Soil Biology & Biochemistry 68 (2014) 133–139

Contents lists available at ScienceDirect

Soil Biology & Biochemistry

journal homepage: www.elsevier.com/locate/soilbio

The effects of natural carbon dioxide seepage on edaphic protozoan communities in Campo de Calatrava, Ciudad Real, Spain

R. Gabilondo [a,b,*], E. Bécares [a,b]

[a] Department of Ecology, Faculty of Biology, University of Leon, 24071 Leon, Spain
[b] Environmental, Natural Resources and Biodiversity Institute (IMARENABIO), University of Leon, 24007 Leon, Spain

ARTICLE INFO

Article history:
Received 18 April 2013
Received in revised form
12 September 2013
Accepted 13 September 2013
Available online 8 October 2013

Keywords:
CO_2 upwellings
Gas vent
Campo de Calatrava
Soil protozoan community
Ciliate diversity
Flagellates
Naked amoebae

ABSTRACT

The effects of natural carbon dioxide [CO_2] seepages or "upwellings" on edaphic protozoan communities at two locations in the Campo de Calatrava district in Spain were studied. The fluxes of CO_2 in the soil ranged from 11.46 g m^{-2} day^{-1} (ambient) to 117.45 g m^{-2} day^{-1} at Cañada site and from 14.64 to 293,508.66 g m^{-2} day^{-1} at Sima location. No significant changes in the total abundance of ciliates, flagellates and amoeba with CO_2 increases were observed at either of the sites. Nevertheless, the composition and structure of the community of ciliates were significantly affected by CO_2. The diversity of the community, calculated by using both the Shannon and the Margalef indexes, decreased as CO_2 increased. Equitability (or evenness) and even more so total richness decreased with higher CO_2. A shift from Polyhymenophorea (Spirotrichea and Heterotrichea) to Colpodea dominated communities and a decrease in the percentage of rapacious ciliates was observed as CO_2 increased. The composition of the ciliate community could therefore be used as a potential indicator of CO_2 concentrations in soils.

1. Introduction

Natural seepages or "upwellings" of carbon dioxide [CO_2] may be considered as natural models for studying and predicting the effects of artificial CO_2 increases on edaphic protozoan communities. Despite the range of CO_2 fluxes in natural seepages is much wider than expected in a potential leak into the biosphere from artificial CO_2 in geological storage, these seepages could be considered as natural experiments on this aspect, especially when the lower range of concentrations are considered for comparison. Protozoan communities might be used as bio-indicators of CO_2 increases in the soil to detect significant and potential changes in soil function and structure, whether these are from atmospheric sources, or gas leakages from geological stores of CO_2 in saline aquifers. Moreover, ancient natural CO_2 upwellings, like those present in Campo de Calatrava, written records of which date back to 1574 (González, 1996), may have promoted the evolution of new protozoan species adapted to the presence of high concentrations of CO_2.

Protozoa react more quickly to physical and chemical changes in the soil than other edaphic organisms, and therefore, are potentially good indicators of soil pollution (Foissner, 1994, 1999). Moreover, protozoa are an essential component of the soil; they inhabit, and are particularly abundant in, soil ecosystems with extreme environmental conditions (Foissner, 1987).

Because of recent findings concerning their role in controlling plant growth (Bonkowski, 2004), there is an increasing interest in micro-edaphic fauna. An increase of CO_2 in the atmosphere, arising, for example, from anthropogenic causes, would stimulate plant activity, promoting the excretion of organic matter into the rhizosphere. This would stimulate bacterial activity, and consequently bacterivores and hence root activity (Bonkowski et al., 2000). An increase in CO_2 directly in the soil could change the physical and chemical conditions of the soil, specifically pH, which could influence its fauna. On a local scale, protozoa are the principal factor controlling bacterial communities (Darbyshire, 1994). Changes in abundances and species of bacteria could be influenced more by bacterivores than by CO_2. There is extensive literature concerning the effects of CO_2 on soil microbial populations and vegetation (including Beaubien et al., 2008; Krüger et al., 2009, 2011; Lakkaraju et al., 2010; Morozova et al., 2011; Oppermann et al., 2010; Pierce and Sjögersten, 2009; Tarkowski et al., 2008; West et al., 2009). However, there is only sparse information in relation

