Effects of elevated CO₂ concentrations on the vegetation and microbial populations at a terrestrial CO₂ vent at Laacher See, Germany

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Abstract

CO_2 capture and geological storage offers an option for reducing man-made greenhouse gas emissions. But one major concern related to geological CO_2 storage is the possibility of leakage from the reservoir and subsequent effects on the environment, which cannot completely be excluded. This study aims at investigating the environmental impact of CO_2 release from reservoirs into near surface terrestrial environments. To understand the effect of CO_2 leakage on such an ecosystem, detailed knowledge on the abundance and diversity of plants and microorganisms is essential. Therefore, an ecosystem study has been conducted within the Network of Excellence "CO_2GeoNet" on a natural CO_2 vent at the Laacher See, Germany. Near surface CO_2 conditions and CO_2 fluxes of the venting area were described by means of conventional soil gas measurement equipment, and brought up the difference between the CO_2 anomalies and their surroundings. A comparison of the soil columns between control sites and the centre of the venting area showed a small but significant change in the soil pH below 10 cm. The botanical survey revealed some remarkable vegetation changes like the investigation of important soil microbial communities showed significant differences between the CO_2-rich sites (up to 90% and more of soil gas), medium CO_2 sites (~20%), and control locations with background CO_2 concentrations. The ecosystem appears to be adapted to the different conditions through species substitution or adaptation, showing a shift towards anaerobic and acidotolerant to acidophilic species under elevated CO_2 concentrations. At the end, this ongoing study should identify possible candidates in the botanical and microbial kingdoms, whose presence or absence provide easily detectable indicators for the leakage of CO_2 from deep reservoirs into near surface terrestrial ecosystems.

Keywords
CCS; CO_2; microbiology; methanogenesis; denitrification; acidification; Laacher See

1. Introduction

The fourth IPCC report on global warming states once again that the rise in average global temperatures observed over the last century is most likely due to the release of anthropogenic greenhouse gases (IPCC, 2007). It turns out that large-scale solutions are needed immediately to quickly reduce greenhouse gas emissions and to mitigate their subsequent environmental effects. CO_2 capture and geological storage in deep saline aquifers or depleted gas and oil reservoirs offers a new option for reducing greenhouse gas emissions in large quantities. To proceed with a responsible large-scale deployment of this technology, all potential risks should have been studied, understood and, finally, minimized to exclude harm to the environment including humans. For this, it is a priori important to assess the potential risks associated with the unlikely leakage of significant volumes of CO_2 from the reservoir into the near surface environment (West et al., 2005; West et al., 2006). Although several studies have been published regarding the effect of increased atmospheric CO_2 concentrations on ecosystems (Jossi et al., 2006), there are only very few that have examined the effects of increasing CO_2 concentrations in the soil column due to upwardly migrating gas. These include a detailed study of a terrestrial CO_2 vent at Latera, Italy (Beaubien et al., 2008; Oppermann et al., 2010), a survey at Mammoth Mountain (California, USA) for the influence of volcanic CO_2 on soil chemistry and mineralogy (Stephens and Hering, 2004) and research at Stavesinci (NE Slovenia) for the influence of high soil-gas concentrations of geothermal CO_2 on plants (Macek et al., 2005; Pfanz et al., 2007).

In order to address some of the above-mentioned issues, this study investigates the potential environmental impact of CO_2 release from deep reservoirs on near surface terrestrial environments. Particularly the effect of CO_2 leakage on the abundance and diversity of plants and microorganisms is investigated in an ecosystem study conducted as a joint activity within the European network "CO2GeoNet" at a natural CO_2 vent at Laacher See, Germany.

