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Soil communities are affected by CO_2 belowground emissions at a natural vent in Spain

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ABSTRACT

Natural CO_2 vents have received growing interest in the last years due to their relation to CO_2 capture and storage (CCS) risk assessment studies. Despite the increasing body of knowledge, mostly focused on microbial communities, scarce information is available on how geological CO_2 affects mesofauna and microfauna, and their interactions. We studied microorganisms, microfauna i.e. protists and nematodes, and mesofauna communities, i.e. collembola and mites and their relationships in a natural CO_2 vent at La Sima (Spain). Four CO_2 flux intensities from Control (7–19 g m^{-2} d^{-1}) to low (40–55 g m^{-2} d^{-1}) and high fluxes (260–1600 g m^{-2} d^{-1}), including extreme emissions (more than 10^4 g m^{-2} d^{-1}) were studied. We found that increasing CO_2 emissions from Control to high fluxes strongly affected biota abundances and richness, cascading from microorganisms to mesofauna, and resulting in reduced and less diverse populations in each of the groups levels assayed. Nevertheless, at extreme fluxes edaphic biota biomass recovered in most of the communities, suggesting that the extreme CO_2 conditions are associated with high abundances of well adapted communities, although with very low diversity. Increases in abundance of bacteria, fungi and amoebae, but not ciliates, were related to increases in mesofauna richness and nematode and mesofauna abundances. Our results help to indicate the CO_2 threshold from which accidental losses from CCS operations can be detected in the long-term.

1. Introduction

Rising atmospheric CO_2 concentrations and the relative interest in studying the consequences on soil ecosystems has stimulated research at CO_2 vents (mofettes) (Paoletti et al., 2005). Mofettes are natural ecosystems where cold, geogenic CO_2 migrates upward through surface water or soil to the atmosphere (Russell et al., 2011). These ecosystems, where CO_2 has been venting for decades or centuries, represent a stable long-term scenario of basic scientific interest that may serve as a model system to assess the effects of anomalous CO_2 concentrations (Šibanc et al., 2014).

The high CO_2 concentrations presented in these areas, sometimes reaching 100% in the centre, leads to changes in soil gas composition, soil hypoxia or even anoxic conditions (Vodnik et al., 2009), resulting in adverse conditions for microbial and animal life in mofette fields (Paoletti et al., 2005; Gosálvez et al., 2010). Nevertheless, the impossibility of surviving above the ground may not apply to belowground life (Russell et al., 2011). In fact, it is known that soil CO_2 concentrations are 10–100 times higher than the atmospheric level (Drigo et al., 2008; Russell et al., 2011) therefore by at least some edaphic biota may be resistant to mofettes' environment. Moreover, several studies have found microbial communities adapted to high CO_2 concentrations in soils (Tarkowski et al., 2009; Oppermann et al., 2010; Beulig et al., 2015).

Natural CO_2 vents have received growing interest over recents years due to emerging CO_2 capture and storage (CCS) applications, as they can be used as a simulation of a potential leakage from a CCS site (e.g. Beaubien et al., 2008; Krüger et al., 2009; McFarland et al., 2013). CCS is a process consisting of the separation of CO_2 from industrial and energy-related sources, transport to a storage location and long-term isolation from the atmosphere (IPCC, 2005). Although storage sites are evaluated to avoid possible leaks, this

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cannot be completely excluded and understanding the consequences on the environment is a major concern (Krüger et al., 2011).

Biological studies in natural CO_2 vents have focused on microbial communities (mainly bacteria) (Frerichs et al., 2013; McFarland et al., 2013; Šibanc et al., 2014), but scarce information is available on higher trophic levels. As an exception, Russell et al. (2011) found mofettophilous Collembola in Cheb Basin (Czech Republic) and Yeates et al. (1999) observed negative effects of high CO_2 emissions on nematode communities in a Northland natural vent (New Zealand). However, no studies have focused on other groups of animals or on the relationship among them.

Our investigation takes advantage of natural CO_2 emissions at La Sima vent, located in Calatrava Volcanic Field (CVF), central-eastern Spain. La Sima covers a wide range of CO_2 fluxes (from 40 g m^{-2} d^{-1} to 300 kg m^{-2} d^{-1}) being one of the sites with the most extreme fluxes ever studied (Beaubien et al., 2008; Krüger et al., 2011; McFarland et al., 2013; Šibanc et al., 2014; Beulig et al., 2015). To date there are been few biological studies in La Sima vent (Gabilondo and Bécares, 2014; Sáenz de Miera et al., 2014), and to our knowledge, no studies have addressed the effects of belowground CO_2 emissions on organisms of different trophic levels. Hence, the aim of this research is to analyse microbial, protozoan and mesofauna populations from La Sima mofette, to better understand the effect of high CO_2 belowground fluxes on edaphic communities and their relationships.

