CO_2 impacts on Microbial Communities in different Near-Surface Geosystems

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Diese Arbeit ist mir selbst gewidmet. In Erinnerung an das was möglich ist…

Summary

Anthropogenic CO_2 emissions increased during the last ~150 years. Subsequently, the atmospheric CO_2 concentration increased with severe effects for the Earth's climate and the global carbon cycle. Although technical progress in energy efficiency, renewable energy sources and industrial CO_2 capture and storage techniques led to decreasing greenhouse gas emissions in many countries, the potential impacts of the still increasing atmospheric CO_2 concentrations for the ocean and terrestrial carbon cycle are still poorly understood.

Hence, this thesis focussed on geochemical processes and the microbial activity, abundance and community structure potentially altered by high CO_2 concentrations in different near-surface geosystems. The main objectives of this thesis were (i) to investigate CO_2-induced geochemical alterations, (ii) to identify potential adaptation mechanisms of the microbial community leading to changes in the microbial community structure and metabolic activity and (iii) to identify potential indicator organisms. Therefore, two long-term CO_2-adapted geosystems (terrestrial and freshwater) and one artificial short-term CO_2 injection facility have been studied.

The main findings of the thesis are: (i) Long-term CO_2 exposure with high CO_2 concentrations led to significant changes in both, plant coverage and community composition as well as geochemistry e.g. the acidification of soil and sediment (pore water), increased soil moisture content and soil weathering.
(ii) Long-term CO_2 exposure with high CO_2 concentrations led to a shift towards anaerobic, acidic tolerant to acidophilic methanogens and autotrophs. The verified predominant archaeal taxa belonged to *Methanomicrobia* (*Euryarchaeota*), *Thermoprotei* (*Crenarchaeota*) and *Pacearchaeota*.
(iii) Long-term CO_2 exposed geosystems with high CO_2 concentrations revealed the potential importance of *Chloroflexi* as potential bacterial indicator species. The very abundant *Chloroflexi* species are presumably connected to anaerobic organic matter degradation and CO_2-fixation.
(iv) Short-term CO_2 injection over a period of 24 months revealed no significant geochemical and microbiological alterations. The CO_2-exposed samples did not show significant changes in microbial CO_2 and CH_4 turnover rates compared to reference samples. Likewise, no alterations in microbial abundances and community composition were detected.
(v) Concerning a possible CO_2 threshold for significant i.e. detectable ecosystem changes, the results of all geosystems in summary suggest a CO_2 concentration of up to 50 % as critical level. CO_2 concentrations below did not lead to significant geochemical and microbiological changes.

Keywords: CO_2, geochemistry, methanogens, autotrophs

Zusammenfassung

Anthropogene CO_2 Emissionen sind während der vergangenen ~150 Jahre angestiegen. Dies führte zu einem Anstieg der CO_2 Konzentration in der Atmosphäre und folglich zu schwerwiegenden Auswirkungen auf das weltweite Klima und den globalen Kohlenstoffkreislauf. Obgleich der technische Fortschritt im Bereich der Energieeffizienz, der erneuerbaren Energiequellen und Techniken der industriellen CO_2 Abscheidung und Speicherung zu einer Verringerung von Treibhausgas Emissionen führte, die möglichen Auswirkungen der auch weiterhin ansteigenden CO_2 Konzentrationen in der Atmosphäre auf den Kohlenstoffkreislauf an Land und im Meer sind auch weiterhin wenig verstanden.

Die vorliegende Arbeit konzentrierte sich daher auf mögliche Veränderungen in den geochemischen Prozessen sowie in der mikrobiellen Aktivität, Abundanz und Gemeinschaftsstruktur oberflächennaher Geosysteme durch hohe CO_2 Konzentrationen. Die Hauptziele dieser Arbeit waren (i) die Untersuchung CO_2 induzierter Veränderungen in den geochemischen Prozessen, (ii) die Identifizierung mikrobieller Anpassungsmechanismen die zu Veränderungen in der mikrobiellen Gemeinschaft und ihrer Aktivität führen und (iii) die Identifikation möglicher Indikatorspezies. Hierfür wurden zwei dauerhafte, CO_2 adaptierte Geosysteme untersucht (terrestrisch wie limnisch) sowie eine künstliche Anlage, in welcher CO_2 kurzzeitig injiziert wurde.

Die wichtigsten Ergebnisse dieser Arbeit sind: (i) Der Nachweis, dass eine langfristige CO_2 Exposition zu signifikanten Veränderung im Pflanzenbestand und -Wuchs sowie in den geochemischen Prozessen führte. Dies beinhaltete u.a. die Versauerung des Bodens/Sediments (bzw. Porenwassers), einen Anstieg der Bodenfeuchte sowie die Verwitterung des Bodens. (ii) Das eine langfristige Exposition mit hohen CO_2 Konzentrationen zu einer Verschiebung hin zu anaeroben, säuretoleranten bis säureliebenden Methanogenen und Autotrophen führt. Die vorherrschenden Taxa der *Archaea* wurden hierbei den *Methanomicrobia* (*Euryarchaeota*), *Thermoprotei* (*Crenarchaeota*) und *Pacearchaeota* zugeordnet. (iii) Geosysteme, die langfristig hohen CO_2 Konzentrationen ausgesetzt waren machten die Bedeutung von *Chloroflexi* als mögliche bakterielle Indikatorspezies deutlich. Diese besonders abundanten *Chloroflexi* Spezies sind mit hoher Wahrscheinlichkeit am anaeroben Abbau organischen Materials sowie an der CO_2 Fixierung beteiligt. (iv) Eine kurzzeitige CO_2 Injektion über einen Zeitraum von 24 Monaten zeigte keine signifikanten geochemischen oder mikrobiologischen Veränderungen. Es wurden keine signifikanten Veränderungen in den mikrobiellen CO_2 und CH_4 Umsatzraten zwischen CO_2 exponierten Proben und Referenzproben festgestellt. Auch die mikrobielle Abundanz und Gemeinschaftsstruktur blieb unverändert. (v) Einen möglichen CO_2 Schwellenwert für signifikante d.h. nachweisbare Veränderungen im Ökosystem betreffend, legen die Ergebnisse aller untersuchten Geosysteme nahe, dass eine CO_2 Konzentration ab 50% als kritisch eingestuft werden kann. Konzentrationen darunter führten in der vorliegenden Arbeit zu keinen signifikanten geochemischen und mikrobiologischen Veränderungen.

Schlagworte: CO_2, Geochemie, methanogene und autotrophe Mikroorganismen

Table of Contents

Abbreviations

*amo*A	Ammonia monooxygenase subunit A
ANOVA	Analysis of variance
ANOSIM	Analysis of similarities
AOA	Ammonia oxidizing *Archaea*
AOB	Ammonia oxidizing *Bacteria*
ASGARD	Artificial Soil Gassing and Response Detection
BLAST	Basic Local Alignment Search Tool
bp	Base pair
CBB	The Calvin-Benson-Bassham cycle
CCS	Carbon Capture and Storage
°C	Degree Celsius
d	Day
DC/HB	The dicarboxylate/ 4-hydroxybutyrate cycle
DOC	Dissolved organic carbon
16S rRNA (gene)	Encoding gene for the small subunit (16S) of ribosomal RNA
EU	European Union
GC	Gas chromatography
Gt	Giga tones (1 Gt = 1 000 000 t)
h	Hour
HPLC	High Performance Liquid Chromatography
HP/HB	The 3-hydroxypropionate/4-hydroxybutyrate cycle
IEA	International Energy Agency
IPCC	International Panel on Climate Change
Km^2	Square kilometre
*mcr*A	Encoding gene of methylcoenzyme M reductase subunit A
mL	Millilitre
n	Number of replicates
NCBI	National Center for Biotechnology Information
OTU	Operational taxonomic unit
PCA	Principal Component Analysis
PCR	Polymerase chain reaction
ppm	Parts per million
pCO_2	Partial pressure of carbon dioxide
p-value	Probability value (limit of significance 0.05)
PyroTag	A sequence derived from 454 pyrosequencing

qPCR	Quantitative PCR
RISCS	Research into Impacts and Safety in CO_2 Storage
ROV	Remotely operated vehicle
RuBisCO	Ribulose-1,5-biphosphate carboxylase/oxygenase
SIMPER	Similarity Percentages
SIP	Stable isotope probing
SOM	Soil organic matter
TC/TOC	Total carbon / total organic carbon
ZERT	Zero Emission Research and Technology field site
µl	Microliter

1. General Introduction

1.1. Climate Change

Climate is a key factor for life on Earth and is determined by these five spheres on Earth: atmosphere, hydrosphere, cryosphere, lithosphere, and biosphere (Change 2007). The atmosphere is the most unstable and rapidly changing part of the earth system and is composed of nitrogen (about 78%), oxygen (about 21%), argon (about 1%) as well as carbon dioxide and other gases in trace amounts. Some of the trace gases including carbon dioxide (CO_2), methane (CH_4), nitrous oxide (N_2O), ozone (O_3), hydrofluorocarbons (HFCs), sulfur hexafluoride (SF_6) and perfluorocarbons (PFCs) absorb and emit infrared radiation. These so-called greenhouse gases play an essential role in the earth's energy budget because they absorb infrared radiation (long-wave) emitted by the earth and prevent it by escaping into space (greenhouse effect). Therefore, the earth's surface and atmosphere are heating up which results into global warming and climate change.

The atmospheric concentrations of greenhouse gases remained relatively constant during the pre-industrial era but increased during the last ~150 years (post-industrial era). For example, the atmospheric concentration of carbon dioxide (CO_2) has increased by 40% since 1750. Simultaneously, the global temperature increased by about +0.85 °C (Stocker *et al.* 2013). Comprehensive investigations in recent years resulted in the scientific consensus that Earth's climate is being affected by human activities (anthropogenic climate change) which led to increasing greenhouse gas concentrations in the atmosphere and therefore e.g. increasing surface temperatures, upper ocean warming, decreasing arctic sea ice, shrinking glaciers. This consensus has been verified by several IPCC reports of the Intergovernmental Panel on Climate Change (IPCC) (Oreskes 2004; Solomon 2007; Stocker *et al.* 2013). Therefore, human activities like fossil fuel burning as well as changing land-use might have the strongest impacts on atmospheric CO_2 concentrations. In consequence, the reduction of greenhouse gas emissions would be necessary to stabilise radiative forcing.