The Laacher See volcanic centre is located in the core of the East Eifel volcanic field, and comprises of about 100 eruptive centres that cover an area of approximately 330 km². The East Eifel volcanic field is located west of the Rhine River in the still uplifting Paleozoic Rhenish Massif. The Laacher See eruption is the only known large explosive eruption that took place in central Europe during late Quaternary time (~12,900-12,880 yrs BP; e.g. Boogard and Schmincke, 1985). The Laacher See volcanic centre is morphologically characterized by a basin filled by a lake (Laacher See), with 3.3 km² area, surrounded by a steep ringwall rising 90 to 240 m above the basin. The ringwall, which can be classified as an extinct volcanic caldera, is made up by different basanitic/tephritic cinder cones and the tephra deposits of the Laacher See eruption. The internal structure of the Laacher See basin is dominated by an Eastnortheast-Westsouthwest striking thrust and four other geological lineaments: 2 running more or less North-South, the other Northeast-Southwest. CO_2 is produced below the caldera, it emerges from degassings of the upper earth mantle and migrates along faults and fractures to the surface (Möller (ed.), 2009). Release to the atmosphere typically occurs from gas vents, characterised by a small core of elevated gas flux.

One defined gas vent was chosen for this study, located in an almost naturally-vegetated pasture field on the western side of the lake (Figure 1). The vent is situated in an area which became dry land very recently. As a consequence of two tunnel constructions in 1164 and 1844, the water level of the lake was artificially lowered by about 10 plus 5 m. The terrestrial development of the studied site is therefore very young. Some organic material from former lake deposits is still to be found in deeper soil horizons. The vent is clearly visible due to a 5 m wide core of nearly bare soil surrounded by an approximately 40 m wide area of variably-impacted vegetation.

Figure 1: Sketch of the investigated CO_2 vent close to the western shore of the lake Laacher See; note the vegetation change from closed, green grassland to patchy *Polygonum arenastrum*-mats towards the centre of the vent.

2. Methods and materials

Surveys were conducted in September 2007 and July 2008 along a 60 m long transect across the vent (Figure 1), providing a spectrum of different CO_2 flux rates, soil gas concentrations and compositions. In addition to the detailed survey of these conditions (soil gas concentrations and gas fluxes), intensive botanical studies and sampling for microbiological, mineralogical and geochemical analysis were performed at the same time.

2.1. Characterisation of the near surface CO_2 conditions

The applied techniques are potential methods for the near surface monitoring of geological CO_2 storage sites; they were used during both field campaigns in 2007 and 2008. First, a rapid surveying of the whole study area was undertaken by means of a newly developed, mobile open path laser system which was mounted at about 30 cm above the ground on a quad bike (for details see Jones et al., 2009). The system detected already known CO_2 vents, and confirmed and discovered suspicious or unknown degassing sites. Afterwards, the 60 m long traverse across the strongest vent was intensively investigated with conventional soil gas concentration and flux measurement equipment: Steel probes and handheld infrared gas sensors (Li-Cor and Dräger instruments) for soil CO_2 concentration, and commercial and custom-made accumulation chambers for the CO_2 flux quantifications. The measurements were carried out in 0.5 m intervals. Some additional gas samples taken along the traverse for comparative laboratory analyses were also used for the determination of carbon isotope ratios ($\delta^{13}C_{CO2}$; by means of a Thermo Delta plus XL mass spectrometer) which give hints on the origin of the CO_2.

2.2. Botanical impact survey

The botanical survey was conducted along the entire length of the transect. The investigations registered the percentage cover of identified plant species and groups at 0.5 m intervals using a 0.5 m x 0.5 m quadrat levels. Field flora books were used to identify critical plant taxa (Blamey and Grey-Wilson, 2003; Fitter et al., 1984) and digital photographs were taken of each quadrat for a complete visual record.

2.3 Microbiological analyses

Basic soil related field work and lab analyses of soil samples followed conventional approaches. Soil pH was measured in a suspension of 10 g of fresh soil in 25 ml of distilled water with a pH-redoxmeter GPRT 1400 AN (GSG Greisinger Electronic). Prior to organic carbon measurements the soil was dried at 105°C and grounded. Inorganic carbon was removed with 50 µL 1N HCl followed by drying the sample on a 40°C heating plate (repeated three times). The content of organic carbon was finally determined using an elemental analyser (VarioMAX Elementar Analysensysteme).

