 2021).

While the response of diatoms to detect eutrophication is very well documented, their tolerance and sensitivity to herbicides is still an open question, especially for benthic species. A diatom biomonitoring index was capable of detecting herbicide impacts on benthic diatom communities in Australia (Wood *et al*., 2019). The authors sampled diatoms from rocks, pebbles, cobbles and plants, and found some species to be strongly associated with sites polluted by herbicides. Pesticide contamination was found to have a more significant impact on the composition of ecological guilds of periphyton in lotic mesocosms, selecting motile, low-profile and mucous tubule diatom guilds in contaminated channels (Rimet & Bouchez, 2011). Additionally, *in vitro* experiments have identified some species as sensitive to pesticide contamination (Debenest *et al*., 2009; Rimet & Bouchez, 2011; Larras *et al*., 2012; 2014; Wood *et al*., 2016; 2019).

Despite rice fields being a component of the subtropical landscape, especially in South America, there have been no studies of the soil diatom flora of organic rice fields nor the effects of herbicides in field conditions. Considering this lack of information in Brazil, we aimed to investigate soil diatom assemblages of rice fields.

This study represents the first effort to provide detailed information about soil diatoms of organic and conventional rice fields, including their response to herbicide application. The effects of the herbicides clomazone or penoxsulam on soil diatom communities are unexplored so far. Moreover, soil diatoms of agroecosystems can provide information about species that are directly exposed to agricultural impacts.

Thus, we aimed to investigate whether the soil diatom assemblages (richness, relative abundance and species composition) of organic and conventional rice fields differ and to detect alterations after the application of herbicides (clomazone and penoxsulam). Considering that most of the literature is based on bioassays, we expected to find differences in the attributes of the diatom assemblages of organic and conventional systems and particularly after the application of herbicides in field conditions.

Material and methods

Study area

The studied organic rice farm is located 22 km away from the urban center of the municipality of Manoel Viana in Rio Grande do Sul State, South Brazil. Four hundred hectares of rice fields are currently cultivated there by 224 families belonging to the Organic Rice Producers Association of Santa Maria do Ibicuí Settlement (Ramos, 2012). The water for the fields comes from the Ibicuí River, which extends 385 km into lowlands and sandy riverbeds. The cultivation system uses pre-germinated seedlings and does not use chemical fertilization or pesticides. Residual plants from the last crop are turned over to promote soil organic content.

The studied conventional rice farm is located 12 km from the urban center of the municipality of Alegrete City in the Rio Grande do Sul state, southern Brazil, and 45 km away from the OF. The area has been used for irrigated rice farming since 1986, employing no-tillage cultivation and chemical fertilization, according to recommendations of the Sociedade Brasileira de Arroz Irrigado (SOSBAI, Brazilian Society of Irrigated Rice). The water for the rice field comes from the Ibirapuitã River, which receives organic loads from livestock and agriculture, predominant activities in the region. The area is in the Pampean ecoregion and has a subtropical climate (type CFa Köppen, Alvares *et al*., 2014), with 1900 mm annual rainfall and well-defined seasons.

Diatom sampling

Sampling of the organic rice field (OF) was conducted at three different sites: OF1 29º30'36" S, 55º39'26" W; OF2 29º30'20" S, 55º39'17" W and OF3 29º30'10" S, 55º39'23" W. The three sites were sampled in three periods in 2018: Period 1 - November 19th, Period 2 - November 21st, and Period 3 December 2nd. Sampling of the conventional field (CF) was also conducted at three different sites: CF1 29º41'47" S, 55º50'44" W; CF2 29º41'34" S, 55º50'49" W and CF3 29º41'23" S, 55º51'07" W in four periods: Period 1 - one day before herbicide application; Period 2 - one day after herbicide application, Period 3 - twelve days after herbicide application and Period 4 - thirty-five days after herbicide application (Fig. 1).

Two soil samples were collected at each site using a PVC core (10 cm diameter; 2 cm depth), placed in plastic bags and carried to the laboratory. Interstitial water pH and temperature were measured using a pH meter (PHTEK®) and a thermometer (Incoterm®), respectively.

Live motile diatoms were isolated from the sediment using the trapping method (Eaton & Moss, 1966, adapted by Laudares-Silva & Cimardi, 1989). Each soil sample was homogenized in a flask and then placed in a Petri dish under natural light and avoiding artificial light during the night. A double layer of Whatman® 105 filter paper (2x2 cm) was put on the surface layer and removed the next morning, trapping live diatoms that migrated toward the light. A subsample was examined microscopically to verify the presence of live cells in the communities at the moment of sampling was carried out. The remaining sample was processed using the oxidation method described by Simonsen (1974) and mounted on permanent slides using Naphrax.

The herbicides penoxsulam and clomazone are widely used to control weeds in Brazilian rice fields. The presence and concentration of residual herbicides in the soil of CF were determined by the Solid Phase Extraction method and analyzed by *Liquid Chromatography with Mass Spectrometry* (LC-MS/MS), according to Donato *et al*. (2012), in the Pesticide Residue Laboratory (LARP) at the Universidade Federal de Santa Maria.

The abundance of taxa was obtained by counting valves on permanent slides using a Zeiss Axioplan Microscope, seeking to reach 80% sampling efficiency, according to Pappas & Stoermer (1996). The relative abundance (RA) of each species was presented as a proportion of the total valves counted. Species considered abundant were those whose population density was higher than the value obtained by dividing the total density by the number of identified species, while dominant species were those with densities greater than 50% of the general density for the entire community (Lobo & Leighton, 1986).

A two-way analysis of variance (two-way ANOVA) was used to detect differences in species richness between

