ISNN 1517-6770

Effects of fire stress to soil protozoan abundance and richness in a Brazilian savanna

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Abstract. Protozoans are important components of the aquatic and terrestrial food webs. Since they are voracious bacterial predators in terrestrial habitats, they can consequently affect soil fertility. Considering that fire is a common and frequent event in agricultural and natural soils, especially in cerrado areas, this work aimed evaluating the effects of fire on protozoan communities occurring in a soil with cerrado vegetation. It was conducted 16 samplings (from august/2011 to august/2012) at a location exposed to a fire event (Burned) and one not exposed (Control). The "Non-Flooded Petri Dish" technique used to evaluate the protozoan cryptic diversity of the samples. Qualitative and quantitative analyses of the active soil protozoans were also done. It was used the Canonic Correspondence analysis (CCA), Jaccard indexes, the Bray-Curtis similarity coefficient, Shannon indexes and Kruskal-Wallis test for data analysis. It was found 102 morphospecies, distributed into 12 protozoan taxonomical groups. From the total, only 32 morphospecies, distributed into 9 taxonomical groups, were active. The CCA evidenced that the significant variables by the Monte Carlo test, that showed association with the biological data were: mean air temperature, mean relative humidity of the air, coarse sand, medium sand, fine sand and clay. The Jaccard index and Bray-Curtis test showed that the Control (C) and Burned (Q) areas are similar. The value chosen to delimit the clusters were respectively 0,25 and 0,60. The Shannon index gave the same results for the two areas. This index and the Bray-Curtis test consider both richness and abundance, but the Jaccard index considers only richness. The Kruskal-Wallis test showed a significant difference between the two areas in only 2 samplings. With the other 14 sampling there was no difference. Protozoan densities did not change at the first three weeks from the fire event, then, the populations starts to reduce. Subsequently, three months after the fire, at more favorable conditions, the protozoan populations increased again. After this phase, three and a half months after the fire, protozoan densities reduced and the densities stayed similar in both regions. The fire did not caused changes in the protozoan community structure, but only caused density reduction. Probably the fire did not affect directly this community, but indirectly, intensifying soil drought. We concluded that these protozoan populations are adapted to the effects of fire.

Key-words: fire, diversity, protozoans, microbiota, soil.

Introduction

In the last decades, the interest in studying microbial diversity increased, not only by the accelerated environmental damage and changes, but also due to recent evidences that high microbial diversity benefits ecosystem function (Laakso & Setälä, 1999; Griffiths *et al*., 2000).

The microbiota is the main responsible for organic matter decomposition, nutrient cycling

and energy flow in soil, influencing organic matter transformation and serving as carbon and mineral stocks (Jenkinson & Ladd, 1981). As part of the soil microbiota, protozoans are very important because they are directly responsible for about 60% of the nutrients ingestion and excretion into the environment (Bardgett, 2005).

Great part of global biodiversity stands in soil, and regarding bacteria, possibly, it comprises more than a half million of species (Torsvik *et al*., 1996a). Soil community analysis using molecular methods obtained an estimated range of 2000-18000 prokaryotic genomes per gram of soil (Torsvik *et al*., 1996b; Daniel, 2005). Most of this diversity is still unknown. Regarding protozoans, 70 to 80% of the soil protozoan biodiversity (around 1600-2000 species) is still not identified (Foissner, 1997). According to Foissner *et al.* (2007), soil samples from Africa, Asia, Australia, South America and Europe showed 964 ciliate species, 320 of which were undescribed. Based on the estimation sample coverage (ACE) from a single abundance sample, these authors proposed that half of the protozoan diversity of all continents is still unknown (1,600 – 2,427 species).

Soil is a heterogeneous environment in time and space and its microbial activity is concentrated in sites near organic residues (Ekelund *et al*., 2002) or the rhizosphere (Paul, 2007). Soil microorganisms are carbon limited (Wardle, 1992) and their growth is activated by excreted carbon from plant roots (Semenov *et al*., 1999) and by the inflow of fresh organic residues in this environment. Beyond these, other factors have been cited as controllers of abundance and distribution of soil protozoans like pH, humidity, porous geometry and type of humus (Stout & Heal, 1967; Acosta-Mercado & Lynn, 2004).

Protozoans frequently found in soil are: ciliates, heterotrophic flagellates, naked and testate amoebas. In these ecosystems, protozoans are conspicuous (Berthold & Palzenberger, 1995), acting both as predators and prey of other microorganisms (Clarholm, 1981) in addition to influencing the development and metabolic activity of bacterial communities (Pussard *et al*., 1994; Griffiths *et al*., 1999). Soil protozoans can regulate and modify the size and type of bacterial community (Gel'tser, 1991), accelerate the turnover of microbial biomass and soil nutrients (Vargas & Hattori, 1990) and provide nutrients from their excretion (Zwart & Brussaard, 1991); which, in turn, increases plant biomass (Kuikman *et al*., 1990; Alphei *et al*., 1996). Approximately 70% of total animal soil respiration can be attributed to protozoans (Sohlenius, 1980; Foissner, 1987). According to Foissner (1987), the ciliated protozoans contribute significantly to soil respiration and are excellent bioindicators of changes in the soil environment.

The cysts, common to soil protozoans, allow them to survive under adverse conditions such as drought and, according to Foissner (1987), under favorable conditions, protozoans can quickly excyst and become active. However, even under optimal conditions, much of them can remain encysted most of the time (cryptic diversity). When active, their growth and reproduction increase with increasing temperatures, and then cease due to encystment (Sleigh, 1973). Considering this, it is expected that the protozoans are very important in warm climates soil communities. On the other hand, tropical soil protozoans are poorly known but the few data available indicate that they have highly diverse communities in these environments (Foissner, 2000).

The Cerrado, also known as Brazilian savanna,

is the second largest biome in Brazil and occupy regions with two well-marked seasons; a wet season, from October to March, and a dry one, from April to September (Klink & Machado, 2005). It is characterized by a mosaic of vegetation types, including grassland physiognomies (campo limpo, campo úmido), savanna physiognomies (campo sujo, campo cerrado, campo rupestre, cerrado típico e cerrado denso) and forest vegetation (cerradão) (Coutinho, 1978a, Ribeiro & Walter, 2008). This biome is considered an important corridor and local breeding areas for species that inhabit the Amazon and the Atlantic Forest (Quirino *et al*., 2009). The Cerrado soil is deficient in nutrients and rich in iron and aluminum. Fire is an ancient and common factor in the Cerrado environments (Coutinho, 1981), which can be natural or from anthropogenic origin. Although the Cerrado is considered adapted to fire, burning regimes aiming to stimulate the growth of new pasture has resulted in soil erosion and depletion (Klink & Machado, 2005).

The burning regime influences population dynamics of plants (Hoffmann, 1999) and structure of the savanna community (Moreira, 2000). In the dry season, most of the grasses are inactive and the majority of the savanna biomass is dry, favoring the occurrence of fires (Klink & Solbring, 1996). The occurrence of fire can extend the effect of drought, because the biomass removal allows a higher incidence of solar radiation, which reduces the water availability in soil layers (Medina & Silva, 1991). Fire not only causes negative effects in this ecosystem, but according to Coutinho (1976), it promotes flowering in many plant species.