1.2. Carbon dioxide and Carbon Cycle

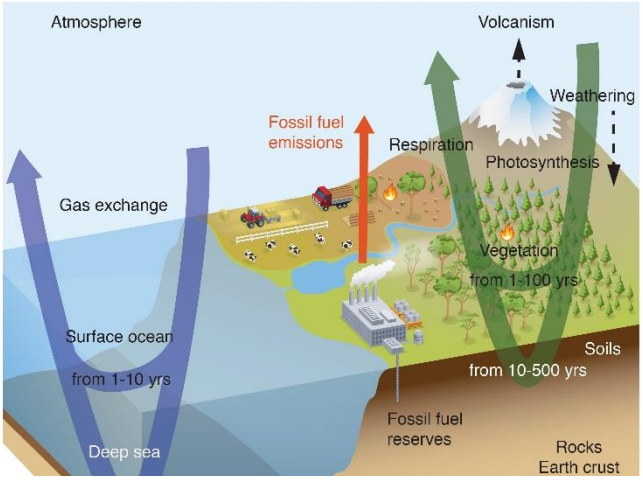

Figure 1:Simplified presentation of the global carbon cycle showing the turnover time scales for carbon transfers through ocean, terrestrial and anthropogenic reservoirs. Source: IPCC, 2013.

Carbon dioxide has a quantity of 0.04 %vol. in the atmosphere and a residence time of 5-200 years. Therefore, carbon dioxide represents the most important greenhouse gas in context of climate change. Atmospheric carbon dioxide concentrations have increased by 40% since pre-industrial times (Stocker *et al.* 2013). However, the atmospheric CO_2 is increasing only at about half the rate of fossil fuel emissions. The rest dissolves in oceans and is taken up by terrestrial ecosystems (Prentice *et al.* 2001). Following recent

reports, there is high confidence that increasing atmospheric CO_2 concentrations will lead to increased CO_2 uptake by the oceans and the terrestrial biosphere with however, uncertain amounts (Stocker *et al.* 2013). Thus, oceans and the terrestrial biosphere are important carbon reservoirs but not the only ones: major carbon reservoirs are the lithosphere (~70,000,000 Gt), oceans (~38,400 Gt), fossil fuels (~4,130 Gt) and terrestrial biosphere (~2,000 Gt) (Falkowski et al. 2000). How much CO_2 can be taken up by oceans and the terrestrial biosphere can be calculated from the changes in the atmospheric CO_2 and O_2 content based on the terrestrial exchange of fixed CO_2 and emitted O_2. Therefore, oceans represent the biggest CO_2 sinks (Prentice *et al.* 2001; Griggs and Noguer 2002).

1.2.1. Ocean Carbon Cycle

The exchange of CO_2 between the atmosphere and the oceans resulted into 50 times higher dissolved carbon concentrations in oceans compared with the atmosphere. Because of its solubility and chemical reactivity, CO_2 is taken up by water very effectively. Therefore, most of the CO_2 (~91%) dissolves in water and forms a weak acid that reacts with carbonate anions and water to form bicarbonate and carbonic ions (Stocker *et al.* 2013):

$$CO_2 + H_2O \longrightarrow \underset{\text{carbonic acid}}{H_2CO_3} + \underset{\text{bicarbonate}}{HCO_3^-}$$

$$\underset{\text{carbonic acid}}{H_2CO_3} \longrightarrow H^+ + \underset{\text{bicarbonate}}{HCO_3^-}$$

$$\underset{\text{bicarbonate}}{HCO_3^-} \longrightarrow H^+ + \underset{\text{carbonate ion}}{CO_3^{2-}}$$

The CO_2 solubility is temperature dependent. Therefore, in contrast to the terrestrial biosphere, the oceans uptake of anthropogenic CO_2 is primarily a physically and chemically controlled process. However, increasing atmospheric CO_2 concentrations will impact the ocean carbon cycle. This includes the buffering capacities of the oceans which will decrease with increasing CO_2 concentration. Furthermore, rising surface water temperatures will lead to increasing surface water pCO_2 and therefore, lower CO_2 solubility. This increase in surface water temperature also affects the vertical stratification of ocean waters and lower the mixing rate between deep and surface water layers which would finally lower the ocean CO_2 uptake (Prentice *et al.* 2001). So far, no stimulating effect of increasing atmospheric CO_2 on biological processes could be determined. In contrast, decreasing ocean pH because of increasing CO_2 dissolution was observed which slow down the calcification of key marine organisms including corals and plankton (Riebesell *et al.* 2000; Orr *et al.* 2005). This is important for the regulation of the ocean carbon cycle (Milliman 1993).

1.2.2. Terrestrial Carbon Cycle

The terrestrial sphere is the second largest CO_2 sink and primarily controlled by biological processes. The amount of carbon accumulated in living terrestrial biomass is approximately three times greater than the CO_2 in the atmosphere (Falkowski *et al.* 2000; Schmidt *et al.* 2011). The terrestrial biosphere accumulates soil organic matter (SOM) via CO_2 fixation of photosynthetic and chemolithotrophic organisms. Carbon is then returned to the atmosphere by (i) respiration of plants, (ii) microbial mineralisation and (iii) disturbances like deforestation and large fires which in turn enhance microbial mineralisation.

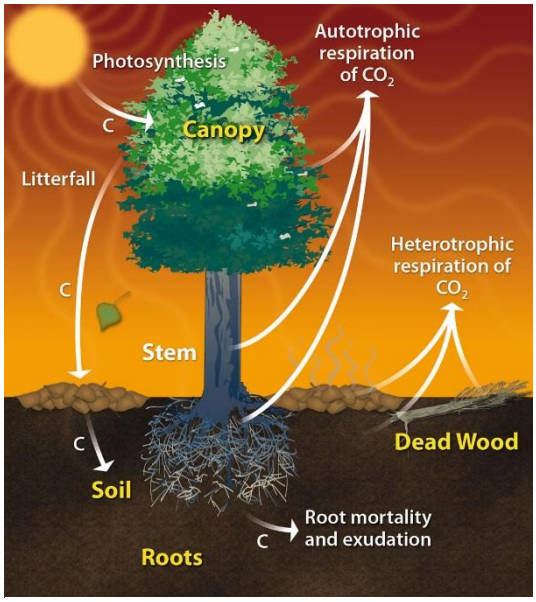

Figure 2:Simplified presentation of the terrestrial photosynthetic carbon cycle. Source: (DOE 2008).

However, similar to the ocean carbon cycle, increasing atmospheric CO_2 concentrations will also impact the terrestrial carbon cycle. It appears very likely, that increasing atmospheric CO_2 concentrations promote photosynthesis, and thus carbon uptake which in turn increases the biomass in vegetation and soils (Masle 2000). The magnitude of this carbon sink however, depends strongly on other factors as well including water and nutrient availability as well as climate extremes (Stocker *et al.* 2013). For example, the plant respond depends on the photosynthetic pathway which is used. Plants with a C3 photosynthetic pathway (all trees, nearly all plants of cold climates, and most agricultural crops including wheat and rice) show increasing photosynthesis. Plants using only the Calvin cycle for carbon fixation are called C3 plants. They fix atmospheric CO_2 using Ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) (Ehleringer and Cerling 2002). In contrast, C4 plants (tropical and many temperate grasses, some desert shrubs, and some crops including maize and sugar cane) show either no or less photosynthetic response. In C4 plants, the light-dependent reactions and the Calvin cycle are physically separated. They fix atmospheric CO_2 in the mesophyll cells and finally form malate.

In the bundle-sheath cells CO_2 is released by decarboxylation of the malate and fixed by RuBisCO (Kellogg 2013). Therefore, the magnitude of the terrestrial carbon reservoir is more difficult to predict.

1.3. Sources and Mitigation of Anthropogenic Carbon Dioxide

Among human activities that produce greenhouse gases, power generation by combustion of fossil fuels represents by far the largest source of greenhouse gases and CO_2 emissions.

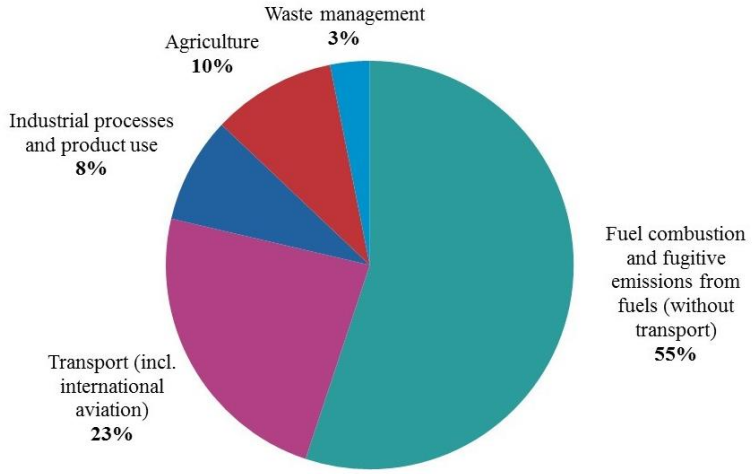

Figure 3:Sources of greenhouse gases by source sector in 2015 (EU-28).
Source: EEA.

According to the International Energy Agency (IEA, 2017), countries worldwide strive economic growth and development which led to an increase in the energy demand of ~150% between 1971 and 2015. However, the improvements in energy efficiency, the use of less carbon intensive fuels (gas instead of coal), the increasing use of renewable energy sources or nuclear energy led to decreasing greenhouse gas emissions in many countries. Therefore, greenhouse gas emissions decreased for countries including China (-32%), the United States of America (-12%) or the European Union (-21%) between 1990 and 2015. The German greenhouse gas emissions also decreased by -22% (IEA, 2017). Another option for the reduction of CO_2 emissions is carbon dioxide capture and storage (CCS) which will be discussed in the following chapter.