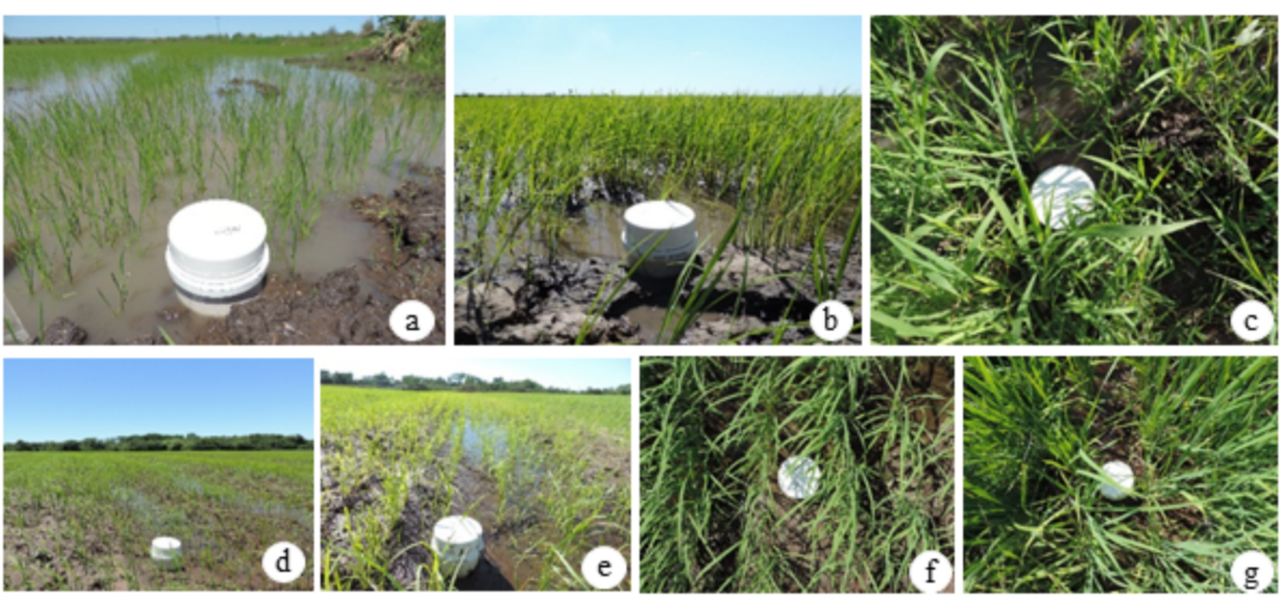


Figure 1. Soil core samples from the studied organic rice field taken during three different periods (a) Period 1 (b) Period 2 and (c) Period 3; and from the studied conventional rice field taken during four different periods (d) Period 1, (e) Period 2 (f) Period 4 and (g) Period 4.

cultivation systems and among periods. Permutational analysis of variance (PERMANOVA) was performed to evaluate differences in diatom composition between OF and CF. Nonmetric multidimensional scaling (NMDS) was applied to the species abundance matrix to investigate species distribution between Of and CF. Species representing less than 2% of the relative abundance in all samples were removed to increase the significance of the data (Birks, 2010). NMDS was also used to detect associations between abundant species and herbicide concentration in the sampling periods for CF. The community matrices were transformed using Wisconsin double standardization and a Bray-Curtis dissimilarity matrix was calculated using the "metaMDS" function of the "vegan" package in R software. Vectors of the environmental variables were adjusted to the resulting bi-dimensional map using the function "EnvFit", also of the "vegan" package of R software. All analyses were performed using the vegan package (Oksanen *et al*., 2020) of R software (R Core Team, 2021).

The samples were deposited in the Herbário Alarich Schultz (HAS), Museu de Ciências Naturais, Secretaria do Meio Ambiente e Estrutura, Porto Alegre, Brazil, under numbers HAS 11062–11073 for CF and HAS 11053–11061 for OF.

Results

Physical and chemical soil parameters

The physical and chemical parameters of the interstitial water of the soil of CF and OF are presented in Figure 2. The temperature ranged from 22.2 – 34.0 °C for OF and 18.4 °C – 32.0 °C for CF, decreasing over time in both cultivation systems. Acidic conditions were observed in OF (pH 4.6 – 5.9) and slightly acidic to neutral conditions in CF (pH 4.8 – 7.4). Changes toward neutral values were observed after flooding (Period 3 and Period 4).

There was no residual herbicide in the soil in Period 1 of CF (one day before herbicide application) (Table 1), nor in OF at any time. The highest concentration of clomazone and penoxsulam was observed one day after herbicide application (Period 2), which decreased over time. Penoxsulam was not detected in some samples of Period 3 and Period 4, as it dissipates faster than clomazone.

Organic versus conventional diatom assemblages

The evaluated attributes revealed important differences between the soil diatom assemblages of OF and CF. Soil diatom species richness differed significantly between the cultivation systems (p=0.03), with it ranging among samples from 13 to 45 species for OF and from 18 to 33 for CF (Fig. 3). Total species richness was greater for OF than CF, with the former having 123 taxa of 33 genera and the latter with 79 taxa of 23 genera (Supplementary Material).

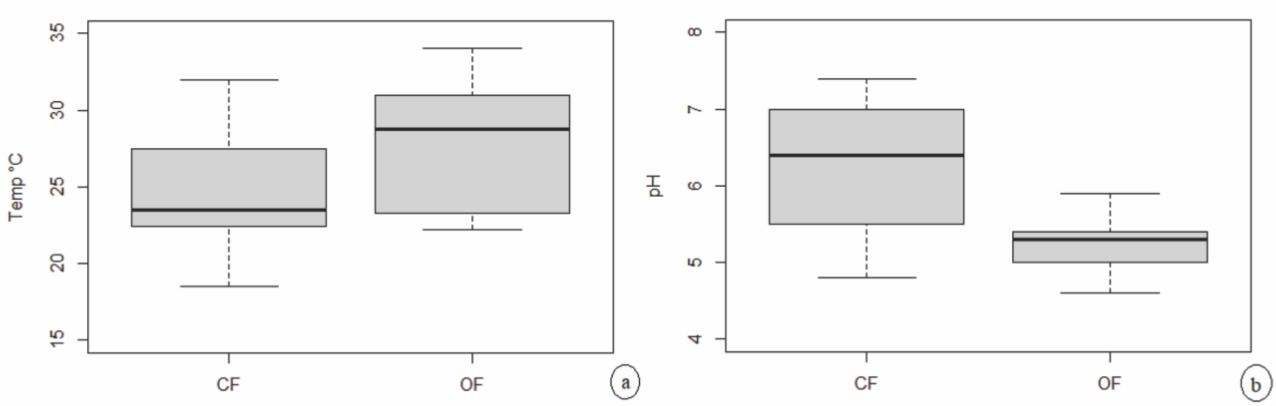


Figure 2. Temperature (a) and pH (b) values of interstitial water in the studied conventional (CF) and organic (OF) rice fields.

Table 1. Residual concentrations of herbicides of soil samples from the conventional rice field in period 1 (19/11/2018); period 2 (21/11/2018); period 3 (02/12/2018) and period 4 (25/12/2018). ND = non-detected, LQ =limit of quantification.