In general, the fire is lethal to microorganisms so changes in size of microbial population are common after this event. However, microbial responses are variable or even unpredictable, depending upon site conditions and intensity / severity of fire (Busse & De Bano, 2005). In this context, the present work aimed to evaluate the qualitative and quantitative effects of fire on the Cerrado soil protozoans, since its effects are still unknown. Considering the lethality of fire, we hypothesized that it can cause size and diversity reduction in the soil protist community.

Material and Methods

Study site and sampling

The study was conducted in Brazil, in a Cerrado legal reserve area (approximately 124.8 ha), located in the countryside of São Carlos-SP, at the Federal University of São Carlos campus. The main soil types in the region are allic Red-Yellow Latosol and dystrophic Red-Yellow Latosol, characterized by sandy texture and great deep. The altitude ranges between 815 and 895m. The climate is tropical, with wet summers and dry winters with minimal precipitation index from April to September. The area used as a control in this study suffered burns in 2006.

The vegetation of the reserve is heterogeneous, including the cerrado grassland and gallery forest, with a predominance of cerrado *sensu stricto* (characteristic vegetation of the savanna, comprised of arboreal-shrubby, stems and thick gnarled branches plants, slightly sparsely distributed and interspersed by a covering of herbs, grasses and semi-shrub species). The work was performed in the area of cerrado *sensu stricto*.

The 16 samplings (between August 2011 to August 2012) were made in two different areas, at approximately 200 m apart: the Control (21º58’04,06’’S; 47º53’02,96’’W), which remained unchanged, and the Burned (21º58’02,33’’S;

47º53'05,36''W), that suffered a fire event. The first sampling was made two days after the burning event. The samplings were made within a year, as follows: four samplings weekly, three with an interval of 15 days, three with an interval of 30 days, three with an interval of 60 days, and the last one with an interval of 30 days.

Soil samples of approximately 300 g were collected in 500 g capacity plastic bags properly identified with the sites and dates using representative acronyms (C = control, Q = burned). The sampling areas were defined as a quadrant of 60 m^2. In this quadrant, the sampling points were distant 2 m from each other. From each, it were taken 3 soil subsamples distant 0,5 m from per other (approximately 100 g soil was collected in each subsample). In laboratory three subsamples were mixed well yielding a sample in each collection point. Samples were taken with the shovel garden in the first five centimeters of soil, because the fire has more effect on this layer. According to Coutinho (1978b), during the passage of a frontal flame in a place with cerrado vegetation, surface soil temperatures reached 65-75 °C and there was no significant temperature increases at depths greater than 1 cm. Additionally, considering that protozoan densities decrease with depth (Bamforth, 1976; Ingham, 1994), Araújo *et al*. (2003) also reported an immediate reduction in microbial activity after controlled fire at three depths studied (0-5 cm, 5.1-15 cm and 15.1-20 cm), specially at the more superficial layers.

Climatological data and soil pH

Temperature, humidity, wind speed, solar radiation, evapotranspiration (Eto) and rainfall data were provided by Embrapa Southeast Cattle Meteorological Station (São Carlos - SP) (Embrapa, 2012). Soil pH measurement was performed according to Embrapa (1979). The proportion of soil for deionized water was 1: 2.5, the suspension was stirred with glass beads and allowed to stand for 1 hour. Before measurement the suspension was stirred again. The pH was measured on the pH meter Micronal (B-374).

Soil granulometric analysis

Soil granulometric analysis was performed according to Suguio (1979). The sample was dried at room temperature then, an aliquot was transferred to a tared flask. The organic matter extraction was made with hydrogen peroxide (H_2O_2) in a water bath, and the sample was cleaned 3-4 times using a syringe with distilled water. The cleaned sample was left in an incubator for 24 hours at 105 °C. The weight difference of the sample represented the content of organic matter of the sample. The separation of coarse (sand) and fine (silt + clay) fractions was performed after stirring the sample for 10 to 15 minutes using defloculant (1N NaOH). The resultant solution was passed through a 0.053 mm diameter diameter and the retained material was dried in an oven for 24 h. After this, the coarse fraction was passed through appropriate sieves and weighed in tared vials. The fine fraction was placed into a 1000 mL beaker and stirred with a glass stick according to Stokes' Law, to obtain the percentage of silt and clay.

Qualitative analysis of the protozoan cryptic diversity

The method used for the qualitative analysis of the protozoan cryptic diversity was the "non-

flooded Petri dish" (Foissner, 1992). Soil samples were brought to the laboratory and left dry for approximately 30 days. After being homogenized, 30 g of each soil sample was placed in a 12 cm sterile Petri dish and saturated with sterilized distilled water so that the soil would not get soggy. These were left to stand for 12 hours, minimum time for complete soil saturation. Slides were mounted with the liquid extracted from the Petri dishes and examined under an optical microscope in order to identify protozoan morphotypes. The inspection was performed on days 2, 6, 12, 20 and 30 after the sample preparation. The morphospecies were separated into taxonomic groups, according to the literature (Foissner & Berger, 1996).

Qualitative and quantitative analysis of the soil active protozoans

The number of active protozoans in the fresh soil samples was estimated by direct counting technique adapted from Lüftenegger *et al*. (1988) and Finlay *et al*. (1979). Sub-samples (0.1 g) of homogenized soil were placed into 5 mL tubes and suspended in 1 mL of sterile soil extract according to Aescht & Foissner (1992). The supernatant was analyzed using an optical microscope. The number of ciliated protozoa observed and identified in each 5 µl volume drop was recorded up to 150 ciliates or 150 drops. The analyses were made in the sampling day to prevent protozoan encystment. Morphospecies were separated into the taxonomic groups according to Foissner & Berger (1996).

Data analysis

Canonical correspondence analysis (CCA) was used to seek correlations between density of protozoan morphospecies and the environmental variables, performed in CANOCO program (ter Braak, 1986). During the analysis, the significance of correlation was tested by a Monte Carlo test with 999 random permutations, and $p \leq 0.5$ as the criterion of significance.

The Jaccard similarity index was used for the comparative analysis between the cryptic protozoan communities found in two locations (32 samples). The similarity value of 0.25 was used to delineate groups. This index gives a measure of the qualitative similarity of the samples, since only evaluates the absence or presence of species (Magurran, 1988).

Bray-Curtis similarity and Shannon index were calculated for the active protozoans. In the Bray-Curtis similarity analysis, for the compositional comparison of the different sampling sites, it was chosen the 60% similarity value to define clusters for the dendrogram construction. The diversity of protozoa in each region was calculated by the Shannon index (H) because it enhances the proportional abundance of species emphasizing the richness and homogeneity. This diversity index considers equal weights to rare and abundant species (Magurran, 1988). The Shapiro-Wilk test was used to test the normality of the data. The Kruskal-Wallis test was used, with the significance of 5%, for total density of protozoa in each sample aiming to observe if the areas become different from each other due to fire. These were calculated in Past program (Hammer *et al*., 2001).

Results

Mean values of climatic variables are shown in Table 1. It was decided to calculate mean values for 10 days before and including the sampling date, because climatic events during this period could be

influencing protozoan community.

Table 1: Mean climatological data from 10 days before samplings (Mean Temp.= Mean Temperature; Mean Rel. Hum.=Mean Relative Humidity; Rain; Mean Wind Speed; Solar Rad.=Solar Radiation; Eto=Evapotranspiration).