1.3.1. Carbon Dioxide Capture and Storage (CCS)

The stabilization of an atmospheric CO_2 concentration of 550 ppmv by 2100 would require a global emission reduction of 7-70% (Solomon 2007). The capture and storage of CO_2 could be a feasible method for a significant reduction of industrial CO_2 emissions. The technology essentially consists of (i) the separation of industrial CO_2 (Capture), (ii) the transportation of CO_2 from the emission source and (iii) the deep geological storage of CO_2. The CO_2 can be captured before or after the fuel is burnt (pre- or post -combustion capture), by burning the fuel with oxygen (oxyfuel combustion) or it can be captured by industrial process streams. After the CO_2 is captured it can be compressed and transported

by pipelines or by ships to a storage site on - or offshore where it can be injected into deep underground. Based on IPCC (2005) (Rubin and De Coninck 2005), the options for CO_2 storage include geological storage, ocean storage, mineralization and the industrial utilization of CO_2. Potential geologic repositories for CO_2 are oil and gas reservoirs, unmineable coal seam formations and the intensively studied deep (often saline) nonpotable aquifers. Feasible storage reservoir rocks are porous and permeable sandstones or limestones overlaid by layers of low permeability 'caprock' to prevent the upward migration of the stored CO_2.

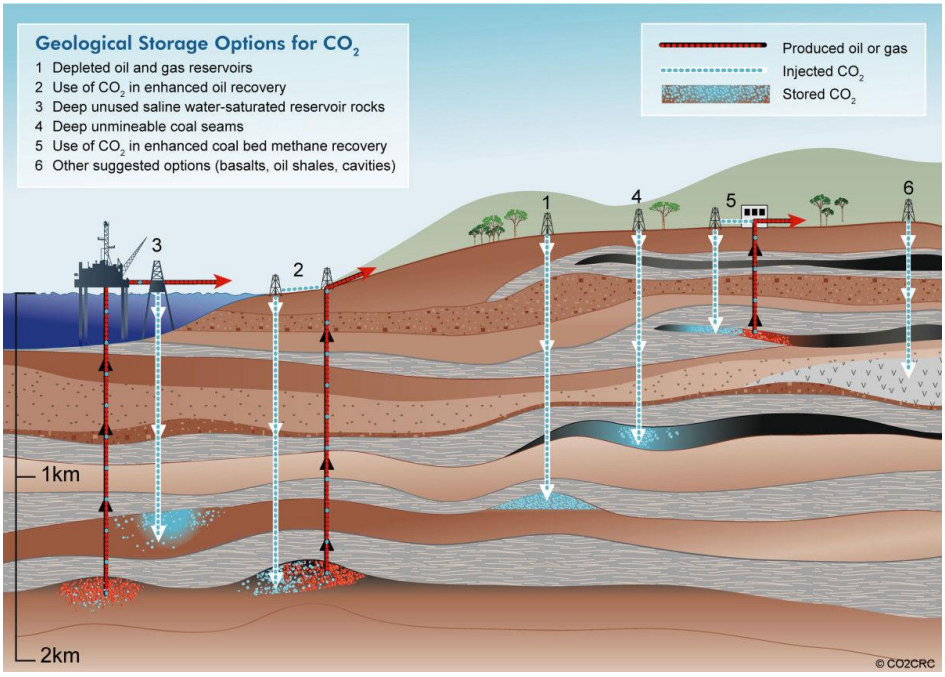

Figure 4: Geologic storage options for carbon dioxide. Sources: IPCC special report 2005 (Cook 1999; Rubin and De Coninck 2005).

So far, CO_2 storage by mineralization and industrial use are, however not considered to be of huge significance because the scale of CO_2 utilization is small or the technique energy-intensive. In Europe, suitable storage formations occur both offshore (North Sea) and onshore including northern Germany, France, Spain and the Netherlands, as well as regions in other countries. For Germany, only two types of CO_2 storage sites are possible: saline aquifers and depleted natural gas fields. The estimated storage volume in potential CO_2 reservoirs in Germany amount ~10 Gt of CO_2 which would equal the industrial CO_2 emissions of Germany for several decades (Knopf *et al.* 2010). However, a major risk factor and potential barrier for the implementation of geologic CO_2 sequestration is a CO_2 reservoir leakage. Leakage scenarios for geological reservoirs include the migration via (abandoned) wells, along fractures and the diffuse release through insufficiently sealing cap rock. Depleted oil and gas fields are typically perforated by a large number of wells which increases the risk of leakage through such wells (Ebigbo, Class and Helmig 2007). Leakages via wells can be easily detected at the surface due to high CO_2 point emissions. Diffuse leakages via fractures or through cap rock with low CO_2 concentrations at the surface

are more difficult to detect and they could also lead to the dissolution of the rising CO_2 into aquifers and lakes which affect the (drinking) water quality (decreasing pH, heavy metal mobilization).

The remaining question however, how potential CO_2 leakages and therefore increasing near-surface CO_2 concentrations affects near-surface ecosystems is of vital importance. Hence, natural CO_2 seeps are important analogue sites which allow the investigation of CO_2 long-term effects on different environments.

1.4. Potential Impacts of Carbon Dioxide on Near-Surface Ecosystems

The importance to understand potential CO_2 impacts for near-surface environments led to numerous studies, particularly at natural CO_2 releases from volcanic or geothermal areas. Studies have also been undertaken at artificial test sites where CO_2 has been injected. The following chapters summarize the obtained information about potential CO_2 impacts for plants, geochemistry and microbiology with special focus on terrestrial ecosystems.

1.4.1. Potential Impacts of Carbon Dioxide on Plants

According to the Intergovernmental Panel for Climate Change, increasing atmospheric CO_2 concentrations are expected to promote photosynthesis and therefore the carbon uptake (Masle 2000). Nevertheless, the response of plants varies depending on e.g. the exposure time, CO_2 concentration, atmospheric or upwelling CO_2, CO_2 fixation pathway, geochemistry or size of the root zone (Macek, I. *et al.* 2009; Noble *et al.* 2012; West *et al.* 2015). For example, the CO_2 emissions from a reservoir of magmatic gas beneath Mammouth Mountain volcano (California, USA) caused extensive tree killing in a 30ha area. Almost 100% of the conifers died at CO_2 concentrations between 30-96% (in 30-60 cm depth) (Kerr *et al.* 1995). Botanical surveys at additional natural CO_2 seeps showed that monocotyledonous plants (grasses) become increasingly dominant where soil gas CO_2 concentrations at 20 cm depth reach ~30–40% (West *et al.* 2015). Similar results were obtained for the artificial test sites ASGARD in Nottingham (GB) and ZERT in Montana (USA) (West *et al.* 2009; Zhou *et al.* 2013). Changes in the plant diversity up to the total killing of plants may occur over months to years. Changes in the photosynthetic pigmentation (showing yellowish to brown leaves) and root respiration rates due to high CO_2 concentrations are more immediate (Pfanz *et al.* 2007; Noble *et al.* 2012). However, threshold concentrations of CO_2 for significant alterations in plant activity, diversity or killing are unknown.

1.4.2. Potential Impacts of Carbon Dioxide on Soil Geochemistry

Major CO_2-induced geochemical alterations in pH (water, soil and pore water), metal concentration and mineralogy were observed in numerous previous studies (Giggenbach 1990; Stephens and Hering 2002; Holloway *et al.* 2007; Lewicki, Birkholzer and Tsang 2007; Gal *et al.* 2011). Therefore, a CO_2-induced decrease in pH was observed because of the dissolution of CO_2 in (pore) water which forms carbonic

acid which, in turn, will partially dissociate to release H^+, HCO_3^- and CO_3^{2-}. The acidification of (pore) water, in turn, leads to metal mobilization in soils and sediment. This mobilization has been attributed largely to the activity of H^+ and HCO_3^-. For example, Wei, Maroto-Valer and Steven (2011) detected an increase of metal concentrations in agricultural soil by up to 500% during three days of CO_2 incubation. Similar results were obtained for various CO_2-induced aquifer sediments which showed increasing concentrations of several metals (e.g. Al, V, Cr, Mn, Zn, and Co) by up to 3 orders of magnitude (Little and Jackson 2010). Increasing metal concentrations were also observed at artificial test sites where CO_2 was injected and natural CO_2 seeps (Blume and Felix-Henningsen 2009a; Kharaka *et al.* 2010b; Mehlhorn *et al.* 2014). The acidification also enhances chemical weathering of soils, rocks and sediment which leads to the dissolution of carbonates and silicates (Blume and Felix-Henningsen 2009a). Common weathering reactions of silicate mineral hydrolysis and carbonate mineral dissolution are as followed (Mortatti and Probst 2003):

Aalbite into kaolinite:

$$2NaAlSi_3O_8 + 2CO_2 + 11H_2O \longrightarrow Al_2Si_2O_5(OH)_4 + 2HCO_3^- + 2Na^+ + 4H_4SiO_4$$

K-feldspar into montmorillonite:

$$2KAlSi_3O_8 + 2CO_2 + 6H_2O \longrightarrow Al_2Si_4O_{10}(OH)_2 + 2HCO_3^- + 2K^+ + 2H_4SiO_4$$

Ca-plagioclase into kaolinite:

$$CaAl_2Si_2O_8 + 2CO_2 + 3H_2O \longrightarrow Al_2Si_2O_5(OH)_4 + 2HCO_3^- + Ca^{2+}$$

Olivine weathering:

$$Mg_2SiO_4 + 4CO_2 + 4H_2O \longrightarrow 2Mg^{2+} + 4HCO_3^- + H_4SiO_4$$

calcite dissolution:

$$CaCO_3 + CO_2 + H_2O \longrightarrow Ca^{2+} + 2HCO_3^-$$

Apart from the major geochemical alterations which would possibly occur, there are also indirect changes like increasing soil moisture concentrations in consequence of CO_2-induced plant death (Beaubien *et al.* 2008a; Pettinelli *et al.* 2008). The increase of soil moisture would, in turn, contribute to the increasing anoxic conditions in soil.

1.4.3. Potential Impacts of Carbon Dioxide on Microbial Processes

Microorganisms play an important role in the terrestrial carbon cycle because they are participating in both, atmospheric CO_2-uptake as well as the CO_2 release during SOM degradation (mineralisation). With increasing atmospheric CO_2 concentrations, microbial processes affiliated with carbon fixation and anaerobic microbial SOM degradation become of crucial importance.