	Period 1			Period 2			Period 3			Period 4		
Herbicide	**S1**	**S2**	**S3**	**S1**	**S2**	**S3**	**S1**	**S2**	**S3**	**S1**	**S2**	**S3**
Clomazone (μg L^{-1})	ND	ND	ND	0.35	0.56	0.41	0.06	0.04	0.03	ND	< LQ	ND
Penoxsulam (μg L^{-1})	ND	ND	ND	0.07	0.11	0.03	ND	< LQ	< LQ	ND	ND	ND

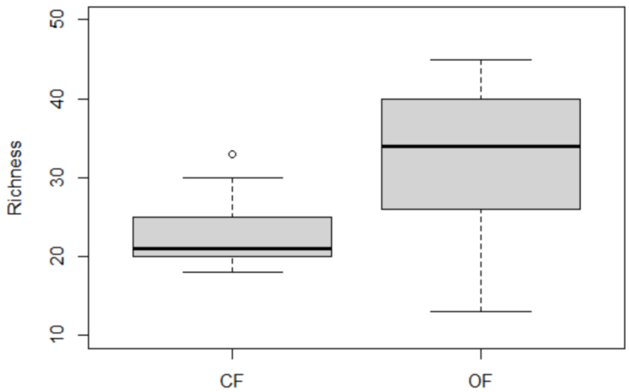


Figure 3. Total richness (number of species) of soil diatom assemblages of the studied organic and conventional rice fields, RS, Brazil.

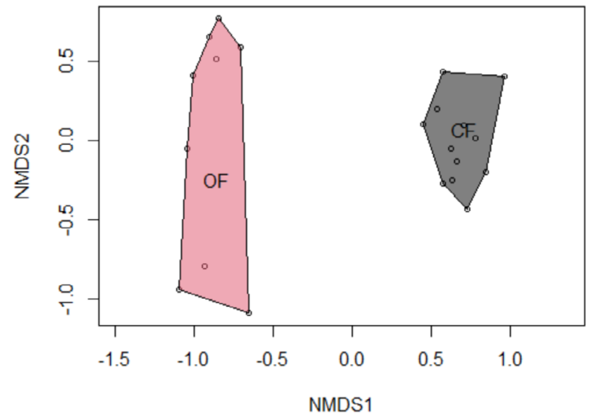


Figure 4. Nonmetric multidimensional scaling (NMDS) ordination of soil diatoms species (> 2% of abundance) (stress value = 0.02) from the studied organic (OF) and conventional rice fields (CF).

For both systems, species richness was not significantly different among samplings over time (Fig. 5). The average richness for OF ranged from 23 species (Period 1) to 36 species (Period 3). The average richness for CF ranges from a low of 22 species after herbicide application (Period 2) to 26 species (Period 4). A selection of the most representative diatom species (>1% abundance) of CF is shown in Figures 7–8 and of OF in Figures 9–10.

Species composition was a significant attribute of the soil diatom assemblages. The PERMANOVA analyses (R^2= 0.34, P = 0.0002) and the distributions of species in the NMDS model (Fig. 4) revealed that the soil assemblages of OF and CF had distinct diatom species compositions (>2% relative abundance).

Pinnularia was the richest genus in both systems with 31 species in CF and 18 species in OF. *Eunotia* was the second-richest genus in OF with 25 species, while *Nitzschia* was the second-richest genus in CF with nine species.

Eighteen taxa were found in both systems: *Diadesmis confervacea, Humidophila contenta, Encyonema silesiacum, Gomphonema parvulum, Hantzschia amphioxys* var. *amphioxys* f. *capitata, Hantzschia* sp. 3, *Luticola intermedia, Nitzschia nana, N. palea, Pinnularia borealis, P. dubitabilis, P. marchica, P. microstauron* var. *nonfasciata, P. rumrichae, P. subcapitata, Stauroneis lapponica* and *S. reichardtii*. The following taxa are some of those exclusive to OF: *Craticula* sp1, *Eunotia longicamelus, E. monodon, E. pseudosudetica, E. intermedia, E. didyma* var. *gibbosa, E. botuliformis, Frustulia saxonica, F. guayanensis* subsp. *ecuadoriana, Navicula leptostriata* and *Surirella tenuissima*. The following 39 taxa were exclusive to CF: *Caloneis fontinalis, C. bronderi, C. tenuis, Capartogramma crucicula, Craticula ambigua, Cymbopleura* sp., *Eunotia pyramidata, Eunotia* sp., *Gomphonema gracilioides, Hantzschia* sp. 2, *Luticola cristinae, L. fuhrmanni, Luticola* sp. 4, *Navicula trivialis, N. rostellata, Neidium* sp. 1, *Neidium* sp. 2, *Neidium* sp. 4, *Nitzschia amphibia, N. nana, Nitzschia* sp. 3, *Nitzschia* sp. 5, *Pinnularia brebissonii* var. *brebissonii, P. obscura, Pinnularia* aff. *schimanskii, P. renata, Pinnularia* sp. 5, *Pinnularia* sp. 8, *Pinnularia* sp. 13, *Pinnularia* sp. 14, *Pinnularia* sp. 18, *Pinnularia* sp. 20, *Sellaphora seminulum, Sellaphora* sp. 2, *Stauroneis phoenicenteron, Surirella* cf. *bouillonii, S. tenuissima* and *Surirella* sp. 2.

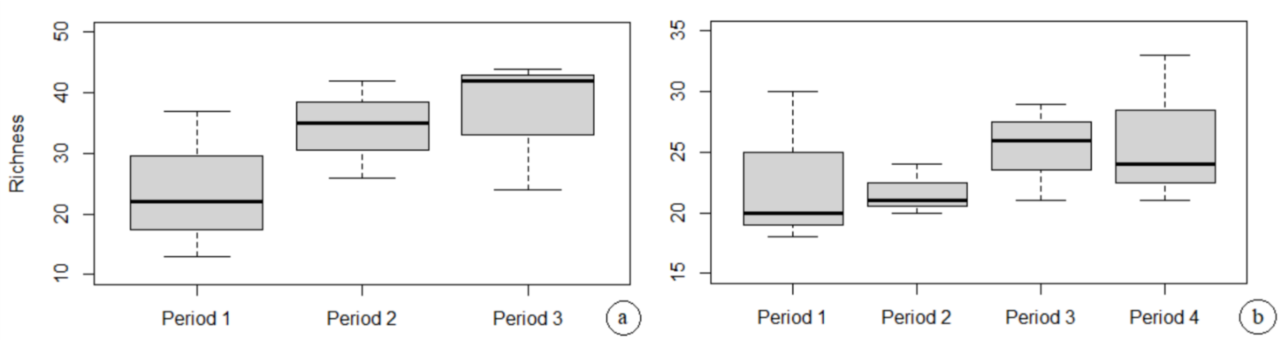


Figure 5. Variation in total richness (number of species) of soil diatom assemblages: during three periods in the studied organic rice field (a) and during four periods in the studied conventional rice field (b), RS, Brazil.