Determination of microbial activities: The collected soil samples were first mixed 1:1 with artificial mineral medium to obtain homogenous slurries (Widdel & Bak 1992). Subsequently, 9 ml of medium were added to 3 ml of soil slurry into sterile glass tubes (20 ml) which were afterwards sealed with butyl-rubber stoppers and screw caps. The headspace was either flushed with N_2 for methane and anaerobic CO_2 production as well as sulphate reduction measurements, with air for aerobic CO_2 production or with air and 2% CH_4 for aerobic methane oxidation.

As important indicators of the gross mineralisation in the soil the CO_2 production (CPR; under aerobic and anaerobic conditions), the anaerobic methane production (MPR), and the sulphate reduction rates (SRR) were quantified. The potential aerobic oxidation of methane rates in the soil samples were determined in vitro as described previously by Krüger et al. (2002). Triplicate tubes were incubated horizontally at 20°C and gently shaken once per day to ensure an even distribution of gases or sulphate within the microcosms. The rates were calculated per gram of dry weight (g_{dw}) as determined after drying at 80°C for 48 h and deviations are expressed as 95% confidence intervals unless stated otherwise. The sulphide content was determined using the formation of copper sulphide after Cord-Ruwisch (1985). Methane and CO_2 were determined using a GC 14B gas chromatography (Shimadzu) as described in Nauhaus et al. (2002), which was additionally equipped with a methaniser to quantify the CO_2.

DNA extraction and quantitative *Real Time* PCR (qPCR): The DNA was extracted from 0.5 to 1 g of a frozen soil sample following the manufactory's manual of the FastDNA Spin Kit for Soil (Bio 101) with addition of 200 µg of poly-adenylic acid (poly A) to the lysis mixture (Webster et al. 2003). The resulting DNA was dissolved in 100 µl ultrapure PCR water and used as target for PCR based analysis.

DNA standards for quantitative real time PCR (qPCR) were prepared as described previously by Engelen et al. (2008). Specific fluorescent probes were used targeting the ubiquitous 16S rRNA genes of bacterial or archaeal organisms (Takai & Horikoshi 2000, Nadkarni et al. 2002). The assays were carried out using the TaqMan PCR Master Mix (Applied Biosystems). Each DNA extract was measured in triplicate and in two to three dilutions to check for PCR inhibition. Conversion factors for DNA copy numbers to cell numbers were: 4.1 for *Bacteria*, and 1.5 for *Archaea* (Lee et al. 2009). The detection limits for qPCR analyses were 10^3 DNA copies g^{-1} dry weight for the assays specific for *Bacteria* and 10^1 DNA copies g^{-1} dry weight for the assays specific for *Archaea*.

Lipid biomarker studies: The microbiological analyses were supplemented by lipid biomarker studies as described in detail by Oppermann et al., 2010.

3. Results and discussion
3.1. Gas monitoring and soil chemistry

The results of the gas surveys are generally very similar from year to year. The soil CO_2 concentration and flux data series of the two years are very homogeneous and well correlated as shown by their coefficients of determination (see Table 1).

Hence, the spatial patterns observed in 2007 could be confirmed in their shapes in 2008. Figure 2 illustrates the soil CO_2 concentrations in 15 and 60 cm depth while Figure 3 shows the CO_2 fluxes from the underground to the atmosphere for the different years.

Table 1: Correlation matrix (coefficients of determination, r^2) of soil CO_2 concentrations in 15 and 60 cm depth and CO_2 fluxes for 2007 and 2008.

Figure 2: Comparison of 2007 and 2008 CO_2 concentrations in soil gas along the traverse across the studied vent (September 2007 and July 2008, using only points measured in both years).

Figure 3: Comparison of 2007 and 2008 CO_2 flux data for the traverse across the vent.

Three main zones of higher CO_2 concentrations and fluxes could be identified along the traverse: Between locations 11-17 m, 21-28 m, and 30-42 m, the latter representing the so-called centre of the vent where peak concentration values of more than 90 vol% CO_2 were registered in 60 cm depth (Figure 2). But already in a very short distance from these distinct anomalies the CO_2 concentrations and fluxes drop back to background values; demonstrating the limited size of natural CO_2 vents. Furthermore, particularly the figures of the CO_2 concentrations show also the relatively high small scale variability which could be quite marked between adjacent measurement points.