* Corresponding author. Department of Ecology, Faculty of Biology, University of Leon, 24071 Leon, Spain. Tel.: +34 987291568.
E-mail addresses: regina.gabilondo.toscano@gmail.com, regabilon@yahoo.es (R. Gabilondo).

0038-0717/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.soilbio.2013.09.028

to its effects on communities of protozoa in the soil (Hungate et al., 2000; Rillig et al., 1999; Rønn et al., 2003; Treonis and Lussenhop, 1997) and on their diversity (Ekelund et al., 2001; Rønn et al., 2001).

In this paper, the effects of CO_2 on edaphic protozoan communities was studied at two different locations in Campo de Calatrava district in Spain. These covered a range of CO_2 fluxes from 11.46 g m^{-2} day^{-1} (ambient) to more than 293,508.66 g m^{-2} day^{-1}, the latter one of the highest ever measured in natural conditions.

2. Material and methods

2.1. Site description

Campo de Calatrava district is in the centre of the province of Ciudad Real in Spain. Volcanic activity took place there between 8.7 and 1.7 million years ago, during the Pliocene and Quaternary Eras. CO_2 is present in the subsurface and aquifers. Its origin lies in degassing processes when magma cools slowly under the surface. CO_2 seepages or "upwellings" to the surface are due to fractures and cracking in rocks (González et al., 2007).

2.2. Soil sampling

Soil was collected at two sites. In the location called Sima near Granátula de Calatrava, Spain (38° 49′ 17.7″N, 3° 45′ 19.1″O; 30S/434432/4297249), the soil was Alfisol Xeralf, according to the United States Department of Agriculture (USDA) soil taxonomy (United States Department of Agriculture, 1999). Alfisols have clay-enriched subsoil, aluminium and iron. It was composed by gravels and polygenic pebbles of quartzitic sandstone and quartz, sands, sandy clays, clays and carbonates (alluvial cones). The other location was Cañada Real (Cañada), near Valenzuela de Calatrava, Spain (38° 51′ 15.1″N, 3° 51′ 23.6″O; 30S/0425677/4300945), where the soil was Entisol Orthent, according to USDA soil taxonomy, with no profile development other than A horizon (United States Department of Agriculture, 1999) and was composed by quartzitic sandstones, sandstones and shales. Chemistry composition of the soils is presented in Table 1. Distribution of CO_2 seepage in soil was measured using a CO_2 field device (LICOR LI820 CO_2 soil flux metre from West Systems). The flux of CO_2 emanating from the soil was measured. Only those zones with the same vegetation and similar soil characteristics were selected for each of the CO_2 fluxes in Sima sampling point. Calatrava sampling zone was completely homogeneous in soil and vegetation as it was a prairie park. Once collected, soil was stored in plastic bags and analysis was conducted within the following two days in the laboratory in Leon, Spain. Humidity, pH, organic matter and conductivity were measured in accordance with the standard protocols of the Spanish Ministry of Agriculture, Fishery and Food (Ministerio de Agricultura, Pesca y Alimentación, 1994). The available fraction of the elemental concentrations of ions and metals in the samples were analysed with an ICP-AES Optima 2000 DV from Perkin Elmer, using the standard protocols of the Spanish Ministry of Agriculture, Fishery and Food (Ministerio de Agricultura, Pesca y Alimentación, 1994) and the Canadian Society of Soil Science (1993).

Table 1
Physical and chemical average characteristics of the soil in each of the sample conditions.