Soil diatoms and herbicides

Richness decreased after herbicide application (Period 2) compared to before application (Period 1). Nonetheless, associations between period, herbicide presence/concentration and the diatom assemblage were detected in CF (Fig. 6). There was a tendency towards significant differences in soil diatom composition among the four sampling periods (permanova p = 0.06). The matrix of abundant species revealed that *Hantzschia amphioxys* var. *amphioxys* f. *capitata, Stauroneis lapponica, Sellaphora* sp. (pupula group) and *Luticola cristinae* were related to the period without herbicides (Period 1), while *Pinnularia borealis, Pinnularia dubitabilis* and *Stauroneis borrichii* were related to the period with highest herbicide concentrations (Period 2). Additionally, *Pinnularia brebissonii, Stauroneis reichardtii* and *Luticola intermedia* were related to Period 3 and *Pinnularia marchica* and *N. palea* to Period 4 (35 days after herbicide application).

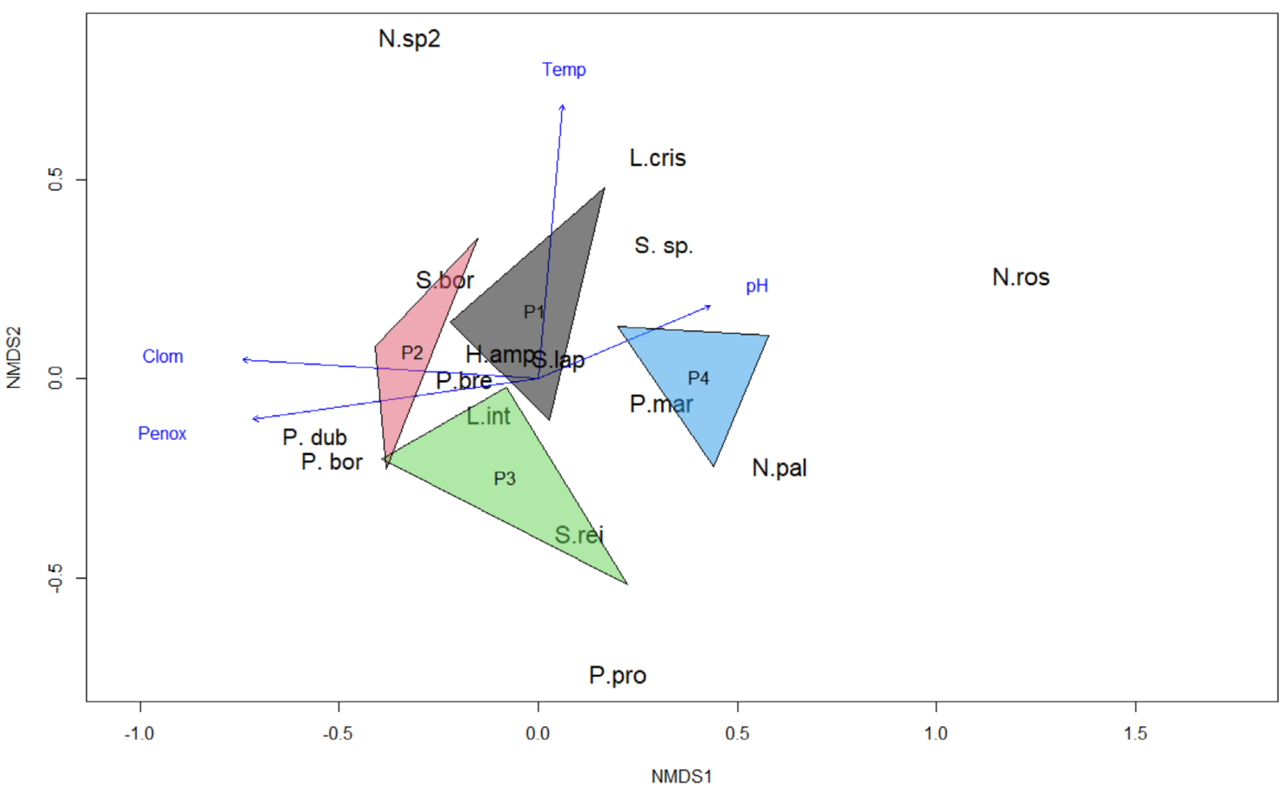


Figure 6. Nonmetric multidimensional scaling (NMDS) ordination of abundant soil diatom species (stress value = 0.11) during four periods in soil rice fields. P1: Period 1, P2: Period 2, P3: Period 3, P4: Period 4. Temp: temperature; pH: potential of hydrogen; Clom: Clomazone herbicide; Penox: Penoxsulam herbicide. Species: H.amp: *Hantzschia amphioxys* var. *amphioxys* f. *capitata*; S. sp.: *Sellaphora* sp. (pupula group); L.cris: *Luticola cristinae*; L.int: *Luticola intermedia*; N.sp2: *Neidium* sp2; N.ros: *Navicula rostellata*; N.pal: *Nitzschia palea*; P.bor: *Pinnularia borealis*; P.bre: *Pinnularia brebissoni*; P.dub: *Pinnullaria dubitabilis;* P.mar: *Pinnularia marchica*; P.pro: *Pinnularia* cf *procera*; S.bor: *Stauroneis borrichii*; S.lap: *Stauroneis lapponica*; and S.rei: *Stauroneis reichardtii*.

