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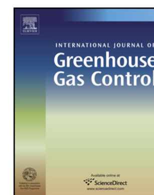

Short-term effects of simulated below-ground carbon dioxide leakage on a soil microbial community

I. Fernández-Montiel[a,b,*], M. Touceda[c], A. Pedescoll[a,b], R. Gabilondo[a,b], A. Prieto-Fernández[c], E. Bécares[a,b]

[a] Environmental Institute, University of León, c/La Serna 58, León, 24007, Spain
[b] Ecology Section, Department of Biodiversity and Environmental Management, University of León, Campus de Vegazana s/n, León, 24071, Spain
[c] Instituto de Investigaciones Agrobiológicas de Galicia, CSIC, Apdo 122, Santiago de Compostela, 15780, Spain

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ABSTRACT

The effects of artificial increments in soil carbon dioxide (CO_2) on microbial communities were studied in an experimental plant in Cubillos del Sil (León, Spain). The impact of two fluxes of CO_2 (20 and 40 l/h) influencing microbial communities and their relationships in two different soils (Cubillos and Hontomín) was evaluated by using three different approaches: community structure by DGGE, qPCR quantification of *Bacteria*, *Archaea* and *Fungi* domains and community-level physiological profile (CLPP). Soil type was the most important determinant factor in microbial activity and abundance: Cubillos soil showed a significantly higher qPCR copy numbers (58.68, 275.92 and 375.4%, for *Bacteria*, *Archaea* and *Fungi*, respectively) with a richer metabolism than Hontomín soil. No significant changes were observed in relation to CO_2 increase with any of the methods employed. Soil microbial communities proved to be resilient to short-term below-ground low CO_2 emissions. Short-term studies are useful for developing methods to detect possible leakages and assessing the likelihood of undesirable consequences on soil ecosystem.

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1. Introduction

The increasing industry of carbon capture and storage (CCS) represents one of the most promising strategies suggested to mitigate greenhouse gas effects on global warming. Deep geological storage of CO_2 has been proposed as a favourable remedy option (Zhou et al., 2013), but it is important to consider all the potential risks associated with an unlikely leakage of CO_2 from the reservoirs (West et al., 2005, 2006). For this reason, studying the effect of elevated CO_2 concentrations on terrestrial ecosystems and understanding the consequences of early CO_2 leaks and associated changes on soil communities are major issues (Noble et al., 2012). Natural environments where CO_2 is released from geological sources have been used in many studies as surrogates to assess the impact of potential leakages from a CCS site (Gabilondo and Bécares, 2014; Sáenz de Miera et al., 2014; McFarland et al., 2013; Frerichs et al., 2013; Krüger et al., 2011, 2009; Oppermann et al., 2010; Beaubien et al., 2008), but the fact that natural seepages have been emitting CO_2 for rather long periods needs to be considered, as the ecosystem could have adapted through species substitution or adaptation (Krüger et al., 2011; Beaubien et al., 2008). Moreover, these sites present specific characteristics (soil type, presence of other gases, soil moisture and temperature) that may not be present in the potential scenario of a CCS leak. Therefore, the results from the studies conducted in natural emission sites cannot be extrapolated to the conditions occurring in other areas after possible escapes from anthropogenic CO_2 storage (Ziogou et al., 2013).

In our study, we focus on microbial communities due to their essential role in soil ecosystems. Microbial communities, through their enormous metabolic diversity and versality, carry out the vast majority of nutrient cycling processes in soil and they participate in the maintenance of soil structure (Prosser, 2007). Changes in microbial community structure may affect both below- and aboveground processes, thus influencing vegetation and key ecosystem functions (Eisenhauer et al., 2011; Drigo et al., 2008). Microorganisms can be extremely sensitive to changes in soil characteristics acting as good indicators of soil quality (Winding et al., 2005). The response of the microbial community to alterations in soil properties could be easily detected by many techniques widely used in soil studies, such as CLPP or methods based on PCR (genetic fingerprints, qPCR) (Oros-Sichler et al., 2007). An increase in CO_2 concentration

* Corresponding author at: Ecology Section, Department of Biodiversity and Environmental Management, University of León, Campus de Vegazana s/n, 24071 León, Spain. Tel.: +34 987 29 34 04.
E-mail addresses: iferm@unileon.es, irenafmontiel@gmail.com (I. Fernández-Montiel).

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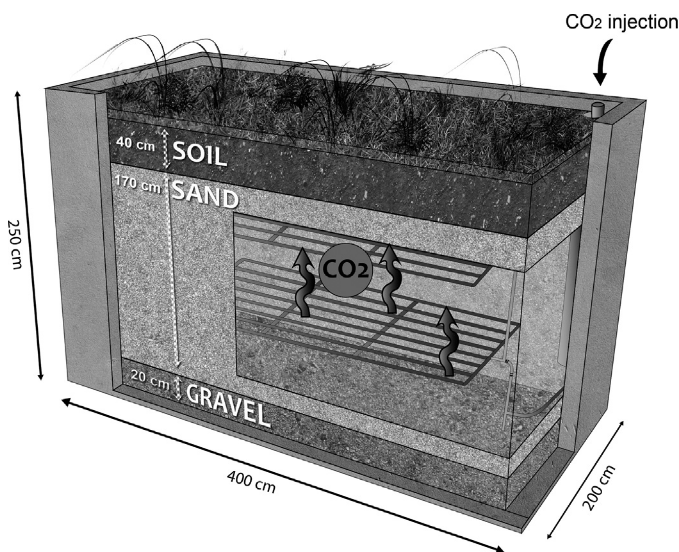