Physical and chemical characteristics	Cañada			Sima		
	Control	Low	High	Control	Low	High
Increased CO_2 flux with respect to ambient	Ambient	X3	X10	Ambient	X3	X79
(g m^{-2} day^{-1})	11.46	35.19	117.45	14.64	47.13	1163.45
Humidity (%)	27.98	24.69	30.85	17.11	27.12	17.46
pH (water)	8.51	8.25	8.27	5.38	5.00	3.89
Organic matter (%)	2.30	1.76	2.42	3.04	3.54	3.65
Total nitrogen (%)	0.17	0.14	0.17	0.22	0.24	0.26
Phosphorus (ppm)	63.45	45.79	72.08	5.88	5.44	21.62
Calcium ($cmol^{(+)}$ kg^{-1})	16.09	15.85	18.14	3.28	5.92	2.62
Magnesium ($cmol^{(+)}$ kg^{-1})	12.18	11.45	13.92	0.65	1.16	0.09
Potassium ($cmol^{(+)}$ kg^{-1})	1.87	2.23	1.98	0.22	0.19	0.17
Sodium ($cmol^{(+)}$ kg^{-1})	0.84	0.79	0.65	0.06	0.04	0.03
Iron (ppm)	18.63	10.02	12.44	102.00	128.71	400.93
Copper (ppm)	2.85	1.06	1.74	0.20	0.29	0.48
Zinc (ppm)	7.73	2.63	5.56	0.41	0.35	0.81
Conductivity (dS m^{-1})	0.21	0.20	0.16	0.02	0.03	0.18

2.3. Analysis of protozoa

The abundance of amoebae, flagellates and ciliates was measured in accordance with the protocol described by Adl et al. (2007). Briefly, 1 g of soil was weighed, mixed with water and the mixture pipetted onto agar in 5 cm Petri dishes and aluminium foil. The number of amoebae was counted 24 h later. Flagellates were counted in a haemocytometer chamber. The number of ciliates was counted with a light microscope (Madoni, 1984) and 4 replicates of 100 μl were measured.

2.4. Diversity of ciliates

Soil was cultured in accordance with the non-flooded Petri dish method described by Foissner (1991). On Day 3, the abundance and the diversity of ciliates were recorded using the light microscope, with 4 replicates of 100 μl. Additionally, 2 ml of the soil supernatant were fixed with Bouin for later study by the Edaphic Quantitative Protargol Staining (EQPS) method (Acosta-Mercado and Lynn, 2003) and 5 ml were stained, using a modified silver carbonate method.

2.5. Modified silver carbonate method

The protocol described by Fernandez-Leborans and Castro de Zaldumbide (1986) was followed in order to prepare silver carbonate: 5 g of silver nitrate were dissolved in 50 ml of distilled water and 7.5 g of sodium carbonate were dissolved in 150 ml of distilled water. The two solutions were mixed. NH_3 was added to the resulting 200 ml mixture, drop by drop. Distilled water was added to make the volume up to 750 ml.

Staining of Ciliates with Silver Carbonate: 5 ml of supernatant from the non-flooded Petri dish method were mixed with 0.5 ml of 40% formaldehyde for 2–3 min 20 ml of distilled water, 0.3 ml of anhydrous pyridine, 0.5 ml of 4% bacteriologic peptone and 6 ml of ammonium silver carbonate were added. The mixture was heated at 65 °C for 10–15 min. Once the mixture turned a cognac yellow colour, the reaction was stopped with 4% sodium thiosulphate (Fernández-Leborans, 1990). The resulting mixture was filtered through a 1.2 μm pore size cellulose nitrate filter in a Millipore column under no more than 100 mm Hg of pressure, to avoid rupturing cells. Filters were removed and placed sample side up on a warm glass slide. A drop of glyceride-albumin was dropped on top of the filter, air dried for 24 h and mounted with Permount™ and cover slips. An example of the staining method is presented in Figs. 4 and 5.

2.6. Diversity indexes

Shannon, Equitability and Margalef indexes were calculated with PAST (PAleontological STatistics) Version 2.17 (Hammer, 2012).