There certainly are also some differences in detail between the two years, but this is to be expected given that the sampling locations will not precisely match within a few centimetres, and there could be changes in the migration pathways of gas to the surface owing to changing underground conditions. One clear difference between the two years are the generally higher gas concentrations and fluxes in 2007 (see Figures 2 and 3). Data correlations with meteorological parameter suggest that factors such as lower atmospheric pressure and higher wind speed drew slightly larger amounts of gas from the ground in the autumn and overrode any impeding effect of higher soil moisture.

Carbon isotope analyses ($\delta^{13}C_{CO2}$) were helpful for the characterisation of the venting areas since the isotope ratios differ from CO_2 rich sites (-4.1 to -2.7 ‰ PDB) to those with medium (-1.7 to -0.2 ‰) to low concentrations (-1.0 to 0.8 ‰). The $\delta^{13}C_{CO2}$ values for the CO_2 rich sites point directly to the upper earth mantle and/or lower earth crust as origin of the CO_2. Contrastingly, the CO_2 gas from medium to low concentration

sites is already affected by mixing processes and isotope fractionation, probably under the influence of some underground carbonate levels.

In terms of bulk mineralogical compositions and soil chemistry, the analysed samples from the centre of the vent (35 m) and the control site (55 m) were relatively similar in the top 70 centimetres. Going deeper into the soil, a small but significant change in the soil pH was observed below 10 cm (Figure 4). This might influence the activity and composition of the microbial communities, as well as the soil mineralogy as also seen at Latera (Beaubien et al., 2008).

Figure 4: Acidity of two sediment cores (pH profiles); in red = area of highest CO_2-seepage, in green = control site; the blue lines show the top of the water table, the black the rock base.

3.2 Botanical investigations

The botanical survey showed that CO_2 soil gas concentrations influence vegetation types with grasses predominating below 20% CO_2. Above this concentration two predominant dicotyledonous plant species were observed and could be used as bioindicators of high CO_2 soil gas concentrations.

Main results of the botanical survey are summarised in Figure 5, which shows the percentage coverage for total moss, total grass (monocotyledonous plants), *Polygonatum arenastrum* and 'other' dicotyledonous flowering plants. *P. arenastrum* is the only observed dicotyledonous plant between 25 and 50 m along the transect where CO_2 concentrations are between ~10-35% at 15 cm depth and ~35-90% at 60 cm depth. Where CO_2 concentrations are below 20% at 15 cm depth, grasses predominate and *P. arenastrum* is not observed (0-25 m and 40-60 m) although other dicotyledonous plants are present. These results can be compared to observations from another natural CO_2 gas vent site at Latera, Italy where only grasses were observed when concentrations of CO_2 were between 5-40% at 10 cm depth (Beaubien et al., 2008). Indeed, dicotyledonous plants did not appear to be able to tolerate CO_2 concentrations over 5% at this site. Other observations at a controlled injection site in an English pasture also suggested that grasses were more tolerant to higher concentrations of CO_2 than dicotyledonous plants (West et al., 2008). The observation of a dicotyledonous plant as a bioindicator of increased soil gas CO_2 is therefore unexpected and also demonstrates that botanical changes are site specific, depending also on other factors such as soil moisture, pH influencing plant ecology, etc. However, monocotyledonous plants appear, in general, to be more tolerant to increased soil gas CO_2.

Figure 5: Effect of CO_2 emissions on the distribution of different botanical groups/species along the transect across the CO_2 vent (centre at approx. 30-35 m); x-axis: location in m from S end, y-axis: coverage.

3.3 Microbial community composition and activities

The determination of environmentally important microbial activities in the soil samples showed significant differences between the CO_2-rich sites (>90 % of soil gas), medium CO_2 sites (20%) and control locations with background CO_2 concentrations. To get some more detailed information, potential sulphate reduction rates as well as methane production and oxidation were determined in sediment samples from the different sites. These measurements with samples from vent and non-vent sites should also provide first information on the influence of elevated carbon dioxide concentrations on selected microbial populations (Figure 6).