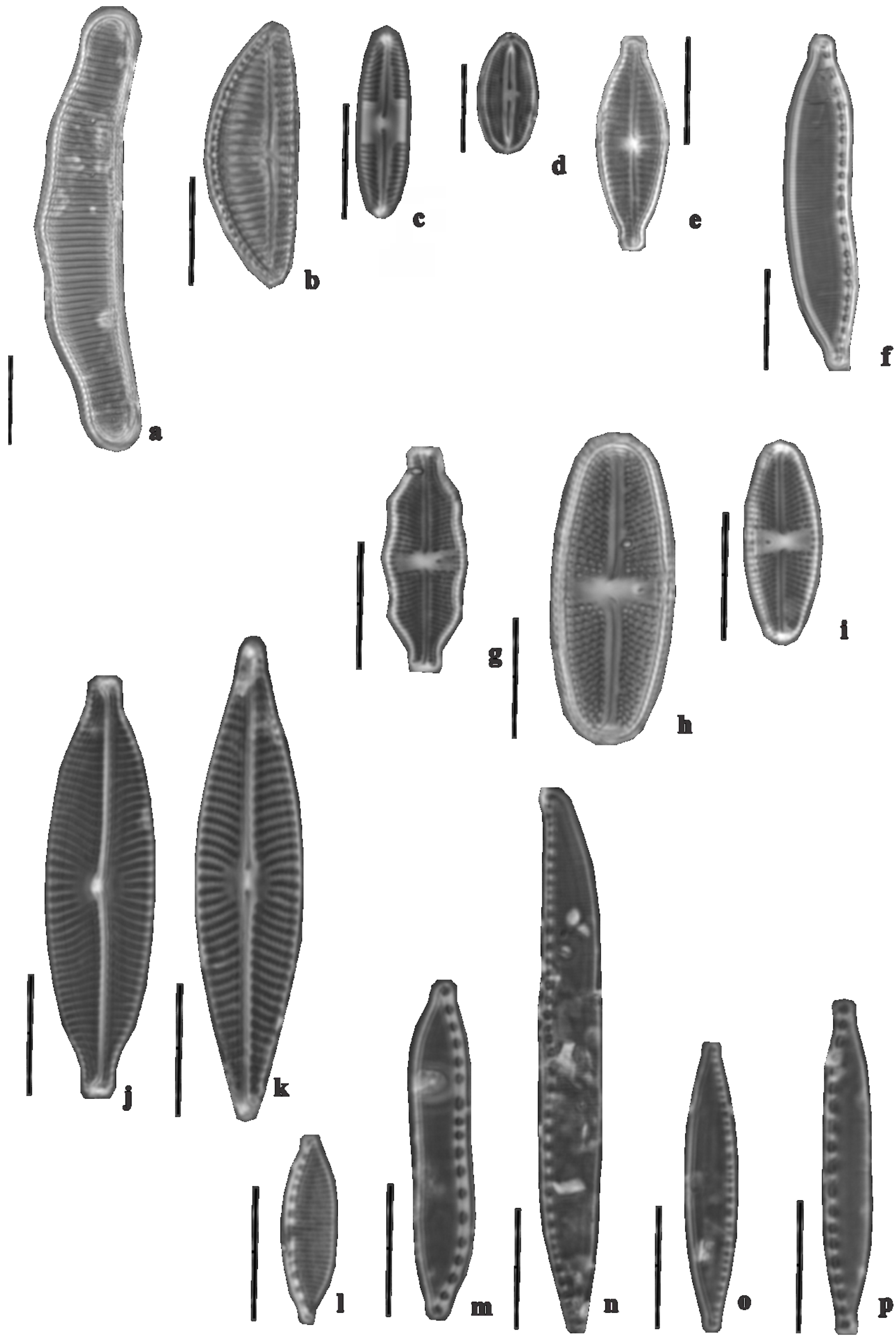


Figure 7. Some soil diatom species representing more than 1% of the relative abundance in the studied conventional rice field. a- *Eunotia pyramidata,* b- *Encyonema silesiacum*, c- *Caloneis fontinalis*, d- *Fallacia insociabilis*, e- *Gomphonema parvulum*, f- *Hantzschia amphioxys* var. *amphyoxis* f. *capitata*, g- *Luticola cristinae,* h- *L. furhmanni*, i- *L. intermedia,* j- *Navicula rostellata*, k- *N. trivialis*, l- *Nitzschia amphibia,* m- *N. brevissima,* n- *N. nana,* o- *N. palea,* and p- *N. perminuta*. Scale bar: 10µm.

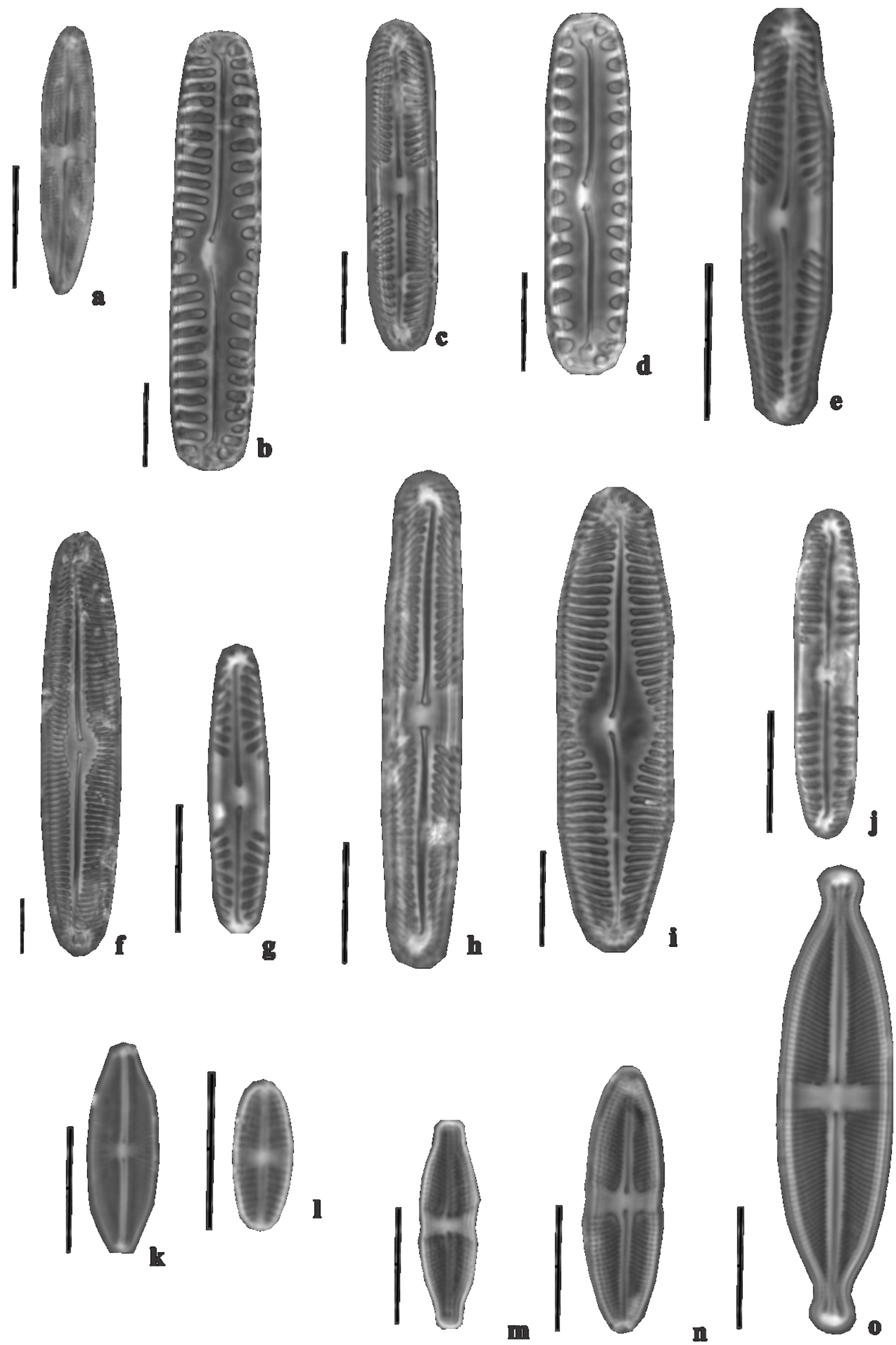


Figure 8. Some soil diatom species representing more than 1% of the relative abundance in the studied conventional rice field. a- *Neidium* sp2, b- *Pinnularia borealis*, c- *P. brebissonii*, d- *P. dubitabilis*, e- *P. marchica*, f- *P. microstaurum* var. *nonfasciata*, g- *P. obscura*, h- *Pinnularia* cf. *procera*, i- *P. renata*, j- *Pinnularia* aff. *schimanskii*, k- *Sellaphora* sp. (pupula group), l- *Sellaphora seminulum*, m- *Stauroneis borrichii*, n- *S. lapponica*, and o- *S. reichardtii*. Scale bar: 10µm.