Sampling dates		Mean Temp. (C°)	Mean Rel. hum. (%)	Rain (mm)	Mean wind speed (m/s)	Solar Rad. (MJ/m^2)	Eto (mm)
August/2011	1	17.2	70.3	11.0	1.02	13.80	24.0
	2	20.1	60.0	0.0	1.1	17.24	31.66
	3	19.4	63.5	5.8	1.26	14.75	26.75
	4	20.6	64.69	15.0	1.62	15.53	29.0
	5	20.3	64.01	15.0	1.12	15.96	29.53
September/2011	6	19.1	53.37	13.8	1.33	19.18	34.22
	7	20.9	56.89	3.2	1.35	17.79	32.73
	8	21.0	56.57	1.2	1.42	20.22	37.26
October/2011	9	21.81	71.69	35.8	1.18	18.93	35.73
November/2011	10	19.85	66.28	2.0	1.42	22.37	40.11
December/2011	11	21.78	78.89	66.6	1.17	23.07	43.56
January/2012	12	21.59	83.37	64.8	0.96	18.45	35.15
March/2012	13	23.23	79.27	37.6	0.89	19.62	38.6
May/2012	14	17.6	78.72	79.2	1.07	13.89	24.03
July/2012	15	17.44	78.1	0.6	0.65	14.24	25.43
August/2012	16	18.02	69.71	0.0	0.81	16.37	29.34

In the control area of soil pH value ranged from 4 to 4.5. In the burned area, these values ranged from 4.5 to 7. In the first eight samplings the pH was 6 or 7 and, in the last eight it was 4.5, with the exception of one day which the value was 5.5.

Table 2 shows values of particle size analysis and amount (g) of organic and inorganic matter; percentage of organic matter, coarse sand, medium sand, fine sand, silt and clay contents.

In the CCA conducted with the morphospecies density and main abiotic variables (Figure 1). It was observed that the first two axes explained 74.3% of the data variability. There is a positive correlation between means relative humidity with 7 morphospecies (sp1, sp5, sp9, sp10, sp12, sp63, sp64). Medium sand was negatively correlated with 3 morphospecies (sp5, sp12, sp16) and positively correlated with 6 morphospecies (sp1, sp9, sp10, sp43, sp63, sp64). Furthermore, there was a positive correlation between coarse sand with 2 morphospecies (sp11, sp12). Fine sand was positive correlated with 4 morphospecies (sp1, sp4, sp6, sp8). The mean temperature was negative correlated with 9 morphospecies (sp4, sp6, sp8, sp11, sp13, sp16, sp29, sp32, sp43). And the clay was positive correlated with 2 morphospecies (sp5, sp31) the latter being the most abundant morphospecies. Regarding the samples, they were scattered and mixed by the diagram.

The cerrado soil showed 102 morphospecies by the non flooded Petri dish method. From those morphospecies, 101 were inactive at least in one soil sample, evidencing the cryptic diversity. Only 1 morphospecies was present in the active form in all collected samples, it is not part of cryptic

Table 2- Values (g) of granulometric analysis: organic matter (O.M.) and inorganic matter (I.M.); and percentage of organic matter (%O.M.), coarse sand; medium sand, fine sand, silt and clay in the soil samples from the two regions studied: Burned (Q) and control (C).

Control								
No. Samples	O.M. (g)	I.M. (g)	%O.M.	Coarse sand (%)	Medium sand (%)	Fine sand (%)	Silt (%)	Clay (%)
1C	0.47	22.73	2.03	0.42	31.38	32.31	6.33	29.56
2C	1.05	21.10	4.74	0.92	35.91	26.34	4.60	32.23
3C	1.22	20.52	6.12	0.76	38.52	22.10	6.01	30.02
4C	1.45	20.75	6.53	0.73	42.91	22.50	4.84	29.03
5C	0.54	24.26	2.17	0.77	41.57	22.15	6.13	30.66
6C	0.78	22.52	3.34	0.57	39.56	22.93	6.60	33.01
7C	0.85	19.15	4.25	0.77	41.75	21.34	2.58	33.59
8C	1.24	21.26	5.51	0.65	38.63	23.67	2.32	32.61
9C	0.55	21.85	2.45	0.68	40.49	22.04	4.53	34.01
10C	1.65	24.25	6.37	0.61	38.97	23.59	6.13	30.67
11C	1.37	24.67	6.55	0.72	40.16	22.43	6.03	32.16
12C	2.09	19.51	9.67	0.91	42.87	19.93	5.07	30.44
13C	1.74	19.96	8.00	0.94	42.95	19.94	7.44	27.28
14C	2.24	21.66	9.37	1.05	40.94	20.49	4.57	34.30
15C	2.08	19.67	9.23	0.93	41.43	19.91	4.65	30.20
16C	1.76	20.31	8.31	1.03	40.32	20.13	6.58	32.01
Burned								
No. Samples	O.M. (g)	I.M. (g)	%O.M.	Coarse sand (%)	Medium sand (%)	Fine sand (%)	Silt (%)	Clay (%)
1B	0.55	19.45	2.75	1.62	46.59	26.53	10.11	15.16
2B	0.73	19.77	3.56	3.66	53.54	20.22	10.04	12.54
3B	0.90	24.80	3.50	0.32	38.01	29.53	12.05	20.09
4B	0.60	21.60	2.70	0.92	44.95	28.53	18.34	11.46
5B	0.57	21.93	2.53	1.85	54.27	28.37	6.77	33.89
6B	0.60	22.50	2.59	1.58	43.65	21.49	15.41	19.82
7B	0.60	20.20	2.88	1.22	43.52	24.31	9.80	22.05
8B	0.50	24.60	2.0	0.44	36.29	28.22	10.08	24.19
9B	0.65	23.05	2.74	0.64	40.86	27.31	8.60	23.65
10B	0.72	23.78	2.93	0.58	37.15	28.44	4.17	31.27
11B	0.86	23.94	3.46	0.66	37.44	26.30	4.14	33.14
12B	0.71	23.19	2.97	0.98	44.71	21.29	6.41	23.51
13B	0.52	21.28	2.38	1.86	45.53	27.37	9.31	13.96
14B	2.10	21.60	8.86	0.91	31.69	24.22	2.29	32.11
15B	0.83	21.41	2.46	0.92	42.67	25.32	6.31	20.14
16B	0.92	21.87	2.87	1.46	43.23	23.67	6.63	23.52

diversity. The 101 morphospecies belong to 12 taxonomic groups: Hypotrichia (33 morphospecies), Hymenostomata (16), Pleurostomatida (11), Heterotrichida (9), Gymnostomatida (8), Oligotrichida (7), Cyrtophorida (7), Colpodea (4), Prostomatida (3), Peritrichia (1); amoeba (1) and testate amoeba (1). Figure 2 shows percentages of protozoan morphospecies within each taxonomic group in both regions studied. We could observe that the control region had 12 taxonomic groups and the Burned one had only 10, being amoeba and Prostomatida absent in this region.