Figure 6: Differences in microbial activity and 16S rRNA gene copies at 10 to 20 cm depth

Gross CO_2 production was under aerobic conditions about 100-fold higher than under anaerobic conditions. Under anaerobic conditions CO_2 production was similar at the vent and the control site, with 4.34 ± 0.25 and 1.03 ± 0.32 $\mu mol\ g_{dw}^{-1}\ d^{-1}$. In contrast, aerbic rates were with 432 ± 57 $\mu mol\ g_{dw}^{-1}\ d^{-1}$ significantly higher at the control site than in the vent centre with 121 $\mu mol\ g_{dw}^{-1}\ d^{-1}$.

Potential methane production rates without substrate addition in the sediment samples from 10-20 cm depth were at about 0.33 ± 0.007 $\mu mol\ CH_4\ g_{dw}^{-1}\ d^{-1}$ and therefore much higher than at the vent centre (location 36 m) than in the control site samples (55 m) where they reached 0.12 ± 0.002 $\mu mol\ CH_4\ g_{dw}^{-1}\ d^{-1}$. Data for methane oxidation under aerobic conditions showed the opposite picture: Higher rates of 5.4 ± 0.42 $\mu mol\ CH_4\ g_{dw}^{-1}\ d^{-1}$ at the control site compared to 2.2 ± 0.35 $\mu mol\ CH_4\ g_{dw}^{-1}\ d^{-1}$ in the intermediate CO_2 positions (14 m) and 0.4 ± 0.11 $\mu mol\ CH_4\ g_{dw}^{-1}\ d^{-1}$ at the centre of the vent. Finally, a remarkable aerobic methane oxidation acitivity was found even in sediment samples down to 1.5 m depths with an identical pattern (data not shown). This points towards a methane supply for the methanotrophic bacteria from deeper sources present in the deepest oxygen-poor, organic deposits of the soil column and tracing back to the limnic evolution of the study site.

Sulfate reduction rates were relatively high, between 1.5 to 2.2 $\mu mol\ g_{dw}^{-1}\ d^{-1}$ in the samples from the centre of the vent, with the highest activity observed in deeper sediment layers below 50 cm depth. Interestingly, sulphate reduction was also detected in deeper samples from the control site, albeit at a much reduced rate. The sources of sulfate and substrates for the sulfate-reducing bacteria is yet to be determined, but might be originating from underground water streams or the decomposition of organic material from lake sediment deposits in the deeper soil horizons.

In accordance with the microbial activities, total numbers of microorganisms showed also significant differences between the individual sites. Cell numbers of *Bacteria* were determined using quantitative PCR (qPCR, [17]): They were highest at the control site and substantially lower towards the vent centre; the values decreased from 9.6×10^9 to 8.7×10^8 gene copies g_{dw}^{-1} of soil. For *Archaea* in contrast, the values

increased from control site towards the centre, with 7.7 x 10^6 and 6.5 x 10^7 gene copies g_{dw}^{-1} of soil. One explanation for the observed changes in the community composition might be the replacement of oxygen in the soil gas with CO_2, leading to first microaerobic and then to anaerobic conditions. This would thus favour e.g. methane-producing *Archaea* or sulphate-reducing bacteria. To analyse this in more detail, group-specific qPCR assays are carried out currently to reveal, whether certain functional groups, like the methane oxidising or sulphate reducing bacteria, were absent or stimulated at the CO_2-rich sites.

Another implication for strong changes in the microbial community came from the lipid biomarker studies. Although the cell numbers of bacteria decreased, the biomarker studies showed that in the CO_2 vent, bacterial non isoprenoidical tetraethers lipids were contained in higher quantities then at the control site (e.g. in 50-60cm depth 503 µg g^{-1} TOC at the CO_2 vent site and 302 µg g^{-1} TOC at the control site). Even though the source organisms of bacterial tetraethers are not known yet, they most likely derive from anaerobic bacteria (see Oppermann et al., 2010 and references therein). This finding is of special interest since ether lipids are more stable than ester lipids that are commonly found in bacteria. Bacteria able to synthesis etherlipids are therefore probably better adapted to the low pH conditions found at the CO_2 vent (Figure 4).