1.4.3.1. Autotrophic Carbon Fixation

Autotrophic CO_2 fixation represents the most important biosynthetic process in nature with a calculated net fixation of $7*10^{16}$ g carbon annually (Berg 2011a). Microorganisms are converting inorganic carbon into biomass via six carbon fixation pathways:

(i) The Calvin-Benson-Bassham cycle (CBB) is the most important mechanism of autotrophic CO_2 fixation in nature including the key enzyme ribulose-1,5-bisphosphate carboxylase/ oxygenase (RubisCO). The CBB cycle was determined in e.g. plants, algae, cyanobacteria, many aerobic or facultative aerobic *Alpha-*, *Beta-* and *Gammaproteobacteria* and members of iron- and sulfur-oxidizing *Firmicutes* (Sulfobacillus spp.,) (Zakharchuk *et al.* 2003; Caldwell, MacLean and Norris 2007; Badger and Bek 2008). So far, three forms of RubisCO had been detected: I (A, B, C, D), II and III. Rubisco forms I and II are participating directly into the CO_2 fixation via the CBB cycle while form III has only been found in *Archaea* e.g. *Archaeoglobus fulgidus*, *Thermococcus kodakaraensis* or *Methanocaldococcus jannaschii* (Kreel and Tabita 2007; Sato, Atomi and Imanaka 2007; Badger and Bek 2008).

(ii) The reductive citric acid cycle (Aron-Buchanan cycle) reverses the reactions of the oxidative citric acid cycle and forms acetyl-CoA from two molecules CO_2. The cycle was determined in numerous anaerobic or microaerobic phyla e.g. *Chlorobi*, *Aquificae*, *Proteobacteria* and *Nitrospirae* (e.g. *Nitrospira*) (Fuchs, Stupperich and Eden 1980; Hügler *et al.* 2005; Takai *et al.* 2005; Hügler *et al.* 2007; Levican *et al.* 2008; Lücker *et al.* 2010).

(iii) The reductive acetyl CoA (Wood-Ljungdahl) pathway is a noncyclic pathway that results in the fixation of two molecules CO_2 to form acetyl-CoA (Berg 2011a). It is used by e.g. acetogens, methanogens, anaerobic ammonia-oxidizing planctomycetes, sulfate-reducing *Bacteria* or autotrophic *Archaeoglobales* (Drake, Küsel and Matthies 2002; Ragsdale and Pierce 2008; Thauer *et al.* 2008; Berg 2011a).

(iiii) The 3-hydroxypropionate (Fuchs-Holo) bi-cycle releases glyoxylate as a first carbon fixation product and was originally and so far, exclusively described for *Chloroflexus aurantiacus* (Herter *et al.* 2002; Berg *et al.* 2010; Berg 2011a).

(v and vi) The 3-hydroxypropionate/4-hydroxybutyrate cycle (HP/HB) & the dicarboxylate/ 4-hydroxybutyrate cycle (DC/HB), two autotrophic CO_2 fixation cycles recently described in *Crenarchaeota*, convert acetyl-CoA and two inorganic carbons to succinyl-CoA. The involved enzymes fundamentally differ in their O_2 sensitivity why the HP/HB cycle is restricted to aerobic microorganisms e.g. the (micro)aerobic *Sulfolobales* (Berg *et al.* 2007, 2010). The DC/HB cycle on the other hand is restricted to anaerobic microorganisms e.g. *Thermoproteales* and *Desulfurococcales* (Ramos-Vera, Berg and Fuchs 2009; Berg *et al.* 2010; Berg 2011a).

1.4.3.2. Anaerobic Organic Matter Decomposition

During SOM decomposition, heterotrophic organisms use SOM as an energy source. The decomposition of organic material is processed initially by the oxidation of organic substances using O_2 as terminal electron acceptor while inorganic N, Mn, Fe and S are mostly present in their oxidized forms. The aerobic SOM degradation is conducted by aerobic and facultative aerobic Prokaryotes and Eukaryotes (Madigan *et al.* 2010). After O_2 consumption subsequently nitrate, Mn(IV), Fe(III) and sulfate are used as alternative electron acceptors during SOM degradation following the maximum energy yields of these oxidation reactions. Therefore, the anaerobic SOM decomposition has four steps: (i) hydrolysis of polymers by hydrolytic microorganisms, (ii) acidogenesis by fermentative *Bacteria* producing e.g. alcohols, propionate, lactate, (iii) acetogenesis from metabolites of fermentation by homoacetogenic or syntrophic *Bacteria* and (iiii) methanogenesis. The final product of the anaerobic organic matter degradation is methane produced by two major groups of methanogenic *Archaea*: heterotrophic methanogens using acetate to produce CO_2 and CH_4 e.g. *Methanosarcina* sp. (acetoclastic methanogens) and autotrophic methanogens using CO_2 and H_2 to produce CH_4 and H_2O e.g. *Methanopyrus* sp. (hydrogenotrophic methanogens). Assuming, that increasing atmospheric CO_2 concentrations result into higher concentrations of substrates for both types of methanogens, methanogenesis generally might be promoted.

1.4.3.3. CO_2 Impacts on Microorganisms – State of Knowledge

Numerous recent studies demonstrated that changes in soil microbial communities are often strongly correlated with differences in soil chemistry, particularly soil pH (Fierer and Jackson 2006; Sait, Davis and Janssen 2006; Nicol *et al.* 2008; Lauber *et al.* 2009; Lozupone *et al.* 2010). Alterations of geochemical parameters, particularly pH were also verified at natural CO_2 seeps and artificial test sites where CO_2 was injected (Kandeler *et al.* 2006; Macek, I. *et al.* 2009; Frerichs *et al.* 2013a; Morales and Holben 2014; Sáenz de Miera *et al.* 2014a). However, the majority of these studies has been conducted using different detection methods e.g. respiration rates (Macek, I. *et al.* 2009), DNA fingerprinting-based approaches (TRFLP, DGGE) (Fierer and Jackson 2006; Nicol *et al.* 2008), phospholipid fatty acid analyses (PLFA) (Fierer, Schimel and Holden 2003; Niklaus *et al.* 2003) or cell counting methods (Gaidos *et al.* 2004; Liu *et al.* 2010). In addition, many studies have focussed on the investigation of single taxonomic groups, for example, ammonia oxidizing *Archaea* and *Bacteria* (AOA, AOB) (Rooney *et al.* 2010; He *et al.* 2012). All these different approaches complicate comparisons between the obtained results and prevent a general conclusion. Furthermore, results to date show inconsistent results regarding CO_2-induced stimulation or inhibition of microbial taxa. For example, Jossi *et al.* (2006) revealed a stimulation of *Actinobacteria* and *Deltaproteobacteria* in the rhizosphere during a field experiment with increasing atmospheric CO_2 concentrations. In contrast, analyses of soil samples from a Slovenian CO_2 vent area showed increasing numbers of *Firmicutes* and *Chloroflexi* sequences with increasing CO_2 (Šibanc *et al.* 2014). Therefore, further comprehensive investigations are needed to clarify the microbial

metabolic versality and alterations in microbial activity, abundance and distribution in CO_2-exposed ecosystems.

1.5. Scope and Objectives of the Thesis

The previous paragraphs displayed the drivers of climate change, the special role of CO_2 and its potential impacts for various environments and the carbon cycle. This information, particularly the gaps of knowledge, were the driving forces for the present thesis. Major gaps of knowledge comprise microbial responses in consequence of increasing CO_2 concentrations and ecosystem specific microbe-geochemistry-interactions. For this reason, the study of the three geosystems was based on the following major hypotheses:

(i) Hypothesis 1: A long-term CO_2 exposure leads to characteristic changes in soil and sediment geochemical parameters which strongly affect microbial and plant communities.

(ii) Hypothesis 2: A long-term CO_2 exposure results in a characteristic shift in the microbial community composition towards methanogenic, acid tolerant and autotrophic representatives, which might serve as possible indicators for CO_2 exposed sites.

(iii) Hypothesis 3: Short-term CO_2 exposure or only low CO_2 concentrations affect soil geochemistry and microbial communities to a lesser degree than long-term CO_2 exposure. Potential CO_2 impacts are therefore, difficult to detect.

1.6. Description of Geosystems

The investigation of the three selected geosystems in this thesis was part of the EU-funded project 'Research into Impacts and Safety in CO_2 Storage' (RISCS). RISCS aimed to improve information about possible implications of CO_2 leakages onshore and offshore. The project comprised industrial and research partners from Europe, Australia and the United States of America. The research included observations at artificial CO_2-injection test sites and at natural CO_2 sites (natural analogues).

The thesis comprises comprehensive microbiological and geochemical investigations at two natural CO_2 geosystems and the artificial CO_2-injection site ASGARD. The following chapters contain a detailed description of each geosystem.

1.6.1. The ASGARD (Artificial Soil Gassing and Response Detection) Field Site

The ASGARD site is located in the University of Nottingham's Sutton Bonington Campus, south of central Nottingham. Previously it was used as sheep pasture and had remained grassland for more than 10 years. The site was established by the University of Nottingham to investigate geochemical and biological alterations during CO_2 releases originally in the context of carbon capture and storage (CCS) and the risks involved. The facility consisted of 30 CO_2-injected and reference plots (15 and 15), each with an area of 2.5 × 2.5 m. Ten plots were kept as pasture for different experiments. The other plots were planted with different agricultural crops. CO_2 was injected into the centre of the plots through permanently installed pipework at a depth of 50-60 cm below ground level. Non-gassed but otherwise identically treated plots were used as references.

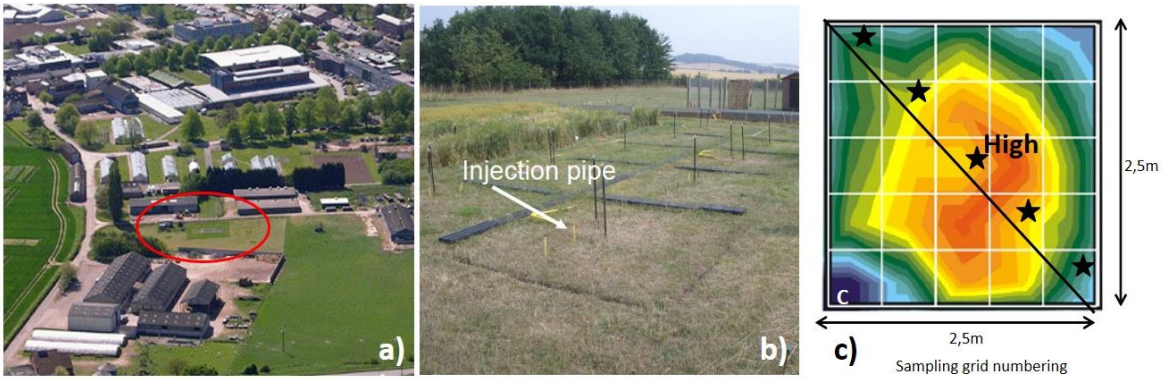

Figure 5: a) The ASGARD field site; b) Experimental CO_2-injected and reference plots; c) Plot size and sampling grid for each experimental plot. Colour map displays the CO_2 concentration with the highest in the middle of each plot (red). Source: RISCS project.