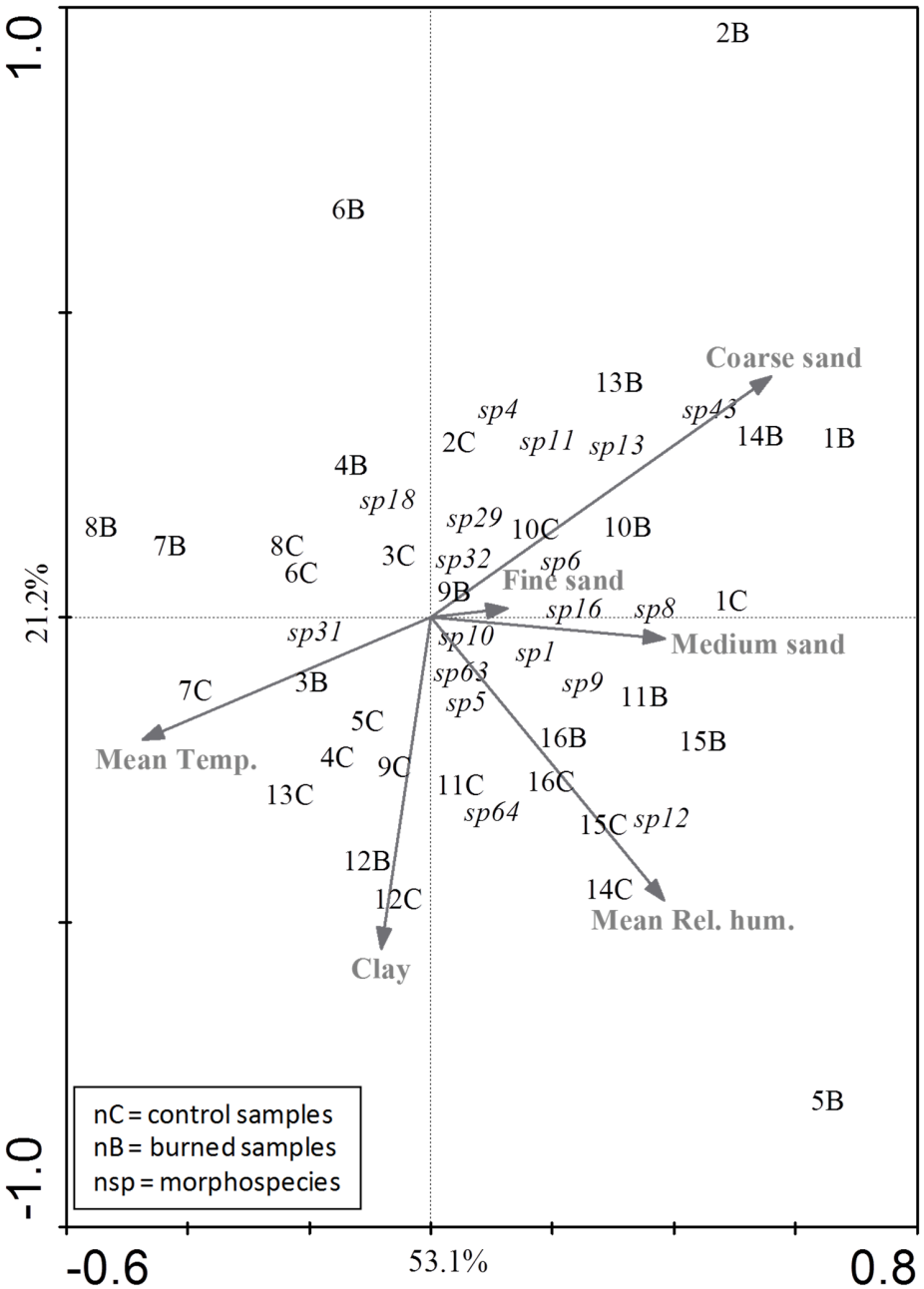

Figure 1. Canonical Correspondence Analysis (CCA) applied the species that occurred in three or more samples and mains environmental variables. The diagram of the abundance of 18 active morphospecies from 32 samples (16 from the control and 16 from the burned area). The diagram shows the distribution of morphospecies, the collected samples and environmental variables. The lines indicate the magnitude and direction of the increased of the environmental variables.

From the active protozoan analysis, it was possible to obtain 32 morphospecies, distributed into 9 taxonomical groups: Hypotrichia (12 morphospecies), Heterotrichia (5), Hymenostomata (4), Colpodea (3), Pleurostomatida (2), Gymnostomatida (2), Oligotrichida (2), Cyrtophorida (1); and testate amoeba (1). Figure 3 shows percentages of active protozoan morphospecies within taxonomic group in both studied regions.

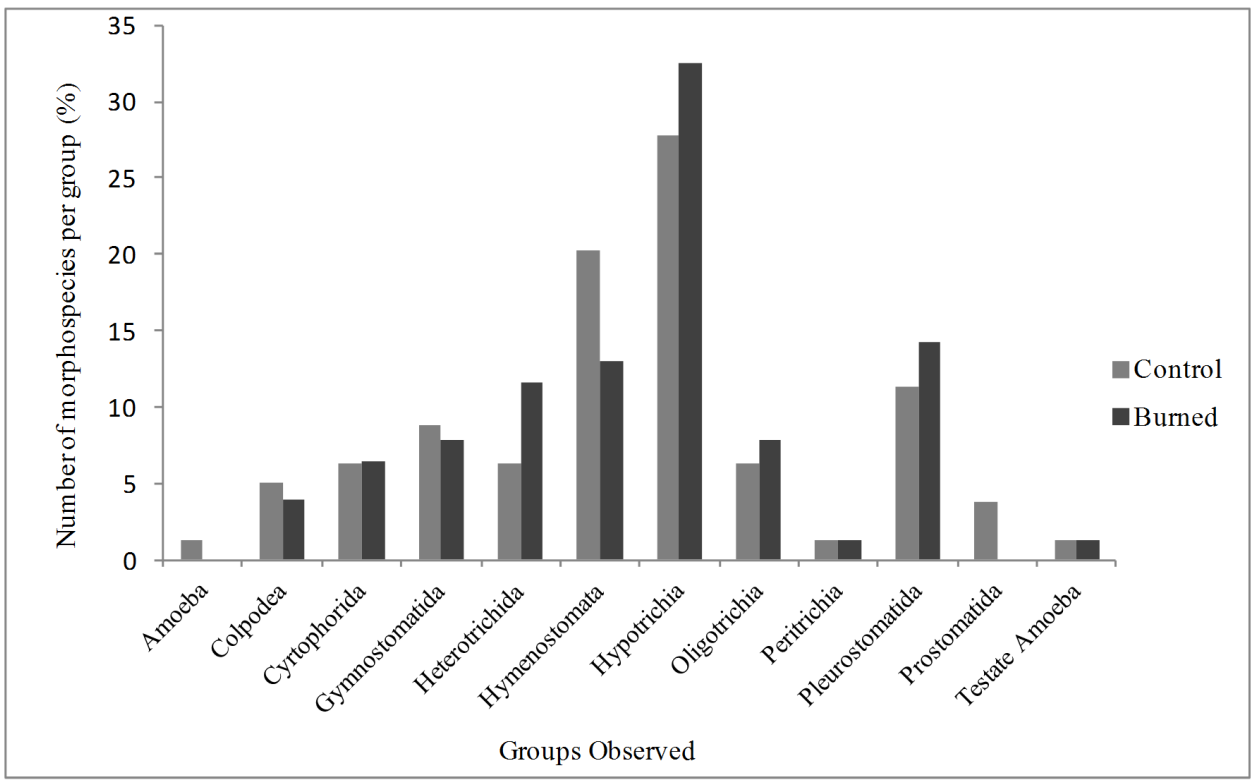

Figure 2. Percent distribution of the 101 protozoan morphospecies to their taxonomic groups at the Burned and Control region.