4. Conclusions

CO_2 gas fluxes into the Laacher See are roughly estimated in the range of about 5,000 tons of CO_2 per year (Aeschbach-Hertig et al., 1996). Additional CO_2 gas seepages from the underground occur permanently at the fringes of the lake. Even if a CO_2 gas release of up to 600 g m^{-2} d^{-1} could be registered along the studied vent, our results indicate that the effects of the gas vents are spatially limited. Nevertheless, some significant effects of high CO_2 concentrations on the terrestrial ecosystem were observed. The ecosystem appears to have adapted to the different conditions through species substitution or adaptation, showing a shift towards anaerobic and acidotolerant to acidophilic species under elevated CO_2 concentrations. The present results have stimulated future research activities which will include an extensive investigation campaign with gas, water and sediment sampling both for the Laacher See and carbonic springs nearby. At the end, this study should identify possible candidates in the botanical and microbial kingdoms, whose presence or absence provide easily detectable indicators for the leakage of CO_2 from deep reservoirs into near-surface terrestrial ecosystems.

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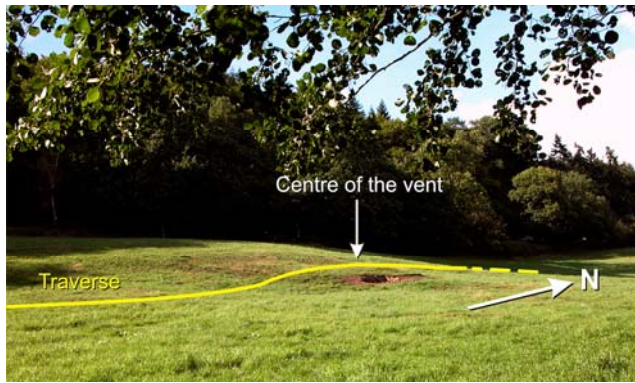
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Table 1: Correlation matrix (coefficients of determination, r^2) of soil CO_2 concentrations in 15 and 60 cm depth and CO_2 fluxes for 2007 and 2008.

CO_2 … (r^2)	15cm, 2007	60cm, 2007	Flux, 2007	15cm, 2008	60cm, 2008	Flux, 2008
15cm, 2007						
60cm, 2007	0.92					
Flux, 2007	0.96	0.87				
15cm, 2008	0.93					
60cm, 2008		0.83		0.82		
Flux, 2008			0.88	0.92	0.73	
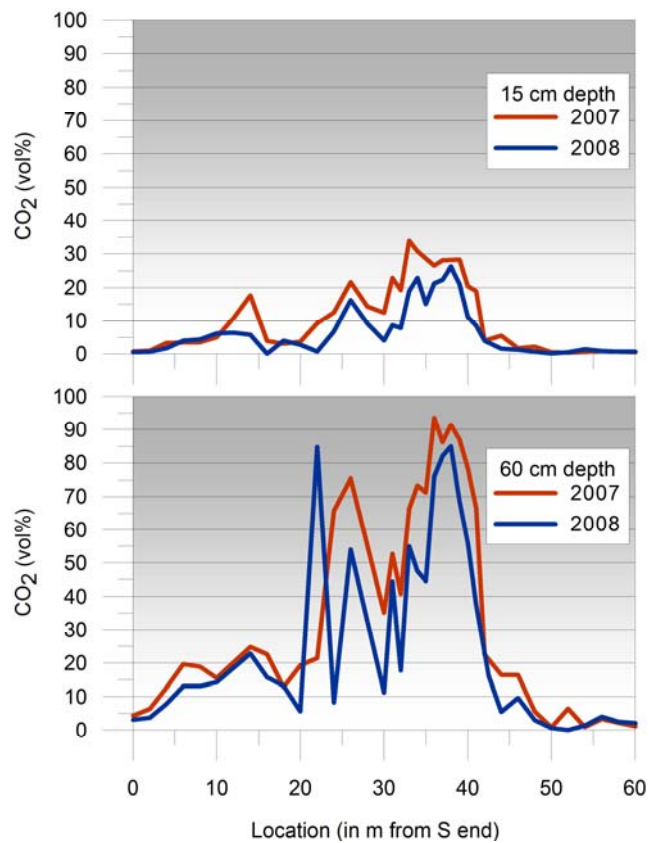
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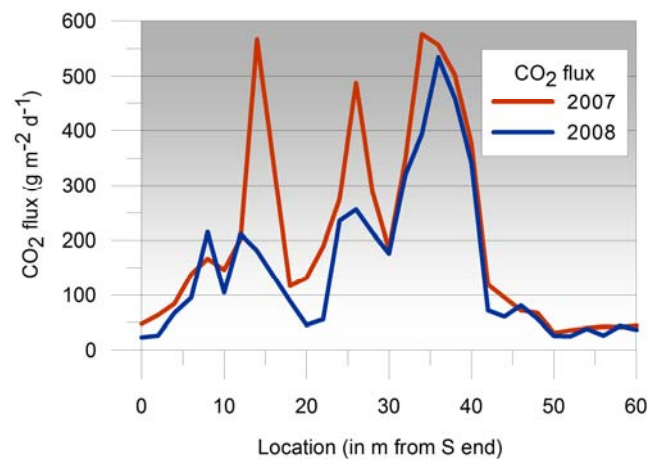