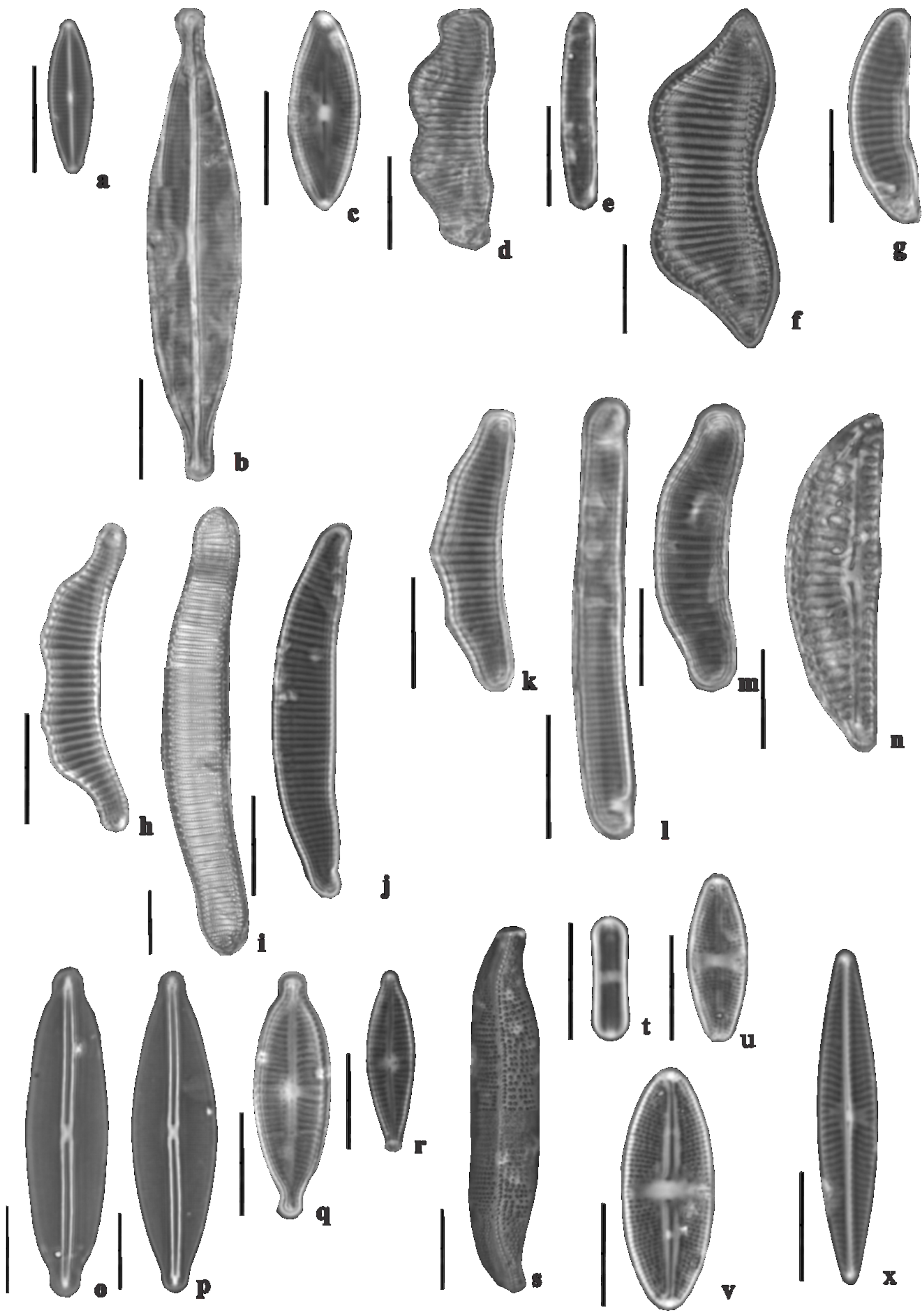
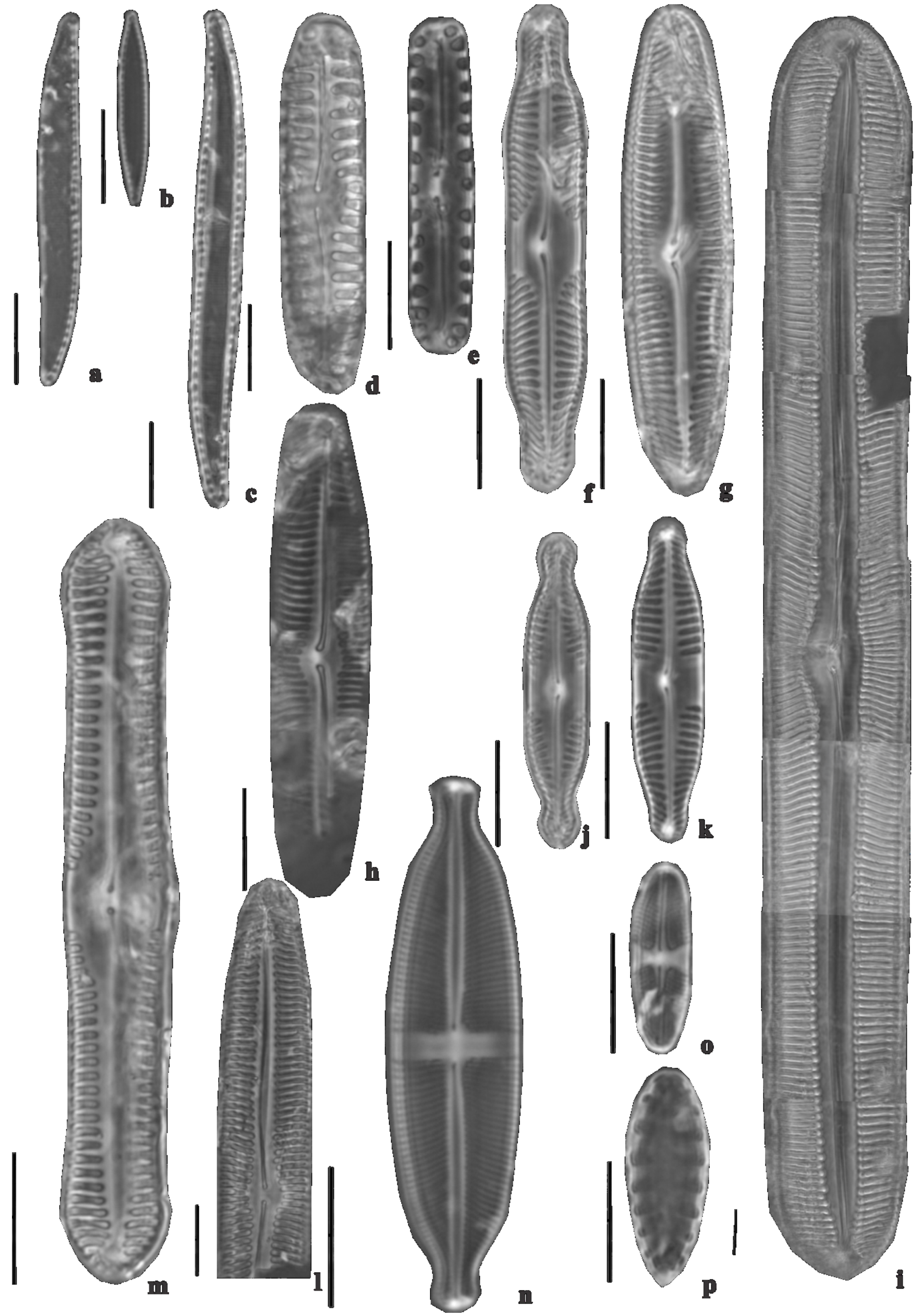