Fig. 1. Cross sectional scheme of the experimental cells.

could cause changes in soil biochemical conditions that would lead to a shift in the functionality or diversity of inhabiting microorganisms (Smith et al., 2013; Patil et al., 2010; Krüger et al., 2011; Oppermann et al., 2010; West et al., 2009)

Instead of using a natural analogue site, where the plant and microbial communities are well established and have been adapted to elevated CO_2 conditions, we used an experimental approach in which the objective was to analyse the first responses of a non-adapted ecosystem to a simulated CO_2 leakage. Our aim was to study the consequences of a below-ground low CO_2 emission via a qualitative and quantitative evaluation of soil microbial community applying the methods described above. For this purpose, we constructed an experimental plant where a continuous below-ground emission was used to simulate a leakage from a geological storage site. To our knowledge, this is the first time that a diffuse and continuous flow of CO_2 is assessed through an injection method based in below-ground releasing grills. This paper focuses on the immediate responses of edaphic microbiota to simulated CO_2 leaks. These short-term studies could be essential for regulatory bodies, stakeholders and society to accept the implementation of CCS projects (Noble et al., 2012).

2. Materials and methods

2.1. Study site and experimental design

The study area was located at the PISCO$_2$ experimental site, within the CIUDEN facilities (42°36′50.04“N 6°34′27.30“O; 578 m.a.s.l.), at Cubillos del Sil (León). The region is characterized by Continentalised Mediterranean climate. Average annual precipitation and temperature are about 668 mm and 12.6 °C, respectively, with an average maximum of 18.1 °C and a minimum of 7.2 °C (AEMET, 2013).

The PISCO$_2$ experimental site consisted of a factorial experiment of 24 plots (8 m^2 each) in which three fluxes of CO_2 were applied to two different soils and four replicates for each treatment were assayed. The plots were filled from the bottom to the top with a 20 cm gravel layer covered by 170 cm of sand and a 40 cm top soil layer. The CO_2 was released by two grills at depths of 1 and 2 m below ground level (Fig. 1). Half of the plots were filled with a sandy loam soil from a meadow in Hontomín (Burgos) (42°34′39.53“N 3°38′6.69“O; 923 m.a.s.l.), where geological storage will be installed, and the rest with a loam soil from a pasture in Cubillos del Sil. Seed bank present on each soil determined the vegetation throughout the experiment. Both soils were subjected to two different CO_2 levels: 20 l/h–low, 40 l/h–high), and a control without CO_2 injection. The treatments were assigned randomly to each experimental cell. These fluxes were chosen as being within the expected range in an early CO_2 storage leakage. In comparison with similar experiments (Smith et al., 2013), in this system a diffuse and continuous flow of CO_2 is assessed through underground CO_2 releasing grills, providing homogeneous fluxes on the surface of the cells.

2.2. Soil sampling and storage

Soil samplings took place for the first time in July 2012, four months after start-up. Samplings were repeated following seasonal variations during a year (July and October 2012 and January and June 2013). Five soil cores of 5 cm diameter, 10 cm depth, were sampled from each plot, and pooled. After samples were taken, the holes were backfilled with the same soil. Soil samples were transported on ice to the laboratory, sieved to 2 mm, removing all plant material, and stored at −20 °C until microbial analysis could be completed (only for DNA extractions). In addition, moisture, pH, organic matter and conductivity were measured using the standard protocols of the Spanish Ministry of Agriculture, Fisheries 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, Fisheries and Food. Physico-chemical analysis were performed at the beginning and at the end of the experiment (July 2012 and June 2013). A net of 18 equidistant points on the surface of each cell was used to evaluate the CO_2 fluxes and to obtain a monthly surface plot of CO_2 fluxes in each one. Gas was measured at each point using a CO_2 field device (LICOR LI820 CO_2 soil flux meter from West Systems). The sampling points were selected according to the CO_2 fluxes measured in the surface plot. Due to the spatial heterogeneity of the CO_2 emission in each cell, only those points with the target CO_2 levels were sampled.

2.3. DNA fingerprints

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.

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, the extracted DNA was amplified using the primer set F984GC/R1378 (Table 1), targeting variable region V6 of 16S rRNA gene. The reaction mixture (25 μl) was as follows: 1 μl 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*, *Betaproteobacteria* and *Actinobacteria*, (semi)nested-PCR was applied; using group-specific primers (Table 1) followed by F984GC/R1378 amplification. Group-specific 16S rRNA gene fragments were amplified as follows: 1 μl template DNA, 1 X *TrueStart* buffer (Fermentas, Lithuania), 2.5 mM $MgCl_2$, 2 mM dNTPs, 5% (v/v) DMSO, 1 mg/ml 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 a template. PCR conditions are given in Table 1. Products were checked by electrophoresis in 1.2% agarose gels and RedSafe™ staining.

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