Of the nine taxonomic groups of active protozoans (Figure 3), 8 were found in control samples and only Cyrtophorida was absent in these samples. In the Burned ones we found also 8 taxonomic groups but representatives of testate amoeba were absent.

Figure 4 shows the abundance of active protozoans within each taxonomical group. The most abundant group was Colpodea in both studied regions, representing 51.81% of total active protozoans in control samples and 50.64% in burned ones.

Two protozoan morphospecies were the most abundant in Control as well as in the Burned samples. They were the sp31 and sp1 with percentages in the control region of 42.63% and 36.35%; and in the Burned region; and 39.78% and 32.48% in the Burned one, respectively. Besides these two morphospecies, other species also stood out in both sampling sites: sp4 (6.78% Control; 7.91% Burned), sp6 (3.53% Control; 6.89% Burned) and sp32 (2.39% control; 2.94% Burned). Thus, in both regions 26 morphospecies were observed. On the contrary, some morphospecies were absent in Control samples (sp17, sp19, sp33, sp42, sp79) and some other in Burned samples (sp61, sp27, sp28, sp39, sp48, sp51 and sp52).

From the total 102 morphospecies observed;

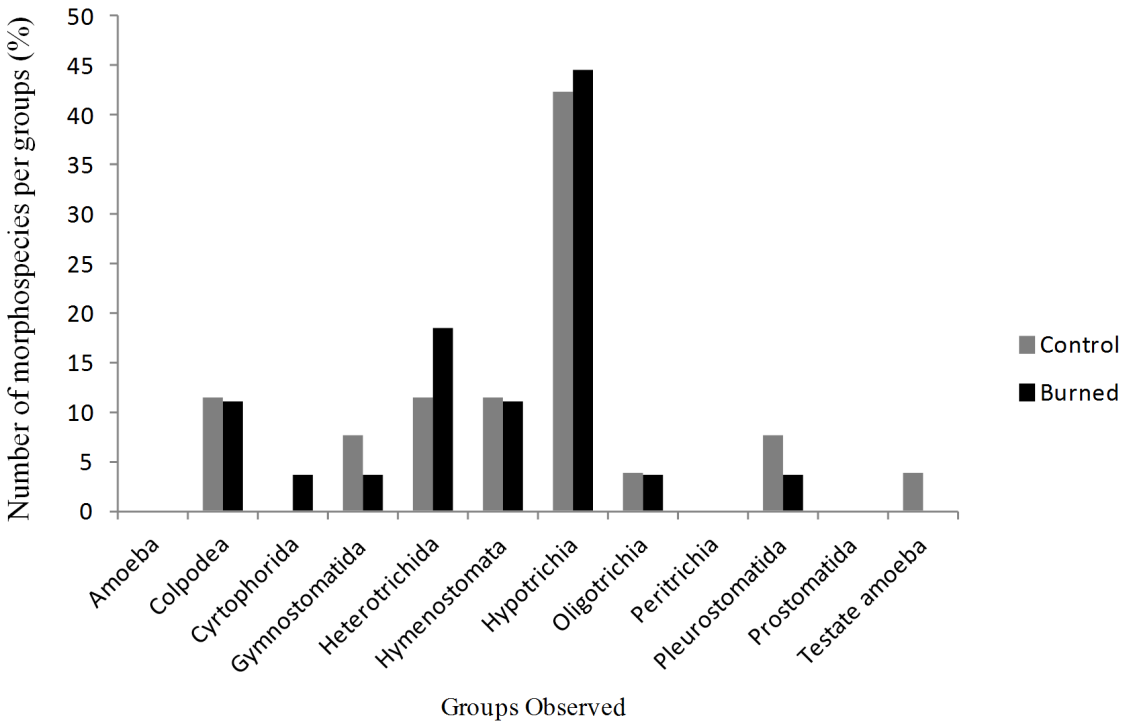

Figure 3. Distribution, in percentage, of the 32 active protozoan morphospecies into their taxonomic groups in the Control and Burned samples.

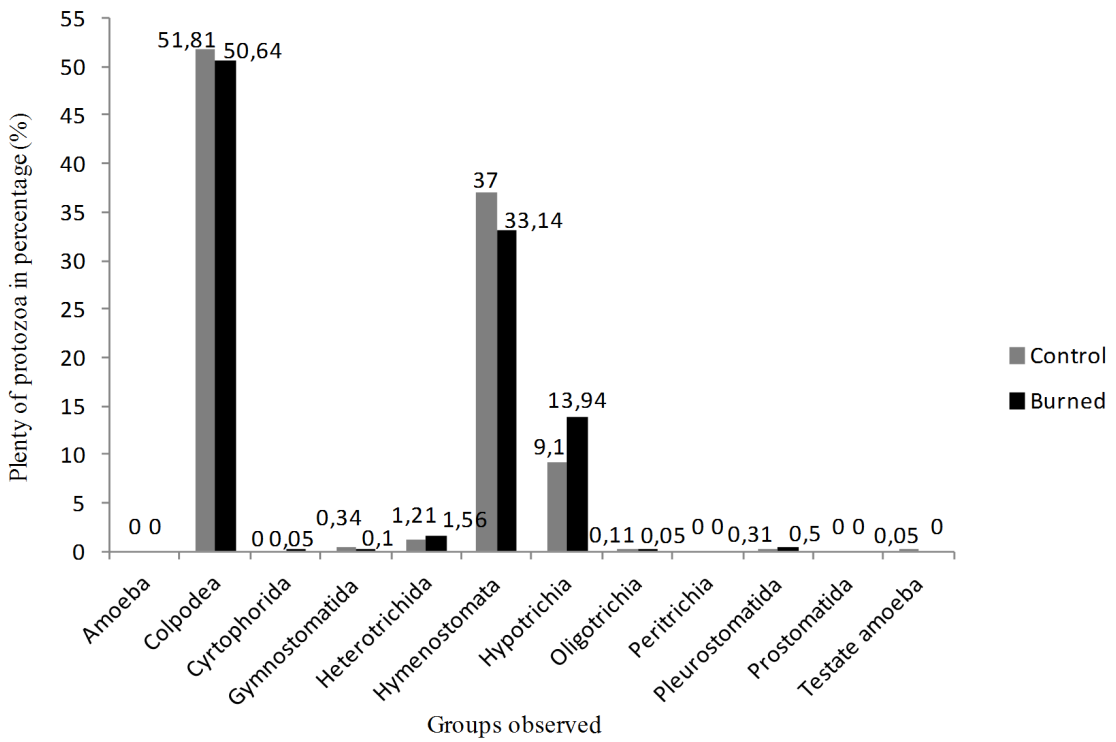

Figure 4. Abundance, in percent, of the 32 active protozoan morphospecies in their taxonomic groups at the Control and Burned samples.

101 were inactive, of which 56 occurred in both regions, 22 occurred only in the control and 23 only in the burned area. Thus, 55.44% of the morphospecies were present in both studied sites. In both regions, 26% of the morphospecies found with the non flooded Petri dish method were also observed in the active protozoan analysis.

The Jaccard index applied to all samples, using the 101 protozoan morphospecies, showed 3 groups (one large and two small) and eight samples that were not grouped. The samples 5C and 9Q were the closest ones, with a similarity value of 0.62 (Figure 5).