Figure 9. Some soil diatoms species representing more than 1% of the relative abundance in the studied organic rice field. a- *Craticula* sp1, b- *Craticula* sp3, c- *Diadesmis confervacea d- Eunotia bidens*, e- *Eunotia botuliformis*, f- *E. didyma* var. *gibbosa*, g- *E. intermedia*, h- *E. longicamelus*, i- *E.monodon*, j- *E. pseudosudetica*, k- *E. tridentula*, l- E. *ursulae*, m- *Eunotia* sp12, n- *Encyonema silesiacum*, o- *Frustulia guayanensis* subsp. *ecuadoriana*, p- *F. saxônica*, q- *Gomphonema parvulum*, r- *G. angustatum*. s- *Hantzschia* sp3, t- *Humidophila contenta*, u- *Luticola intermedia*, v- *Luticola* sp8, and x- *Navicula leptostriata*. Scale bar: 10µm.



'Figure 10. Some soil diatoms species representing more than 1% of the relative abundance of the studied organic rice field. a- *Nitzschia nana*, b- *N. palea*, c- *N. scalpelliformis*. d- *Pinnularia borealis*, e- *P. dubitabilis*, f- P. *marchica*, g- *P. peracuminata*, h- *P. microstauron* var. *nonfasciata*. i- *Pinnularia neomajor*, j- *P. rumrichae*. k- *P. subcapitata*, l- *P. viridiformis*, m- *Pinnularia* sp27, n- *Stauroneis reichadtii*, o- *S. lapponica*, and p- *Surirella tenuissima*. Scale bar: 10µm.

Discussion

Physical and chemical soil parameters

The flooding of rice farms causes rapid physical, chemical, and biological changes (Bambaradeniya *et al*., 2004). Changes from wet to dry conditions promote an intense decomposition process in irrigated systems. Aerobic and anaerobic activity produces organic acids as a result of organic matter decomposition on the soil surface (Bohnen *et al*., 2005). This occurrence could be related to the acidic conditions registered in OF and CF just after flooding (Period 3 – Period 4). Such a change in pH from neutral (7.4) to slightly acidic (6.2) was also observed in Japanese conventional rice paddy fields after flooding (Otsuka & Fujita, 2001). Consequently, the maintenance of wet conditions during Period 1 and Period 2 could be related to the neutral conditions of interstitial water in CF.

Other areas exposed to agricultural activity on cultivated soil without flooding were characterized by acidic and slightly acidic soil conditions, such as a mean pH of 4.7 under oat farming (Stanek-Tarkowska *et al*., 2017) and of 5.4 under winter wheat (Stanek-Tarkowska *et al*., 2018). Thus, in general, agricultural activity is a determinant factor due to acidic soil conditions.

Soil temperature decreased during the time of the experiment in both OF and CF. Incident solar radiation in the water decreases over time due to the growth of the rice plants, as observed in the present study (Fig. 1) and in the mesocosms approach of Cassol *et al*. (2013). This evidence could explain why the highest temperatures observed in interstitial water of OF and CF were during the initial periods.

Organic versus conventional soil diatom assemblages

The present study found important differences in the attributes of the diatom assemblages OF and CF, such as in richness, species composition and relative abundance. The results corroborate differences in the local environment generated by historical exposure to OF and CF over the last two decades. The effects of intensive use of conventional practices can remain present in the soil for a long time (Almeida *et al*., 2023).

Considering environmental conditions, pH has an influence on the species richness of soil algae as well as on higher plants. Neutral and slightly acidic soils are generally richer in diatom species than acidic soils (Stanek-Tarkowska et al., 2017; Poradowska, 2020). However, the present study found higher richness in acidic conditions (OF) than in neutral conditions (CF).

Diatom richness in agricultural fields could vary in response to repetitive and intense practices such as flooding, draining, plowing, transplantation and the application of fertilizers and herbicides. Organic Japanese rice fields had a richness of 104 species and 28 genera (Fujita & Ohtsuka, 2005), which is lower than that found here for OF (123 species and 33 genera). Besides, some taxa were found in both OF and CF, such as *Diadesmis confervacea, Humidophila contenta* and *Nitzschia palea*.

The richness found for CF (79 taxa and 23 genera) was higher than that of a conventional Japanese rice field with 53 species and 17 genera (Negoro & Higashino, 1986) and lower than that reported for Laotioan conventional rice fields (92 taxa belonging to 28 genera - Ohtsuka & Fujita, 2001). Some typical species of Japanese conventional rice fields, such as *Hantzschia amphioxys, N. palea, G. parvulum, Navicula rostellata, Navicula trivialis, N. amphibia* and *N. perminuta* (Negoro & Higashino, 1986; Ohtsuka & Fujita, 2001), were also recorded in CF in the present study.