Previous analyses of the mineralogical composition showed that quartz was the main component of the soil (>90% of the dry weight) followed by K-feldspar and albite along with trace amounts of mica, kaolinite, chlorite and hematite. The top soil layer (~0.1 m) contains 9% clay, 23% silt and 68% sand, with no differences between the A horizon (0.15–0.30 m depth) and B horizon (0.45–0.50 m depth) (Ford 2006; West *et al.* 2009). During short-term, intermittent CO_2-injection periods between 2006 and

2008, first investigations took place focused on CO_2 flux rates and concentration in soil, geochemistry, plant coverage and alterations in microbial biomass, activity and cell numbers (Pierce and Sjögersten 2009; West *et al.* 2009). The authors detected a significant increase in soil CO_2 concentration and changes in the plant coverage but no significant changes in mineralogy. Microbiological results revealed decreasing bacterial cell numbers and microbial activity during CO_2 injection time.

In the course of this work, CO_2-injected and control plots of the ASGARD site were monitored for 3 years with 24 months of continuous CO_2 injection and a subsequent recovery phase of 5 months. Soil samples were taken before, during and after the CO_2 injection period (2010 to 2012). Samples were taken twice a year (May and October) at 15–30 cm depth, in the middle of the investigated CO_2-injected and reference pasture plot. For the first time, the impacts of long-term CO_2-injection for geochemistry and microbiology (activity, abundance and diversity) could be investigated. Comparisons between soil samples before, during and after CO_2 injection comprised analyses of soil properties (e.g. TOC, trace elements), potential microbial activities using gas chromatography (GC), molecular-biological analyses using quantitative PCR (qPCR) and 16S rDNA clone library construction.

1.6.2. The Florina Basin

The Florina Basin is part of the Florina-Ptolemais-Aminteo graben system in Northern Greece. The graben is composed of metamorphic rocks and formed as a result of the Alpine orogenesis. The 800-1000 m overlying deposits consist of conglomerate, marl, Sandstone, loam, peat and limestone with clayey caprocks (Metaxas *et al.* 2007). As a result of the slow upwelling of magmatic, hydrothermal CO_2 along faults and fractures, carbonate-rich springs and CO_2-rich gas vents appear throughout the Florina Basin (Ziogou *et al.* 2013). The studied field site contains a number of gas vents of undetermined age and is used as pasture for sheep, goats and horses.

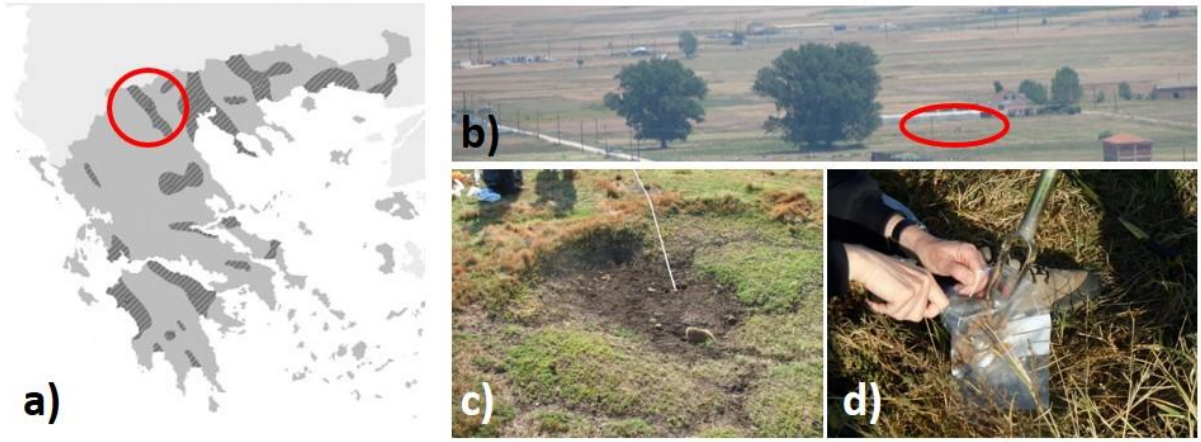

Figure 6: a) Florina-Ptolemais-Aminteo graben system (Greece); b) the Florina 25 m transect; c) Florina CO_2 vent; d) Sampling procedure along the 25 m transect. Source: British Geological Survey; CERTH.

In 2011 and 2012, one of the gas vents was subject for a comprehensive survey. Therefore, soil samples were taken along a 25 m transect from the CO2 vent, medium and reference site in 65-70 cm

depth. Investigations along the 25 m transect comprised analyses of (i) geochemical alterations (moisture, TOC, trace elements), (ii) alterations in the archaeal and bacterial abundance (qPCR) and (iii) alterations in the archaeal and bacterial community composition (16S rDNA clone libraries). In addition, a $^{13}CO_2$ incubation experiment with vent samples of 2012 was conducted to describe the active, CO_2-utilizing bacterial community using stable isotope probing (SIP) and 454-amplicon pyrosequencing (PyroTag). It was the first comprehensive microbiological survey of a mountainous, mediterranean environment with magmatic-hydrothermal CO_2 releases.

1.6.3. The Laacher See

The East Eifel volcanic field, located west of the river Rhine consists numerous natural CO_2 vents. Located in the centre of the volcanic field is the largest water filled caldera, Laacher See. The lake covers an area of approximately 3.3 km^2 and has a maximum depth of 52 m (Giggenbach 1990; Aeschbach-Hertig *et al.* 1996). Destratification occurs once a year why the lake is classified as mesotrophic to eutrophic (Aeschbach-Hertig *et al.* 1996). Discharge of gaseous CO_2 along the (north-) eastern shore as well from the lake bottom are well known.

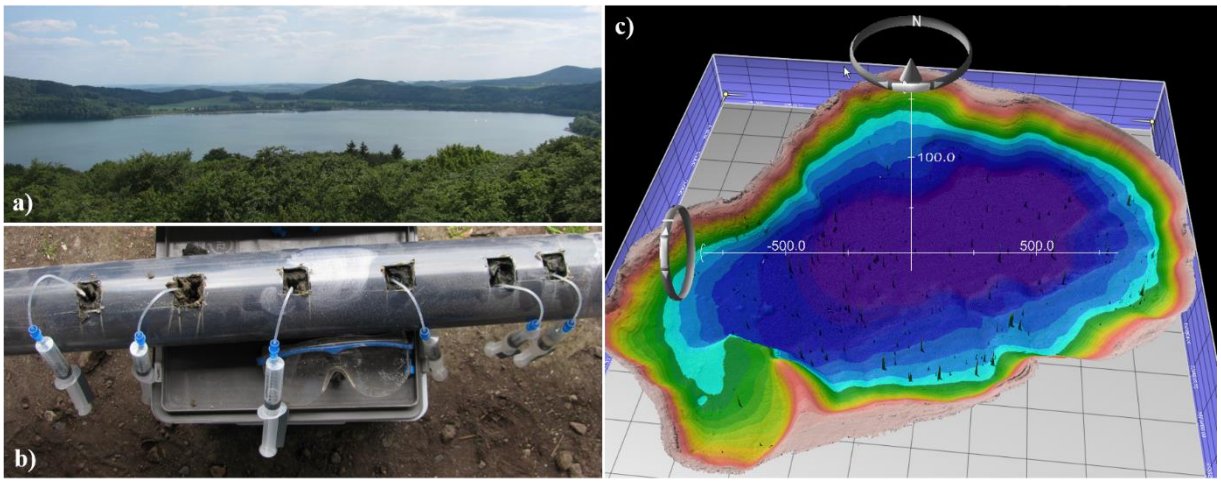

Figure 7: a) The lake Laacher See (Germany); b) Sediment core with Rhizon sampler for the retrieval of pore water; c) Evidence of CO_2 vents at the Laacher See lake bottom by Multibeam Echosounder (3D). Black Peaks represent CO_2 vents. Source: Federal Institute for Geosciences and Natural Resources (BGR); Northern Institute of Advanced Hydrographics.

Analyses of the emerging geogenic gas revealed a high content of CO_2 (99%) and the annual CO_2 flux was estimated with about 5'000 tons of CO_2 (Aeschbach-Hertig *et al.* 1996). In addition, an isotopic analysis of the CO_2 indicated its magmatic origin (Giggenbach 1990). In previous microbiological surveys focused on the pastures surrounding the lake, a shift towards anaerobic and acid tolerant to acidophilic species was detected with however, different results in the bacterial and archaeal abundance using qPCR (Oppermann *et al.* 2010a; Krüger *et al.* 2011; Frerichs *et al.* 2013b).

In the course of this work, CO_2 seeps at the lake bottom and potential control areas were located using several hydroacoustic measurements and subsequently sediment cores were taken in August 2011. The sediment cores were taken at a reference site (without detectable CO_2 bubbles rising), low CO_2 site

(less CO_2 bubbles) and high CO_2 site (high amount of rising CO_2 bubbles). It was the first time, that Laacher See lake sediment from three different CO_2-induced sites were geochemically and microbiologically investigated. Therefore, analyses of the pore water, sediment geochemistry and microbial abundance and community composition using qPCR and 16S rDNA clone libraries were conducted.

2. Results and Discussion

The following chapters summarises and discusses the results of the individual manuscripts and the general conclusions.

