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# Microbial communities in a range of carbon dioxide fluxes from a natural volcanic vent in Campo de Calatrava, Spain

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ABSTRACT

A natural carbon dioxide ($CO_2$) vent sited in Ciudad Real (Spain) was studied to understand how $CO_2$ emissions affect microbial communities along a $CO_2$ gradient. We used different molecular methods (quantitative PCR, DGGE and Biolog EcoPlates™) to assess changes in abundance, diversity and functionality of the main groups of soil microbiota (bacteria, archaea and fungi). A general decrease for all studied variables (gene copies and band richness of bacteria, archaea and fungi, and Biolog activities) was observed from control (7–19 g $m^{-2}$ $d^{-1}$) to high $CO_2$ fluxes (260–1600 g $m^{-2}$ $d^{-1}$). On the contrary, at extreme fluxes (more than 10 kg $m^{-2}$ $d^{-1}$) the microbial community increased their abundance and activity, though remained less diverse. PCA from carbon use substrate pattern and DGGE dendograms clearly differentiated low fluxes from high and extreme. This paper proves that high $CO_2$ fluxes (between 260 and 1600 g $m^{-2}$ $d^{-1}$) cause losses in both structural and functional community diversity, and a decrease in metabolic activities.



## 1. Introduction

Natural $CO_2$ seepages, also known as mofettes, are an example of an extreme surface ecosystem where we can study microbial diversity. Mofettes could be defined as natural ecosystems where cold, geogenic $CO_2$ migrates upward, through surface water or soil fractures to the atmosphere (Russell et al., 2011). In comparison with experimental research (laboratory scale or mesocosms), natural $CO_2$ emission sites make it possible to study key microbial taxa and the factors addressing their activities in a relatively undisturbed and stable long-term scenario (Šibanc et al., 2014; Maček et al., 2011; Oppermann et al., 2010). In this context, extreme environments represent a proved resource of unique microorganisms that exhibit interesting metabolic strategies and could help to elucidate the role of microbial taxa in ecosystem function (Maier and Neilson, 2015).

Moreover, mofettes have recently been used as natural analogues to study the effects of a leakage from a carbon capture and storage (CCS) system (Frerichs et al., 2013; McFarland et al., 2013; Oppermann et al., 2010; Krüger et al., 2011, 2009; Beaubien et al., 2008). Although $CO_2$ storage sites are selected to avoid any possible leak, this cannot be completely excluded and understanding the consequences of the surrounding environment is of major concern (Krüger et al., 2011). In this sense, several studies have taken place in natural analogues, such as Latera in Italy (Oppermann et al., 2010; Beaubien et al., 2008), Laacher See in Germany (Frerichs et al., 2013; Krüger et al., 2011, 2009), Cheb Basin, Czech Republic (Beulig et al., 2015) or Stavešinci, Slovenia (Šibanc et al., 2014) showing a marked shift in the microbial community composition towards higher abundance in anaerobic and acidophilic microorganisms. Other studies have advanced the understanding of specific functional group responses to upwardly migrating $CO_2$; however, the overall effects on microbial community structure are still not fully understood.

To evaluate the impacts of increasing $CO_2$ fluxes on structural and functional properties of microbial communities, we conducted a study at La Sima, a naturally occurring gas vent located in the Calatrava Volcanic Field (CVF; central-southern Spain). Our aim was to evaluate the consequences of long-term exposure to different fluxes of geological $CO_2$ on soil microbiota, including extreme fluxes that have never been measured at other sites (Gabilondo and Bécares, 2014). Although geological characteriza-

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tion is well documented (Elío et al., 2015; Vaselli et al., 2013; Calvo et al., 2010; Gosálvez et al., 2010; Peinado et al., 2009) little is known about the community structure and metabolic profile of microbial communities in La Sima. Our hypothesis was that elevated soil $CO_2$ caused a profound effect of high $CO_2$ emissions on edaphic microbiota, through losses of diversity, changes in microbial structure and activities due to adverse conditions in such an extreme ecosystem. In this paper, we reviewed this hypothesis by assessing changes in functionality, diversity and abundance of the main groups of soil microbiota (bacteria, archaea and fungi) through Biolog EcoPlates™, DGGE and qPCR analysis.

## 2. Materials and methods

### 2.1. Site description

The Calatrava Volcanic Field (CVF) is situated in the centre of the province of Ciudad Real (Spain). It occurs in a circular area of about 3000 $km^2$, one of the largest tectonic basins in central-southern Spain (Stoppa et al., 2012) which constitutes one of the largest and more recent volcanism zones of Spain. The CVF, with more than 300 emission centres, represents one the highest $CO_2$ discharge areas in peninsular Spain (Vaselli et al., 2013; Calvo et al., 2010). Active volcanism existed between 8.7 and 0.7 million years ago, during the Pliocene and Quaternary Eras (Higueras and Gallardo, 2011). $CO_2$ presence in subsurface and aquifers has its origin in magma degassing and cooling processes and it discharges to the surface through fractures in rocks (Elío et al., 2015; Calvo et al., 2010). Samples were collected at the location called La Sima, near Granátula de Calatrava, Spain (38° 49′ 17.51" N, 3° 45′ 19.80" W, 656 m.a.s.l.), in the eastern sector of CVF (Fig. 1). The local climate is Mediterranean, with hot dry summers and cool wet winters. Average annual precipitation and temperature are about 402 mm and 15.6 °C, respectively, with an average maximum of 21.8 °C and a minimum of 9.3 °C (AEMET, 2015).