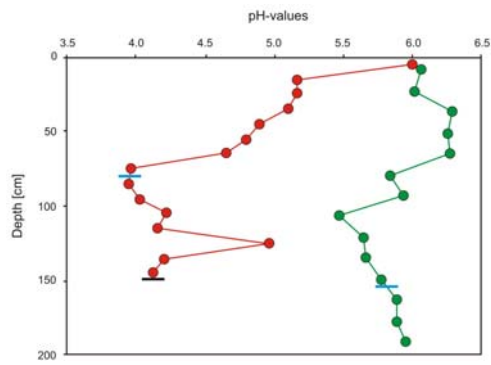Figure 3: Comparison of 2007 and 2008 CO_2 flux data for the traverse across the vent.

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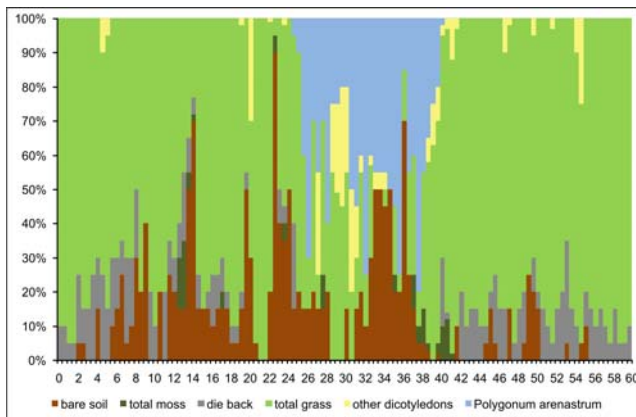

Figure 5: Effect of CO_2 emissions on the distribution of different botanical groups/species along the transect across the CO_2 vent (centre at approx. 30-35 m); x-axis: location in m from S end, y-axis: coverage.
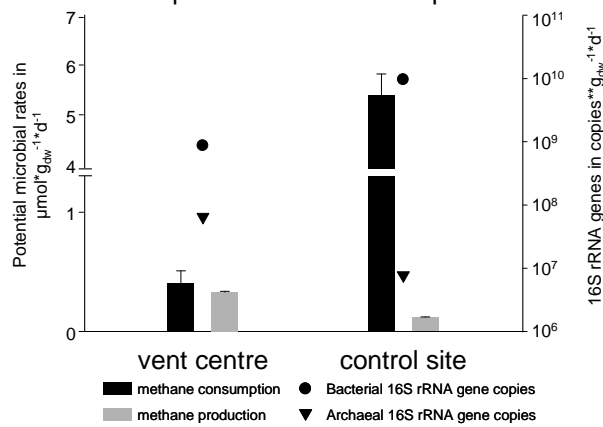
Figure 6: Differences in microbial activity and 16S rRNA gene copies at 10 to 20 cm depth.