The high number of species of the genera *Eunotia* and *Pinnularia* recorded in OF also may be explained by the acidic conditions (pH 4.8–5.8), which undoubtedly favored the development of these genera (Rower *et al*., 2021). Soil acidification (pH 4.4–5.6) and high diversity of *Eunotia* and *Pinnularia* were also found in pasture soils in Poland (Poradowska, 2020).

On the other hand, the genera *Pinnularia* and *Nitzschia* had higher species richness in CF. Higher contributions of these genera were also found in conventional Japanese rice paddy fields (Ohtsuka & Fujita, 2001) and in soil datasets from the Attert River basin, Luxembourg (Foets *et al*., 2021). Both of these genera are sediment-dwelling, but *Pinnularia* prefers acidic water (Krammer, 2000) and some species of *Nitzschia* are found mainly in alkaline waters (Bes & Torgan, 2010). Both of these pH conditions were observed in the rice fields studied here. Besides, species of *Nitzschia* were also found to be dominant in the subsurface of soil rice fields (Fujita & Nakahara, 2006).

Some terrestrial diatoms typical of agricultural areas were registered in CF and not in OF, such as *Stauroneis borrichii* (Stanek-Tarkowska *et al*., 2013 and Noga *et al*., 2014). Similarly, *Pinnularia borealis* and *Hantzschia amphioxys*, also considered typical soil diatoms, were registered in CF, but in low relative abundance in OF. Flooding must be considered an influential factor in diatom assemblages. Fujita & Nakahara (2006) observed the abundance of some species, such as *Hantzschia* spp., increased markedly during drainage, while *Nitzschia* spp. were dominant in relative frequency during rice field flooding.

Three abundant species were found in OF and CF simultaneously: *Luticola intermedia, Nitzschia palea* and *Pinnularia marchica*. Despite *N. palea* being widely distributed in soils and an ecological generalist (Trobajo *et al*., 2009), *L. intermedi*a and *P. marchica* were infrequently recorded in soils. *Pinnularia marchica* was registered growing especially in soils covered with mosses in Poland (Poradowska, 2020). The taxon has been rarely reported in Brazil, just in planktonic and periphytic samples in São Paulo State (Rocha 2008).

Soil diatoms and herbicides

A few studies have highlighted soil diatom species in agricultural fields but rarely in rice paddy fields. The sampling method usually involves taking a thin soil layer (0–2 cm) using core samples (Ø 5–10 cm) with further preparation with some oxidation technique (Negoro & Higashino, 1986; Ohtsuka & Fujita, 2001; Barragán *et al*., 2017). Consequently, live and dead cells are counted and identified on the slide. This differs from the trapping method employed here, which collected live and motile cells that were trapped in cellulose paper when migrating to the surface (Laudares-Silva & Cimardi, 1989). This method is important to access representative living species before and after herbicide application. There have been no comparative studies using this trapping method in freshwater environments in Brazil, just in mangroves (Laudares-Silva & Cimardi, 1989) and salt marshes (Talgatti, 2014). The limited number of studies, and the lack of differentiation between live and dead cells, make it difficult to compare with other areas. Furthermore, dead cells in soil could imply an inappropriate analysis of results related to diatom tolerance to herbicides.

The present study detected associations between species, period and herbicide concentration. The NMDS analysis associated the increase in abundance of *Pinnularia borealis, P. dubitabilis, P. brebissoni* and *Stauroneis borichii* with periods of high penoxsulam and clomazone concentrations (Period 2 and Period 3). *Pinnularia borealis* is a typical subaerial form (Round *et al*., 1990), frequently found in conventionally cultivated soils in Poland (Noga *et al*., 2014; Stanek-Tarkowska & Noga, 2012; Stanek-Tarkowska *et al*., 2018); however, it has also been found to be dominant in fallow fields (Stanek-Tarkowska *et al*., 2015). *Stauroneis borrichii* has also been found to be dominant in fallow fields (Stanek-Tarkowska *et al*., 2015) and has been selected for use as an indicator species for agricultural fields (Foets *et al*., 2020a).

The NMDS analysis related *Nitzschia palea, Sellaphora* sp. (pupula group) and *Luticola cristinae* to the period without herbicides; these taxa were absent from most of the sites in Period 2. Bioassays found *Nitszchia palea* to display different tolerances to herbicides in biofilms (Larras *et al*., 2012), depending on the herbicide family tested (Larras *et al*., 2014). The tolerant strain exhibited an increase in carotenoid content, a molecule involved in short-term photoprotection in diatoms, after dosing with the herbicide atrazine (Esteves *et al*., 2018). Clomazone belongs to the isoxazolidinone chemical group and acts as an inhibitor of carotenoid biosynthesis (Senseman & Armbrust, 2007). The slower degradation rate of clomazone, compared to penoxsulam, could have affected the carotenoid content of *Nitszchia palea* in Period 2.

The *Sellaphora pupula* group is not a natural group but a familiar and recognizable series of species. According to Mann *et al*. (2008), molecular analyses will reveal synapomorphies in aspects of frustule morphology and structure. Species of *Sellaphora* have been recorded as indicative of undisturbed grassland soils, such as *S. nana*, and forested soils, such as *S. harderi* in Luxemburg (Foets *et al*., 2020a).

The abundance of *Hantzschia amphioxys* var. *amphyoxis* f. *capitata, Stauroneis lapponica* and *S. reichardtii* seems to be associated with periods of lower herbicide concentration. Other studies reported the presence of these species in agricultural areas. *Hantzschia amphioxys* was found to be the most abundant diatom in soils under oilseed cultivation (Stanek-Tarkowska *et al*., 2021) in agricultural areas of the Attert River basin in Luxembourg (Foets *et al*., 2020a; 2021), and in soils under conventional maize fields (Bérard *et al*., 2004). However, *S. reichardtii* has only been recorded in limno-terrestrial environments in the Antarctic (Zidarova, 2008) and Arctic (Van de Vijver *et al*., 2004), and in ponds and wetlands in North America (Bahls, 2010).

In summary, the findings of this study represent a pioneering contribution to the knowledge of soil diatom diversity of organic versus conventional rice fields. Differences were evident in species richness, composition and relative abundance. Therefore, it can be concluded that the diatom assemblage of organic rice fields is more diverse, with some exclusive taxa, characteristics of acidic conditions. Furthermore, species composition is likely associated with herbicide application. It would be interesting to evaluate other organic and conventional rice fields to further compare diversity and environmental conditions.

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Authors' contribution

APVC conceptualized the research, conducted field and laboratory work, analyzed the data and wrote the manuscript. LCT conceptualized the research and critically revised the manuscript. APVC and LCT identified the species. RZ performed the chemical analyses.

Conflict of Interest

There is no conflict of interest in this paper.

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