2.1. Long-term CO_2 Exposure Induces Changes in Geochemistry and Plant Coverage

2.1.1. The Florina CO_2 Vent

Distinct differences in plant coverage and geochemistry along the 25 m CO_2 gradient in the Florina Basin were observed. Reduced plant coverage with increasing CO_2 concentration and a total killing of plants at the vent were detected. Similar observations of plant death at natural CO_2 vents have been described previously (Beaubien *et al.* 2008a; Pettinelli *et al.* 2008). However, how CO_2 may impact the vegetation cover at natural CO_2 vents varies. Several studies in the past reported different conclusions including plant stimulation, inhibition and adaption to high CO_2 concentrations (Hättenschwiler *et al.* 1997; Maček *et al.* 2005; Vodnik *et al.* 2006; Rennert and Pfanz 2015). For example, highest soil moisture concentrations detected in CO_2 vents were previously related to e.g. an increased water use efficiency of some plants in consequence of CO_2-stimulated plant growth and increasing root biomass (Moore and Field 2006). At Florina, the high soil moisture concentration in the CO_2 vent is more likely associated to a lack of evapotranspiration in consequence of plant death. Although no pH measurements at Florina were conducted, soil analyses and the concentrations of trace elements and metal oxides confirm the suggested CO_2-induced soil acidification and soil weathering as described previously for natural CO_2 vent systems (Altevogt and Jaffe 2004; Beaubien *et al.* 2008a; Blume and Felix-Henningsen 2009a; Harvey *et al.* 2013; Mehlhorn *et al.* 2014). Therefore, up to 16 times higher concentrations of metal oxides, trace elements and total (organic) carbon (TC/TOC) within the vent were observed. It seems likely, that in consequence of the detected plant death and increased soil moisture concentration, accelerated soil weathering took place. The increase of metal oxide concentrations due to CO_2 exposure however, can vary. Wei, Maroto-Valer and Steven (2011) for example, detected an increase of several metals by up to 500% in agricultural soil due to the CO_2-induced weathering of soils during a three days CO_2 incubation time.

The geochemical results together with the plant observations give evidence for distinct CO_2-induced alterations during long-term CO_2 exposure. In addition, the high TC/TOC concentrations in vent samples suggest restricted microbial organic matter decomposition with increasing CO_2 concentrations and therefore, give first information about CO_2-induced alterations in the SOM degradation (Beaubien *et al.* 2008a; Beulig *et al.* 2014; Nowak *et al.* 2015; Rennert and Pfanz 2015).

2.1.2. The Laacher See High CO_2-influenced Sediment Core

Similar to the Florina CO_2 vent, significant CO_2-induced differences between the sediment cores taken at a reference, low and high CO_2 influenced site in lake Laacher See have been detected. Therefore, significantly higher concentrations of dissolved CO_2 and CH4 and a drop in pH were detected in the pore water of the high CO_2 influenced sediment core. The process of decreasing pH with increasing CO_2

concentrations based on the dissolution of the rising CO_2 in the pore water and its formation of carbonic acid has been described previously (Bhattarai *et al.* 2012a; Harvey *et al.* 2013). The analyses of the dissolved gases give also evidence for a potential correlation between CO_2 supply and increasing CH_4 concentrations in consequence of anaerobic microbial activity i.e. methanogenesis. Anoxia in freshwater sediments generally contributes to high CH_4 emissions by methanogens utilizing CO_2 as a product of organic matter decomposition (Bastviken *et al.* 2004; Tranvik *et al.* 2009). In case of natural CO_2 vents, emitted CO_2 can additionally enhance methanogens. Previous CH_4 $\delta^{13}C$ analyses of rising gas bubbles in the Laacher See lake indeed proved the biogenic origin of the CH_4 (Möller *et al.* 2011). The dissolution of minerals and increasing amounts of metals (-oxides) in consequence of increasing CO_2 concentrations as previously described was not observed for the Laacher See sediment cores (Kharaka *et al.* 2010b; Lu *et al.* 2010; Harvey *et al.* 2013). In contrast, while in the sediment chemistry no changes could be detected, cations and anions in the pore water significantly decreased. This might be explained by an accelerated removal of dissolved cations/anions by a gas bubble driven transport and therefore, a continuous exchange between sediment and lake water body.

In conclusion, the results revealed an acidification of the pore water and suggest sediment weathering whereby the dissolved cations/anions might be transported into the water body of the lake. However, the results provide evidence for enhanced methanogenesis with increasing CO_2 concentration.

2.2. Long-term CO_2 Exposure Induces Changes in Microbial Communities

2.2.1. Methanogens and Autotrophs Dominate Florina Vent Samples

The majority of the identified archaeal 16S rDNA clone library sequences in the Florina CO_2 vent were affiliated to *Methanosarcina* sp. and *Methanomassiliicoccus luminyensis* of the class *Methanomicrobia* (*Euryarchaeota*). The acetoclastic *Methanosarcina* members are predominant in many natural environments because acetate, as a metabolic intermediate during SOM degradation is the quantitatively more available substrate (Liu and Whitman 2008; Thauer *et al.* 2008). In contrast, the hydrogenotrophic *Methanomassiliicoccus luminyensis* are not able to utilize acetate but produce CH_4 by reducing methanol with H_2, although it cannot produce CH_4 when H_2 or methanol is the sole energy source (Dridi *et al.* 2012). Investigations of a CO_2 vent in the Czech Republic by Beulig *et al.* (2015) also determined the predominance of *Methanosarcinales* and *Methanomicrobiales* in the CO_2 vents (50-90%). In addition, they identified active, CO_2-utilizing *Archaea* (using $^{13}CO_2$ incubation experiments and DNA-SIP) which were predominantly affiliated to the two euryarchaeotal orders which already dominated the pyrosequencing libraries. Besides the *Euryarchaeota*, the vent also contained *Crenarchaeota* sequences which were almost exclusively found in the CO_2 vent. The ability of anaerobic, autotrophic CO_2 fixation using the oxygen sensitive key enzymes involved in the DC/HB cycle was recently described for *Thermoproteales* which might explain the detection of unclassified crenarchaeotic *Thermoprotei* exclusively within vent samples (Berg 2011a; Pratscher, Dumont and Conrad 2011a). Although *Thermoprotei* were exclusively found in the Florina vent samples, their 16S rDNA sequence abundance

was however, low suggesting that their distribution and abundance must be driven by other factors as well.

In contrast to the archaeal community composition of the Florina vent, the bacterial community was highly diverse and significantly decreasing bacterial 16S rDNA gene copy numbers were detected with increasing CO_2. Decreasing bacterial abundance with increasing CO_2 due to e.g. hypoxia and changing redox conditions was previously reported for several natural CO_2 vent systems (Beaubien *et al.* 2008a; Fernández-Montiel, Pedescoll and Bécares 2016a). The majority of the bacterial 16S rDNA clone library sequences of the Florina vent were affiliated to *Proteobacteria*, *Actinobacteria*, *Acidobacteria*, *Firmicutes* and *Verrucomicrobia* (41-88%). Lower abundances were detected for *Bacteroidetes*, *Chloroflexi*, *Gemmatimonadetes*, *Caldiserica*, *Candidatus Saccharibacteria*, *Armatimonadetes*, *Planctomycetes* and candidate division WPS-2. According to previous reports, soils from different habitats roughly consist of the same major bacterial taxa as described for Florina, independent of the local environmental conditions (Janssen 2006; Lauber *et al.* 2009). However, quantitatively decreasing sequence numbers of the major phyla *Actinobacteria*, *Acidobacteria*, *Firmicutes* and *Verrucomicrobia* with increasing CO_2 concentrations were observed most likely due to decreasing O_2, soil acidification and weathering. Similar results were detected by Sáenz de Miera *et al.* (2014a) with an overall decrease for most major bacterial phyla with increasing CO_2 concentration along a natural CO_2 vent in Spain. However, statistical SIMPER analyses revealed, that at species level *Chloroflexi* representatives (unclassified *Ktedonobacteria*) contributed most to differences between CO_2 vent, medium and reference site.

Similar results were obtained during microcosm experiments with Florina vent soil to identify CO_2-utilizing *Bacteria*. Therefore, vent samples from 2012 were incubated with ^{13}C-CO_2 and analysed using stable isotope probing (DNA-SIP) and pyrosequencing (PyroTag). After 50 days of incubation major CO_2-utilizing organisms were affiliated to *Chloroflexi*, *Proteobacteria*, *Actinobacteria* and *Acidobacteria*. Therefore, *Chloroflexi* were most abundant in the vent soil before and after 50 days of incubation. Furthermore, statistical SIMPER analyses of the PyroTag sequences revealed that *Ktedonobacteria* (*Chloroflexi*) and *Conexibacter* (*Actinobacteria*) contributed most to differences between the vent samples before and after 50 days of $^{13}CO_2$ incubation similar to the bacterial 16S rDNA clone libraries. *Ktedonobacteria* have been found in various environments including extreme environments like volcano deposits, volcanic caves or CO_2 vents (Weber and King 2010; Northup *et al.* 2011; de Miera *et al.* 2014; Tebo *et al.* 2015). The occurrence of *Ktedonobacteria* in various, even extreme environments suggests a broad metabolic versality of that class. However, most of the studies did not focus on *Ktedonobacteria* and therefore, little is known about their diversity, abundance, metabolic capabilities and ecological significance (Cavaletti *et al.* 2006; Yabe *et al.* 2017). Recent reports demonstrated a potential role of *Chloroflexi* for acetogenesis and the metabolic potential to utilize CO_2 which might explain the high abundance of *Chloroflexi* and *Ktedonobacteria* affiliated

sequences in the vent samples and in the $^{13}CO_2$ incubation experiment (Chan *et al.* 2013; Hug *et al.* 2013; Wasmund *et al.* 2014a; Beulig *et al.* 2015). The results suggest that *Chloroflexi* might be important anaerobic SOM degraders producing important metabolic intermediates for e.g. methanogens, while they are fixing CO_2.

In addition to *Chloroflexi*, *Conexibacter* representatives were of interest because of their increasing abundance during $^{13}CO_2$ incubation time. Similar to our results, Hunger *et al.* (2011) detected increasing abundances of *Conexibacter*-affiliated 16S rRNA gene sequences during $^{13}CO_2$ incubation experiments with fen soil which indicated that these phylotypes assimilated CO_2. Originally, *Conexibacter* representatives were described as strictly aerobic and chemoorganotrophic *Bacteria* (Albuquerque and da Costa 2014). However, the results from Florina in addition to Hunger *et al.* (2011) suggest that *Conexibacter* benefit directly or indirectly from elevated CO_2 and that further investigations are needed to clarify the metabolic capabilities of *Conexibacter*.