La Sima is a $CO_2$-rich discharge (up to 2 t $d^{-1}$) consisting of a 5 m diameter depression (Elío et al., 2015). Since a seismic crisis in the area in 2007, $CO_2$ fluxes increased from 30,000 to 200,000 ppm. Gases also contains traces of $H_2S$, HCl, $CH_4$ and Ra and $O_2$ measurements remain under 7% in the gas vent leading to aerobic to microaerophilic conditions (Gosálvez et al., 2010; Peinado et al., 2009). Due to the carbonitic nature of CFV magma $CO_2$ is the dominant gas in the volcanic system (Stoppa et al., 2012). Vegetation presence was radially reduced ca. two m around the vent, as persistent $CO_2$ leak has led to vegetation mortality.

### 2.2. Soil sampling and storage

Soil sampling took place in November 2012 (monthly average precipitation 153.8 mm, temperature 10.7 °C) and May 2013 (22 mm, 16.6 °C; METEO Ciudad Real, 2016). Before each sampling, distribution of $CO_2$ fluxes in La Sima vent was measured using a $CO_2$ field device (LICOR LI820$CO_2$ soil flux meter from West Systems). Sampling points were chosen according to selected fluxes (Fernández-Montiel et al., 2015; Gabilondo and Bécares, 2014), establishing a gas gradient from the centre of the seepage. $CO_2$ values ranged from more than 10 kg $m^{-2}$ $d^{-1}$ in extreme (E) fluxes to 260–1600 g $m^{-2}$ $d^{-1}$ in high fluxes (H) and 40–55 g $m^{-2}$ $d^{-1}$ in low fluxes (L). Finally, control (C) samples (7–19 g $m^{-2}$ $d^{-1}$) were taken from a zone without a $CO_2$ leak effect (Fig. 1). Three replicates of each $CO_2$ level were taken, each of them consisting of five soil cores (5 cm diameter, 10 cm depth) that were pooled. At both samplings, samples were collected in the same locations. At November 2012 sampling, extreme samples were only sufficient for DNA based analysis (qPCR and DGGE). Soil samples were transported on ice to the laboratory, sieved to 2 mm, removing all plant material, and stored at −20 °C until microbial DNA extractions could be completed.

### 2.3. Soil chemical analyses

Soil chemical analyses were performed for the samples taken at both sampling times. Soil texture was determined by Bouyoucos hydrometer method and USDA classification (United States Department of Agriculture, 1999). Soil pH and conductivity were measured in the suspension of soil in water (1:2.5; 1:5, respectively) (Ministerio de Agricultura, Pesca y Alimentación, 1994). Carbonate content was determined using the Bernard calcimeter method (Ministerio de Agricultura, Pesca y Alimentación, 1994). Organic matter was calculated by Walkley-Black method, total nitrogen was determined using the Kjeldahl method, available phosphorus was extracted by Olsen method and Boron was extracted with warm water (Ministerio de Agricultura, Pesca y Alimentación, 1994). Cations (Ca, Mg, Na and K) were extracted with AcONH4 (1N, pH 7), cation exchange capacity (CEC) was measured by extraction with barium chloride 0.1 M and oligo-elements (Fe, Mn, Zn and Cu) were extracted with DTPA pH 7.3. All of them were analysed with an ICP-AES Optima 2000 DV from Perkin Elmer following Canadian Society of Soil Science's protocols (Carter, 1993).

### 2.4. DNA fingerprints

Microbial community richness was assessed by Denaturing Gel Gradient Electrophoresis (DGGE). We analysed total Eubacterial community, *Alpha-* and *Beta-proteobacteria* to provide a screening on specific groups to evaluate the $CO_2$ effect on microbial community. 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 RedSafeTM (Intron Biotechnology, Korea) and visualised under UV light. PCR was performed to amplify universal and group-specific 16S rRNA gene fragments in a thermal cycler TC-512 (Techne, UK). To analyse total Eubacterial community, DNA extracted was amplified using the primer set F984GC/R1378, targeting variable region V6 of 16S rRNA gene. The reaction mixture (25 μL) was as follows: 1 uL template DNA, 1 X DreamTaq buffer (Fermentas, Lithuania) 0.2 mM dNTPs, 0.2 μM each primer and 0.625 U DreamTaq polymerase (Fermentas, Lithuania). For Alpha- and Beta-proteobacteria, (semi)nested-PCR was applied; using group-specific primers (F203/R1492 and F984/R1492, respectively) followed by F984GC/R1378 amplification. Group-specific 16S rRNA gene fragments were amplified as follows: 1 uL template DNA, 1 X TrueStart buffer (Fermentas, Lithuania), 2.5 mM MgCl2, 2 mM dNTPs, 5% (v/v) DMSO, 1 mg $ml^{-1}$ bovine serum albumin, 0.2 μM primers and 0.625 U TrueStart Hot Start Taq polymerase (Fermentas, Lithuania). For F984GC/R1378 PCR 1 μL of group-specific products was used as template. PCR conditions are detailed in Fernández-Montiel et al., 2015. Products were checked by electrophoresis in 1.2% agarose gels and RedSafeTM staining. DGGE was performed with phor$U_2$ apparatus (Ingeny, Goes, The Nederlands), using a double gradient consisting of 46.5-65% denaturants and 6.2–9% acrylamide (Gomes et al., 2005). For total community analysis 10 μL of PCR product was loaded on the gel and ca. 5 μL in group-specific DGGEs. Electrophoresis was carried out in 1 X TAE buffer at 58 °C, at a constant voltage of 140 V for 17 h. A marker composed of a mixture of F984GC/R1378 PCR products of 6 bacterial isolates was loaded in the extremities and the centre of the gels. DGGE fingerprints were analysed with the "GelComparII" v6.0 programme (Applied Maths, Ghent, Belgium). Similarities were