The Bray-Curtis similarity coefficient applied to the active protozoan in all samples showed that there are 6 groups (one large and five small) and two samples were not grouped. The samples that showed highest similarity value (0.91) were 8C and 8Q (Figure 6).

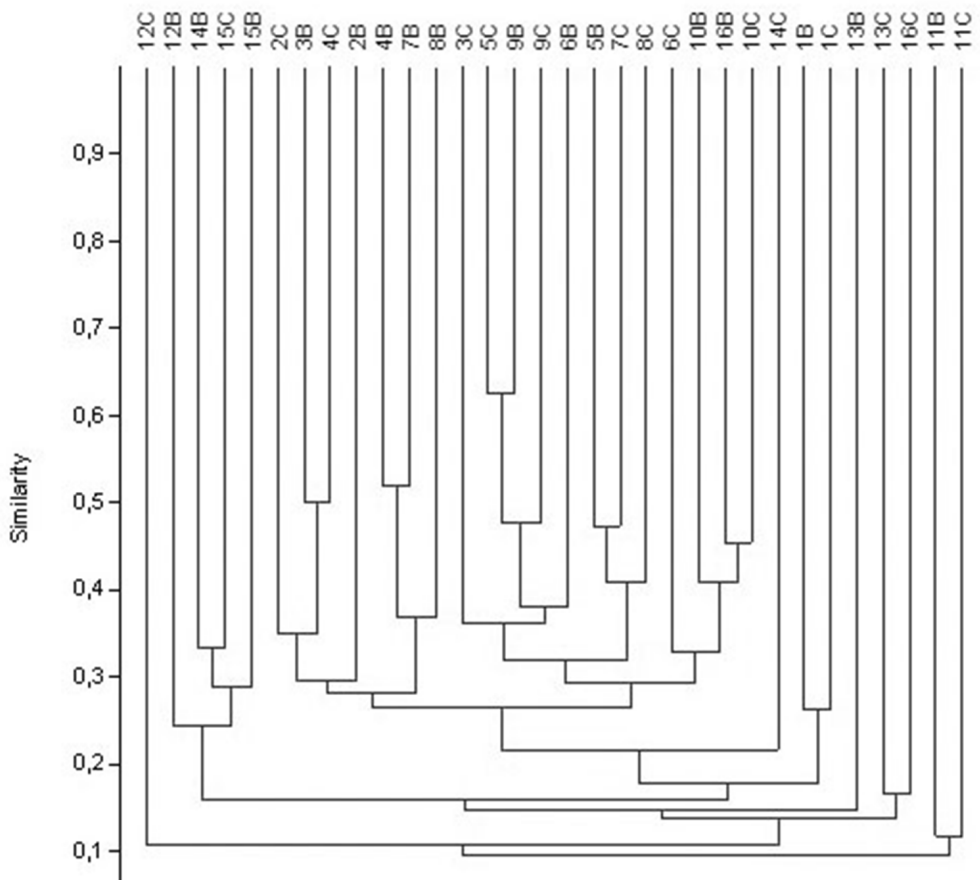

Figure 5. Dendrogram constructed after the Jaccard similarity index. The cutting line used to define the groups similarity value was 0.25.

The mean Shannon diversity index and the standard deviation (SD) for the control area was 1.216 (± 0.335) and 1.302 (± 1.303) for the Burned area.

The number of active protozoans in both areas is shown in Figure 7. In the first two samplings, after the fire event, there was no significant protozoan community change. In the third sampling, protozoan decreased in the burned area and increased in the control area. In the next four samples (4, 5, 6 and 7) protozoans decreased in the control region, equalizing their numbers with the burned region. In the sample 8 there was a protozoan peak in both

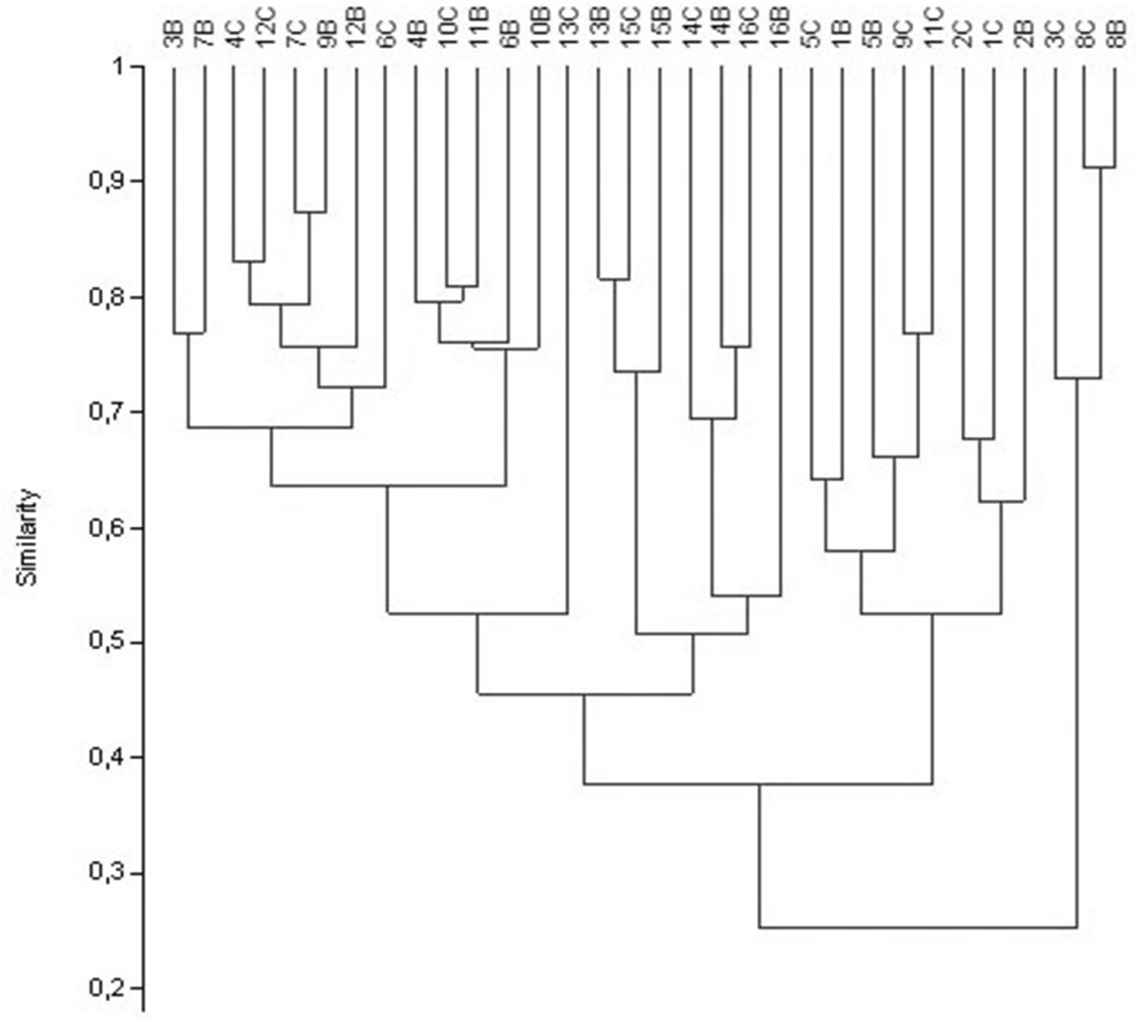

Figure 6. Bray-Curtis Dendrogram. The value used to define the groups was 0.60.

regions, but this peak was higher in the burned area. Finally, in the last eight samples protozoan densities decreased and were equivalent in both regions.