The results for the Florina vent give evidence that the acidification of vent soil and the high availability of CO_2 as a substrate in the vent (geogenic CO_2/produced during SOM degradation) enhances a shift towards hydrogenotrophic and acetoclastic methanogens, acetogens as well as acidophilic autotrophs e.g. *Thermoprotei* and *Chloroflexi*, able to utilize CO_2 as an energy and/or carbon source.

2.2.2. Autotrophs Dominate High CO_2 Influenced Laacher See Sediment Core

For the high CO_2 influenced sediment core of the Laacher See lake, archaeal 16S rDNA clone libraries revealed the presence of euryarchaeotal methanogens (*Methanomicrobiales* and *Methanosarcinales*) as a minor fraction of the archaeal sediment populations. The majority of the archaeal sequences were affiliated to unclassified crenarchaeotal *Thermoprotei* which dominated the three sediment cores with however, decreasing 16S rDNA sequence abundance with increasing CO_2. Statistical analyses confirmed that *Thermoprotei* also contributed most to differences between the three sediment cores. The thermophilic and moderate acidophilic to acidophilic CO_2 fixing *Thermoprotei* have also been detected by Raulf *et al.* (2015a) along natural CO_2 gradients at a benthic volcanic vent. In contrast to the results for the Laacher See sediment cores, they detected a significant increase of *Thermoprotei* with decreasing pH. Frerichs *et al.* (2013b) has also been detected high abundances of *Thermoprotei* in terrestrial vent samples of the pasture area surrounding lake Laacher See. The different results for the *Thermoprotei* occurrence indicate that additional, so far unknown drivers for the distribution of *Thermoprotei* representatives exist.

Besides the predominance of *Thermoprotei* representatives, sequences affiliated to *Pacearchaeota* stand out because of their high abundance within the high CO_2 influenced core. The metabolic versality of *Pacearchaeota* presumably comprises a saccharolytic and fermentative lifestyle with the ability to degrade and utilize complex carbon compounds and to fix CO_2 (Castelle *et al.* 2015a). Therefore,

Pacearchaeota might be an important group of anaerobic organic matter degraders in CO_2 exposed sediments.

The majority of the bacterial 16S rDNA clone library sequences were affiliated to *Geobacter* spp. (*Proteobacteria*), *Thermanaeromonas* spp. (*Firmicutes*) and *Dehalococcoidetes* spp. (*Chloroflexi*) (67-86%) with *Dehalococcoidia* (*Chloroflexi*) as most abundant organisms. *Chloroflexi* have been frequently identified in marine and freshwater sediments (Kadnikov *et al.* 2012; Hug *et al.* 2013; Wasmund *et al.* 2014a). Recently Hug *et al.* (2013) revealed the potential role of *Chloroflexi* in anaerobic sediment carbon cycling beyond organohalide respiration including respiration of sugars, fermentation, CO_2 fixation, and acetogenesis. They detected evidence for both anaerobic and aerobic mechanisms of energy generation suggesting the ability of *Chloroflexi* to adapt to changing sediment redox conditions. The findings of Wasmund *et al.* (2014a) confirm these results. They have sequenced the genome of a marine subsurface *Dehalococcoidia* and found evidence for a broad metabolic versatility e.g. the ability to use the reductive acetyl-CoA pathway for the oxidation of organic compounds or CO_2 fixation.

The results for the high CO_2 influenced sediment core of lake Laacher See confirm terrestrial findings that high CO_2 concentrations and the induced acidification promote anaerobic, acidic tolerant to acidophilic microorganisms, able for CO_2-fixation. Furthermore, the results suggest that *Chloroflexi* representatives can play an important role in anaerobic organic matter degradation particularly because of their adaption capabilities due to their broad metabolic versatility.

2.3. Impacts of Short-term CO_2 Exposure and Low CO_2 Concentrations

2.3.1 The Artificial ASGARD Facility without Geochemical or Microbiological Changes

Geochemical analyses showed no significant CO_2-induced alterations within the CO_2 injected plot as well as no significant differences between CO_2 exposed and reference samples. Soil pH, soil moisture, metals (-oxides) and trace elements showed no significant differences during the 3-year observation period. Therefore, soil acidification due to CO_2 dissolution in pore water and consequently weathering of soils as previously described was not detected (Altevogt and Jaffe 2005a; Beaubien *et al.* 2008a; Harvey *et al.* 2012a). Similar geochemical alterations were detected for natural CO_2 vents suggesting that the achieved CO_2 maximum of 23% at ASGARD was not high enough to induce geochemical alterations. Instead, the results demonstrate the extent of soil buffering capacities which are determined by e.g. the carbonate content, silicate minerals, the cation exchange capacity of the soil and the aluminium content in silicates (where clay minerals react rapidly) (Ulrich and Sumner 2012). During CO_2 injection, not only alterations in the degradation of soil organic matter was considered but also the influence of the rising supply of CO_2 as a direct microbial electron acceptor. However, the microbial activity, abundance and community structure of the CO_2 injected plot showed no significant alterations during CO_2 injection. Therefore, the CO_2-exposed samples did not show significant changes in microbial CO_2 and CH_4 turnover rates compared to reference samples. Likewise, no significant CO_2-induced

variations were detected for the abundance of *Bacteria*, *Archaea* (16S rDNA) and gene copy numbers of the *mcrA* gene, *Crenarchaeota* and *amoA* gene. The majority of the bacterial sequences (75%–95%) were assigned to *Firmicutes*, *Proteobacteria*, *Actinobacteria*, *Acidobacteria* and *Bacteroidetes*. The majority of the archaeal sequences (85%–100%) were assigned to *Candidatus Nitrososphaera* (thaumarchaeotal cluster I.1b; soil group). Therefore, common soil taxa were detected for ASGARD as described previously (Janssen 2006; Lauber *et al.* 2009; Bates *et al.* 2011; Eilers *et al.* 2012). In contrast to the predicted CO_2 impacts, seasonal conditions including temperature and precipitation influenced the geochemistry and microorganisms most. Thus, autumn samples cluster together with rainfall in contrast to most of the spring samples which correlate with temperature and high carbon availability (TC, TOC), due to high rainfall in autumn and the growing season in spring. The impact of climate parameters on near-surface ecosystems as detected within our study was also reported in previous near-surface studies (Kreyling *et al.* 2008; Schlömer, Möller and Furche 2014). Several recent studies show that microbial communities in general are highly dynamic. Their distribution depends on various parameters e.g. inter-annual and seasonal variations, land-use, oxygen availability, vegetation cover, geochemical parameters (e.g. pH) or nutrient availability. Previous findings suggest that the composition of bacterial communities of different ecosystems can vary on the scale of days, seasons and years (Buckley and Schmidt 2003; Lipson 2007; Zhang *et al.* 2011). For example, Lauber *et al.* (2013) examined soil bacterial diversity and community composition of three land-use types using barcoded pyrosequencing of the 16S rRNA gene. They revealed that the bacterial community composition and its shift over time significantly depended on land-use and within the land-use types on the parameters soil moisture and temperature suggesting that these factors directly or indirectly regulate the structure of soil bacterial communities.

Natural CO_2 vents like the Florina Basin or the Laacher See area in turn demonstrated, how seasonal and inter-annual variations become overlapped by long-term CO_2 exposure and its induced implications for vegetation, geochemistry and therefore microbiology (Pfanz *et al.* 2007; Oppermann *et al.* 2010a; Frerichs *et al.* 2013b; Sáenz de Miera *et al.* 2014a; Taylor *et al.* 2014; Raulf *et al.* 2015a; Fernández-Montiel, Pedescoll and Bécares 2016a). At artificial CO_2 injection sites like ASGARD, distinctive CO_2-induced microbial responses are missing, and the results so far are diverse and difficult to generalize although in most cases a stress response of the vegetation was visible (Ko *et al.* 2016). The results from ASGARD and other artificial sites suggest that potential microbial responses to elevated CO_2 might be masked or quenched by natural responses to seasonal and climate variations (Morales and Holben 2013; Schlömer, Möller and Furche 2014; Fernández-Montiel *et al.* 2015). Therefore, the achieved CO_2 fluxes and concentrations or the injection period did not reach the systems threshold for initiating distinct changes and the soil matrix was able to buffer the increasing CO_2 concentrations. Potential future investigations of various near-surface ecosystems for CO_2-induced alterations especially in microbial abundance and diversity requires the analysis of a relatively large number of samples to discriminate

between temporal changes (due to e.g. seasonal, inter-annual variations) and CO_2-induced, constant changes.

2.3.2. Microbial Community Composition on the Florina Medium and Reference Site

In contrast to the Florina vent, the majority of the identified archaeal sequences in the Florina medium and reference samples were affiliated to the ammonia oxidizing *Nitrososphaera viennensis* (62-88%). Representatives of the genus *N. viennensis* showed a mesophilic and mixotrophic lifestyle and it requires organic acids (e.g. pyruvate, oxaloacetate, α-ketoglutarate or glyoxylate) for growth (Tourna *et al.* 2011; Stieglmeier *et al.* 2014). Although, all characterized *Thaumarchaeota* are encoding genes for CO_2 fixation via the hydroxypropionate/ hydroxybutyrate cycle, meta-genome analyses and environmental studies indicated that ammonia oxidizing *Archaea* might be able to switch from autotrophic ammonia oxidation to a mixotrophic and heterotrophic lifestyle (Pester *et al.* 2012; Stahl and de la Torre 2012; Könneke *et al.* 2014). This metabolic versality might explain the global dominance of the *Nitrososphaera* cluster (thaumarchaeotal I.1b group) in soils (Ochsenreiter *et al.* 2003; Leininger *et al.* 2006; Bates *et al.* 2011; Gubry-Rangin *et al.* 2011; Pester *et al.* 2012). It might also explain the presence of *N. viennensis* in reference and particularly in medium soil samples where CO_2 concentrations between 10-50% were detected (2011 and 2012). A comprehensive study of AOA distribution in soils at global, regional, and local scales by Gubry-Rangin *et al.* (2011) revealed that the only parameter significantly influencing AOA abundance and diversity was soil pH with increasing AOA abundance and diversity with increasing soil pH. Therefore, it seems most likely that the presumably low pH due to soil acidification in the Florina vent and other CO_2 vents led to lower abundances of *N. viennensis* compared with the medium and reference site. The bacterial community of the Florina medium and reference site showed high diversity and common bacterial phyla without significant differences (see also chapter 2.2.1.)