2. Materials and methods

2.1. Study site

The Calatrava Volcanic Field (CVF) is one of the highest CO_2 discharge areas in Spain, consisting of more than 300 emission centres throughout an area of about 3000 km^2. Details on the area can be found in Elío et al. (2015). The region is characterized by a continental Mediterranean climate. Average annual precipitation and temperature are 396 mm and 14.7 °C, respectively (AEMET, 2015). La Sima mofette is a CO_2-rich discharge (up to 2 t d^{-1}) consisting of a 5 m diameter depression (Elío et al., 2015), located at Granátula de Calatrava (Ciudad Real, Spain). Since a seismic crisis in the area in 2007, CO_2 concentrations have increased from 30,000 to 200,000 ppm, with traces of H_2S, HCl, CH_4 and Rn (Peinado et al., 2009; Gosálvez et al., 2010). Oxygen concentrations remain under 7% in the gas vent leading to aerobic to microaerophilic conditions. CO_2 atmospheric values range from 150,000 to 200,000 in the spring centre (Gosálvez et al., 2010). CO_2 dominance may be due to the carbonitic nature of the CVF's magma (Stoppa et al., 2012). Vegetation is absent in the vent centre and very reduced at the surroundings, as CO_2 venting has led to plant mortality.

Sampling took place in November 2012 and May 2013. Before sampling, distribution of CO_2 fluxes in La Sima vent was measured using a CO_2 analyser (LICOR LI820 CO_2 soil flux meter from West Systems). CO_2 fluxes reached rates of 300 kg m^{-2} d^{-1} at its centre zone, one of the highest ever measured in natural conditions (Beaubien et al., 2008; Krüger et al., 2011; McFarland et al., 2013). Samples were collected along a CO_2 fluxes gradient from the centre of the vent to surrounding area – non CO_2-affected –, with values ranging from 7 to 19 (Control), 40 to 55 (Low) and 260 to 1600 g m^{-2} d^{-1} (High). An extreme locality with more than 10^4 g m^{-2} d^{-1} was also studied. All sampling points were selected in areas without vegetation to avoid specific vegetation influences on soil communities. Sampling consisted of five soil cores of 5 cm diameter to a depth of 10 cm, which were pooled and then used for microbial (100 g), protozoan (200 g) and micro/mesofaunal (ca. 800 g) analysis. Microbial soil samples were thoroughly homogenized and transported on ice to the laboratory, sieved to 2 mm and immediately stored at −20 °C until further analysis. In addition, moisture, pH, organic matter and conductivity were measured 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 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. At November 2012 sampling, extreme samples were only sufficient for microbial and protozoal analysis.

2.2. Microbial community

Microbial community richness was assessed by Denaturing Gel Gradient Electrophoresis (DGGE) and bacterial, archaeal and fungal abundance was quantified through qPCR. Total microbial community DNA was extracted from 0.25 g of soil samples, using the Power Soil DNA isolation kit (Mo Bio Laboratories, Inc., CA, USA), according to the manufacturer's instructions. DNA yield was assessed by electrophoresis in 1.2% agarose gels stained with RedSafe™ (Intron Biotechnology, Korea) and visualised under UV light. For DGGE, PCR was performed to amplify universal and group-specific 16S rRNA gene fragments in a thermal cycler TC-512 (Techne, UK). The quantitative PCR was performed using SYBR Green PCR Master MIX (Applied Biosystems, Foster City, CA) on the Step One Plus system (Applied Biosystems). PCR conditions and primers used, as well as a more detailed description of the methods used, can be found in Fernández-Montiel et al. (2015).

2.3. Protozoan community

Protozoa were determined as described in Gabilondo et al. (2015). The abundance of amoebae, flagellates and ciliates was measured using the protocol described in 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. Amoebae abundance was counted 24 h later. Flagellates were counted in a haemocytometer chamber. To determine the number of ciliates 4 replicates of 100 ml were measured and counted with a light microscope (Madoni, 1984). For the analysis of diversity of ciliates, 2 ml of the soil supernatant were fixed with Bouin for posterior Edaphic Quantitative Protargol Staining (EQPS) method (Acosta-Mercado and Lynn, 2003) and 5 ml were stained with a modified silver carbonate method as described in Gabilondo and Bécares (2014). Protozoa were only analysed in November.

2.4. Micro- and mesofauna

Soil mesofauna was extracted using modified Berlese funnels, counted (abundance kg^{-1}) and classified at the order level for all animals, and at the family level for the mite orders Prostigmata, Mesostigmata and Oribatida, following Andrés et al. (2011). Holometabolous insect larvae and adults were pooled to a single group. For the calculation of microarthropod taxa richness, only morphospecies were distinguished. Nematodes were extracted from 100 g soil (fresh weight) in sterile distilled water using the Baermann funnel technique. After an extraction time of 10 days, nematodes were preserved in ethanol 70%, counted, and related to g soil dry weight.

2.5. Statistical analysis

Data were log-transformed when necessary to meet the assumptions of parametric statistical tests (normality and