The Shapiro-Wilk test showed the value of p =2,206x10^{-20} as lower p-value and p =5,044x10^{-18} as the higher p-value, showing the data of active protozoans were not parametric. The Kruskal-Wallis test showed that the protozoan density showed significant difference between control and burned areas only in two samples (3 and 8 samples) with p =0.049. For the other 14 samplings the p-value was not significantly different between regions, with the lowest lowest value of p =0.076.

DISCUSSION

Environmental and biological variables

According to the Canonical Correspondence Analysis, the average values of temperature, relative air humidity, coarse sand, medium sand, fine sand and clay were those that most influenced positively and negatively the abundance of active morphospecies in both regions. The relative humidity of the air may have contributed to the maintenance of soil moisture making the

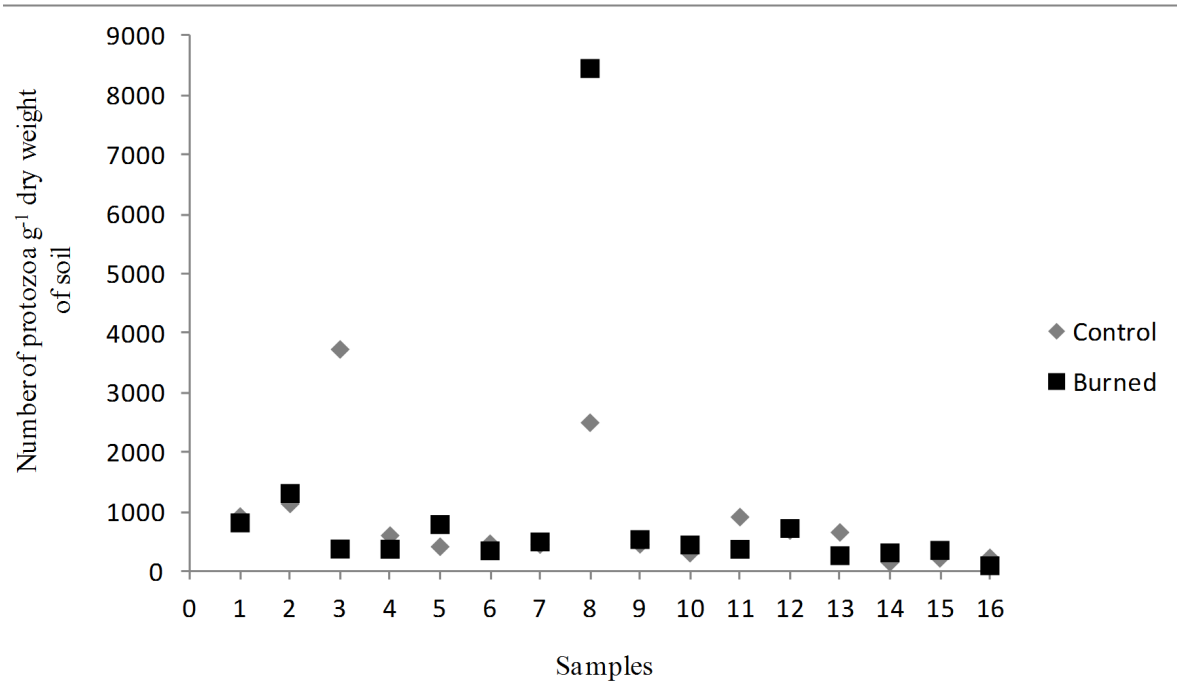

Figure 7. Number of soil active protozoans (dry weight g-1) at Control and Burned regions for each sampling date.

environment favorable the protozoan excystment and activity, therefore, protozoans can become active and reproduce, given that its generation time is short. Relative humidity is also correlated with maintenance of soil moisture, which form a water film around the sand grains, increasing niches to protozoans. Despite the rain to be related to relative humidity this was not significant during the studied period. The rain possibly caused cysts displacement from sites that have not suffered the burning to others that were burned, facilitating recolonization. The rain may have also contributed to organic matter reintroduction.

The temperature increase makes the environment favorable to protozoan excystment, activity and consequently reproduction. According to SLEIGH (1973) active protozoan growth and reproduction increase with increasing temperature, then cease due to encystment. However, very high temperatures would also negatively affect protozoans by the decrease of water film around the sand grain leading to a decrease in niches and causing encystment, especially of large species because they would lose water through evaporation.

The increase of clay in percentages, coarse, medium and fine sand possibly provides protozoan protection against predation, reducing space among the soil particles and causing a physical barrier, hindering the mobility of the predators. The mesofauna (mainly enchytraeids, springtails and mites, which are considered protozoan predators) depends on existing soil macropores for their movements (EKSCHMITT *et al*., 2008), thus the reducing of soil spaces favors protozoan protection from predators. Furthermore, small soil particles such as clay may be negatively correlated with some protozoan species because the niches available for large size morphospecies can be diminished by the reduction of space between grains. And, in this case, the population of small sized morphospecies can be facilitated by the niches availability.

Diversity, abundance and richness

A small proportion of total protozoan richness was found active during the study and the majority was inactive most of the time. These data corroborate the statements made by Foissner (1987) that the majority of the soil protozoa are inactive most of the time. According to Esteban & Finlay (2010) the cryptic diversity (inactive protozoa) can be used to evaluate the restoring capability of the environment. These authors stated that the greater the diversity, the greater the chance of recovery after an impact or environmental stress.

The number of 102 morphospecies found in 32 samples can be considered relatively low compared with a study of Foissner (1998) conducted in Shimba Hills in Kenya (Africa), in which 125 ciliate taxa were recorded in nine samples. It is also low in comparison with Blatterer & Foissner (1988) who obtained 139 ciliate species in 21 samples from Australia. One possible explanation for the lower richness found could be the stress that cerrado inhabitants suffers due to fire (common in such areas), low pH and high concentration of aluminum in the soil. This stress can lead to the colonization only by species adapted to these conditions. It is known that reduced diversity occurs in extreme environments. Brock (1978) defined extreme environments as those where only a limited number of species can live and reproduce. Although the cerrado is not considered an extreme environment, the fact that it suffers with those frequent stresses may explain the relatively low diversity found.

Another explanation for the low number of morphospecies may be the fact that the samples were collected in a limited size region (approximately 60 m^2), so other cerrado areas were not sampled. This is important, especially considering that the cerrado is characterized by a mosaic of vegetation types. In this work only cerrado *sensu stricto* was sampled and its gallery forest and grassland regions were not. Foissner (1998) obtained a higher number of morphospecies from a much smaller number of samples, originated from different regions (covering marshland, forest, grassland) from the same studied area. Probably the areas with different cerrado vegetation that were not sampled could have different richness of that was observed. Besides, perhaps these areas could be suffering differently with those mentioned stresses.

Even if there was no significant difference between the burned and control areas, it was possible to observe a slightly higher diversity in the burned area. This could have occurred probably by the higher availability of niches to be explored in the burned area due to the higher percentage of grain sand, except clay, present in this area

Considering both regions (Control and Burned) and methods (non-flooded Petri dish and quantitative active protozoans) in this study, the taxonomic group with the higher number of morphospecies reported was Hypotrichia. Acosta-Mercado & Lynn (2002), in a study conducted in a Puerto Rico forest, also found the Hypotrichia and Stichotrichia subclasses as the most frequent and dominant ones. The presence of structures such as cirri and the flat body shape may explain the success of these organisms in soil environments allowing them to move on surfaces.

On the other hand, the most abundant group of active protozoans was Colpodea, followed by the Hymenostomata, in both regions. Representatives of these groups tend to be small, which may give them an advantage in situations with little water and space in the soil. So, they can be present in the active form, even in soil with little moisture. The

most abundant morphospecies in these two groups have 50 µm and 25 µm respectively, so probably, when environmental conditions are favorable, they can quickly excyst, start reproduction and consequently increase the number of organisms exploiting the niche with less competition. Furthermore, the cerrado suffers from the above mentioned stresses (such as fire) and this could be the reason of Colpodea be the most abundant group because they are considered colonizers that possess an r-selected survival strategy (Foissner, 1987).

The explanation for the low number of Hypotrichia after the stress can be related with their large size. Under favorable conditions, they probably take more time to excyst and start reproduction then their smaller competitors. The large size can also hinders access to soil deeper layers where moisture is likely higher. So, competition with smaller protozoans can also be a factor that maintains these organisms in low numbers after the stress.

According to the similarity analyses, even with the impact of fire, the burned region can be considered similar to the control one. This was concluded because, considering the five groups that have the highest similarity values, four occurred in the two regions (Control and Burned). The similarity index of Bray-Curtis was even higher than that obtained with Jaccard thus, confirming the similarity between these regions. It is known that fire is a common factor in the cerrado (Coutinho, 1981), therefore, the similarity found for the Burned and Control regions showed that the protozoan community was adapted to this stress. The fire appears to be an important factor because the increase in the percentage of sand grains enhances the available niches which can lead to a small increase in protozoan diversity.

The data obtained by the Shannon diversity index, cryptic diversity and abundance analysis also indicate that the studied regions are similar. However the Kruskal-Wallis test showed significant difference in only two samplings, so the difference was punctual. The data indicate that protozoan community is adapted to the environment and have high ability to resist the stresses because the community structure was maintained.

The protozoan density data showed that the fire had no direct effect on this parameter. In the first two samplings, the fire caused no significant density change but in the third one the density decrease in the burned area probably occurred due to the biomass removal by fire and a consequently increase in soil exposure to solar radiation. According to Pereira *et al*. (1999) the vegetation protects soil from the exposure to solar radiation. The solar radiation may have led to a decrease in soil moisture (Medina & Silva, 1991) which caused an increase in encysting protozoa, and a momentary decrease in active protozoans. Another factor that may have contributed to this protozoan density decrease may be the low food availability by bacterial density reduction by fire. It is known that soon after the fire event there is a reduction in the number of bacteria, fungi and total microbial biomass at the top layer of the soil (Van Reenen *et al*., 1992). Fire can kill part of the soil microorganisms; among them, fungi are most sensitive than bacteria (Dunn *et al*., 1985). So, indirectly, the Colpodea may have had its abundance also affected by fire since the Grossglockneridae, belonging to this group, and described by Foissner (1980), feeds exclusively on fungi and yeast (Petz *et al*., 1985; Petz *et al*., 1986).

In the following four samples after the fire event, the protozoan density decreased and was

similar in both studied regions. This reduction was probably a result of the climatic conditions of the period, such as increased temperature and reduced humidity leading to the enhancement of soil drought. However, in the sample 8 there was a protozoan density peak in both regions maybe resulted of the increased bacterial density possibly due to decreased predation by protozoa in samples 3, 4, 5, 6 and 7. This fact probably occurred together with increased quality of fresh organic matter, because grasses regrowth began approximately one month after the occurrence of fire. So, both decreased predation and increased quality of fresh organic material may have contributed to the increased bacterial density and the increase in protozoan density in sample 8 (Figure 8). We may consider that increased pH contributed also to bacterial increase. Rousk *et al*. (2009) stated that it is possible that the pH increase favors the bacterial growth; moreover, many studies that analyzed heating effects on microorganisms showed that bacteria have a fast recovery after a heating event (Bollen, 1969; Vázquez *et al*., 1993; Guerrero *et al*., 2005; Mabuhay *et al*., 2006). In the last eight samplings protozoan densities were equivalent in both regions. There was a decrease in density, probably due to the control of bacterial population by protozoa and competition, indicating that protozoans have a high capacity to restore and recolonize.

It is known that free-living amoebae are the dominant bacterial consumers in soil (Elliot, 1977; Clarholm, 1981), accounting for 60% of their total populations (Clarholm, 1981). Amoebae are also known as the major protists in the microfauna of the tropical forest soil ecosystems (Krashevska *et al*., 2007). However, it was noted the near absence of amoebae morphospecies during this work. According to Altenburger *et al*. (2010) amoebae have a late colonization compared with flagellates and ciliates, which may explain the results obtained in this study. Another possible explanation for the low amount of amoebae observed in the present study may be the combined effect of high temperatures and increased precipitation. Darby *et al*. (2011) observed, in the desert of the Colorado Plateau in Utah, that amoebae abundance was reduced with the combination of increased temperature and rainfall frequency.

Throughout the samplings, an increase in the amount of organic matter was not observed; especially in the burned area, as observed by Heyward & Barnette (1934). They observed that frequent burning of grass beneath the pines in the coastal plain of the southeastern United States increases the organic matter content in the first 10-15 cm of the soil profile. However, the quality of organic matter may have increased because, approximately one month after fire, the grasses have begun to sprout.

Data from the present work revealed that the fire was not capable of causing changes in protozoan community structure; it caused only a decrease in abundance due to the indirect effects, however the richness is maintained and later the abundance is recovered, thus indicating the restoration ability of this environment, and eventually adaptation of protozoa in relation to stress (fire). The cryptic diversity may have contributed to this restoration, since the greater the diversity, the greater the chance of recovery after environmental stress (Esteban & Finlay, 2010).

One explanation for this rapid protozoan community recovery may be its adaptation to the stress caused by the fire heat. The savanna area studied undergoes a recurrent burning regime;

i.e., on average, once every two years. Due to this burning regime the protozoan community could have been adapted to it. The environment may have selected protozoans with rapid cyst formation and excystation and, due to this fact, recover fast and easily from this stress, once, three months after the fire occurrence, the Burned area was similar to the Control region.

Conclusions

In this study it was possible to obtain 102 protozoan morphospecies divided into 12 taxonomic groups among ciliates, naked and testate amoebas. The protozoan density in most samples remained between 300-900 cells per gram of dry weight. It was observed that even with the stress caused by the fire the protozoan community structure has not changed significantly since the richness was maintained and only the abundance decreased, but was soon recovered. We observed that the fire had no direct effect on the protozoan community, but rather an indirect one, intensifying the effect of drought, through biomass removal. So, the two regions remained similar throughout samplings. The protozoans showed to be adapted to this environment that suffers frequent fire events.

Acknowledgements

We would like to thanks FAPESP (Process n° 2011/15624-4) for the grant afforded to Mayara Pierozzi and CAPES for the financial support.

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