2.3.3. Microbial Community Composition on the Reference and Low CO_2 Influenced Sediment Cores

The archaeal 16S rDNA clone libraries of the reference and low CO_2 influenced sediment cores were dominated by crenarchaeotal *Thermoprotei* with minor abundances of *Euryarchaeota*, *Thaumarchaeota*, *Pacearchaeota*, *Woesearchaeota* and *Aenigmarchaeota*. A detailed description of *Thermoprotei* as most abundant class in all three sediment cores is given in chapter 2.2.2.

In the reference and low CO_2 influenced sediment cores, *Geobacter* spp. (*Proteobacteria*) and *Thermanaeromonas* spp. (*Firmicutes*) dominated the bacterial 16S rDNA clone libraries. The *Geobacter* spp. distribution within the sediments cores correlates with Fe II concentrations in the pore waters and indicate that Fe(III) oxide minerals might be reduced by *Geobacter* resulting in high concentrations of Fe II particularly in the reference core. As previously described, this reaction seems to be coupled to the oxidation of acetate (Lovley, Holmes and Nevin 2004; Weber *et al.* 2006; Mahadevan, Palsson and

Lovley 2011). Acetate is the primary product of the fermentation of organic matter and Fe(III) is anaerobically the most abundant available electron acceptor for organic matter oxidation (Lovley, Holmes and Nevin 2004). In soils, Fe occurs as iron oxides like goethite (FeO(OH)), hematite (Fe_2O_3) and magnetite (Fe_3O_4) or it forms other compounds like siderite ($FeCO_3$). Under anaerobic or acidic conditions as described for the low and high CO_2 influenced sediment cores, Fe(III) is reduced to Fe II. The results for the reference and low CO_2 influenced sediment cores show a distinct decrease of *Geobacter* abundance with increasing CO_2 concentrations indicating a correlation between *Geobacter* abundance and the CO_2-induced weathering of the sediment and therefore decreasing available Fe(III).

Besides *Geobacter*, *Thermanaeromonas* spp. (*Firmicutes*) was highly abundant in reference and low CO_2 influenced sediment cores. In contrast to *Geobacter* however, less information are available for the presumably strictly anaerobic, thermophilic and fermenting type specie of the genus *Thermanaeromonas* spp. (Mori *et al.* 2002). The potential contribution of *Clostridia* and *Firmicutes* representatives in anaerobic hydrocarbon-degrading processes previously described might demonstrate their potential importance in complex organic matter degradation in sediments (Beckmann and Manefield 2014).

2.4. General Conclusions

Addressing the formerly expressed hypotheses, this thesis proved (i) significant CO_2-induced changes in plant coverage (until plant death) and geochemistry e.g. acidification of soil and pore water, and soil weathering during long-term CO_2 exposure in natural CO_2 vent systems;

(ii) a shift towards acidic tolerant to acidophilic methanogens and autotrophs e.g. *Thermoprotei* and *Chloroflexi* during long-term CO_2 exposure in natural CO_2 vent systems;

(iii) that short-term CO_2 injection as well as low CO_2 concentrations did not lead to changes in geochemistry, microbial activity, abundance and community structure.

This thesis also proved the importance of seasonal and inter-annual variations and their impacts for near-surface ecosystems (short-term and long-term). Considerable, site specific variations of near-surface CO_2 concentrations due to seasonal and inter-annual impacts and subsequently geochemical and microbiological variations have been described previously (Schlömer, Möller and Furche 2014).

Although this thesis provides important findings, further comprehensive geochemical and microbiological surveys are needed to clarify the metabolic capabilities of the identified archaeal and bacterial taxa and to assess their potential response to varying environmental impacts.

3. Overview of the Manuscripts

Manuscript 1

Simone Gwosdz, Julia M. West, David Jones, Jana Rakoczy, Kay Green, Tom Barlow, Marco Blöthe, Karon Smith, Michael Steven and Martin Krüger (2016)

Long-term CO_2 injection and its impact on near-surface soil microbiology

FEMS Microbiology Ecology, 92, 2016, fiw193

Soil samples from the artificial CO_2 injection facility ASGARD have been analysed for two consecutive years, 2010 to 2012. Samples were taken before, during and after CO_2 injection twice a year (May and October) and have been analysed for CO_2-induced geochemical and microbial alterations over time. Geochemical data revealed no CO_2-induced changes. In addition, microbial activity, abundance and diversity did not significantly changed during CO_2-injection instead, seasonal variations influenced the soil samples mostly.

Simone Gwosdz was involved into the sampling campaigns, conducted the activity measurements and the molecular biological investigations (qPCR, 16S rDNA clone libraries), and evaluated the respective results.

Julia M. West, David Jones, Kay Green, Tom Barlow, Karon Smith and Michael Steven contributed to the sampling campaigns, conducted field measurements of CO_2 flux and soil concentrations and analysed CO_2-induced changes in the vegetation.

Marco Blöthe and Jana Rakoczy contributed to 16S rDNA clone library evaluation and preparation of the manuscript.

Martin Krüger contributed to the planning of the experiments, the data discussion and preparation of the manuscript.

Manuscript 2

Simone Gwosdz, Vasiliki Gemeni, Fotini Ziogou, Julia M. West, David Jones, Jana Rakoczy, Kay Green, Marco Blöthe, Tom Barlow, Nikolaos Koukouzas and Martin Krüger

Euryarchaeota and Chloroflexi representatives dominate terrestrial CO_2 vent

FEMS Microbiology Ecology (submitted)

Geochemical and microbiological variations along a 25 m CO_2 transect in the Florina Basin (Greece) have been investigated. Soil samples were taken from the CO_2 vent, medium and reference site and have been analysed for (i) geochemical alterations, (ii) alterations in the archaeal and bacterial abundance and (iii) alterations in the archaeal and bacterial community composition and (iiii) CO_2-utilizing vent *Bacteria*. The results showed distinct geochemical and microbiological variations along the 25 m transect. In addition, *Chloroflexi* representatives have been detected as major CO_2-utilizing vent *Bacteria*.

Simone Gwosdz was involved into the sampling campaigns, conducted the $^{13}CO_2$ incubation experiments and the molecular biological investigations (qPCR, 16S rDNA clone libraries, pyrosequencing), and evaluated the respective results.

Julia M. West, David Jones, Kay Green, Tom Barlow contributed to the sampling campaigns, conducted field measurements of CO_2 flux and soil concentrations and analysed CO_2-induced changes in the vegetation.

Vasiliki Gemeni, Fotini Ziogou and Nikolaos Koukouzas contributed to the sampling campaigns and conducted field measurements of CO_2 flux and soil concentrations.

Marco Blöthe and Jana Rakoczy contributed to 16S rDNA clone library evaluation and preparation of the manuscript.

Martin Krüger contributed to the planning of the experiments, the data discussion and preparation of the manuscript.

Manuscript 3

Simone Gwosdz and Martin Krüger

Vertical microbial stratification in freshwater lake sediment of the Laacher See caldera

In preparation

Sediment cores of the Laacher See caldera were taken from a reference site (without detectable CO_2 bubbles rising), low CO_2 site (few CO_2 bubbles) and high CO_2 site (high amount of rising CO_2 bubbles). pore water and geochemical analyses of the sediment cores revealed high concentrations of dissoluted CO_2 and CH_4 and affiliated CO_2-induced geochemical alterations. Real time PCR revealed that *Archaea* outnumbered *Bacteria* within all sediment samples. The archaeal communities consisted mostly of *Crenarchaeota*, *Euryarchaeota* and *Pacearchaeota* without significant differences between the sediment cores. Significant differences have been observed for the bacterial community where Thermoprotei, *Pacearchaeota*, *Deltaproteobacteria* and unclassified *Clostridia* contributed most to differences between the reference, low and high CO_2 influenced core.

Simone Gwosdz was involved into the sampling campaign, conducted the molecular biological investigations (qPCR, 16S rDNA clone libraries), and evaluated the respective results.

Martin Krüger contributed to the planning of the experiments, the data discussion and preparation of the manuscript.

4. Manuscripts

Manuscript 1

Long-term CO_2 injection and its impact on near-surface soil microbiology

Please see: https://doi.org/10.1111/1574-6941.12040

Manuscript 2

Euryarchaeota and *Chloroflexi* representatives dominate terrestrial CO_2 vent

Euryarchaeota and *Chloroflexi* representatives dominate terrestrial CO_2 vent

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Running title: Microbial activity and community composition along a 25 m CO_2 gradient

Keywords: CO_2 vent, *Bacteria*, *Archaea*, pyrosequencing, DNA-SIP

Abstract

Impacts of elevated CO_2 on near-surface soil geochemistry, microbial activity, abundance and diversity are almost unknown. In the present study, we investigated geochemical and microbiological variations along a 25 m transect in the Florina Basin (Greece). Furthermore, the active bacterial community composition within the Florina vent was studied using ^{13}C-CO_2 incubation experiments, DNA-SIP and pyrosequencing. Therefore, soil samples were taken in 65-70 cm depth from the CO_2 vent (100% CO_2), medium (10-50% CO_2) and reference site (<6% CO_2) in 2011 and 2012. Along the 25 m transect, soil moisture, TOC content and dissolved metals/trace elements significantly increased with increasing CO_2. Archaeal 16S rDNA clone libraries showed that the community was dominated by methanogenic *Euryarchaeota* (49-58%) within the vent and mesophilic *Thaumarchaeota* (62-88%) within medium and reference site. In contrast, the bacterial 16S rDNA clone libraries showed higher diversity without a distinct predominance of any bacterial taxon. Bacterial pyrosequences revealed $^{13}CO_2$ utilization by all major soil taxa. The *Chloroflexi* representatives *Ktedonobacter* (5%) and *Thermosporothrix* (7-9%) as well as the actinobacterial *Conexibacter* representatives (7%) were most abundant $^{13}CO_2$-utilizer. Our data suggest the metabolic adavantage of *Euryarchaeota*-affiliated acetoclastic methanogens and *Chloroflexi* representatives within soil CO_2 vents.

Introduction

The capability of the atmosphere to absorb ultraviolet solar radiation strongly depends on its well-balanced gas composition especially of the greenhouse gases CO_2, CH_4 and N_2O. Since the industrial era, fossil fuel combustion and land use have led to substantially increased greenhouse gas emissions. As a consequence, the concentration of CO_2 as most important greenhouse gas increased by 40% from 1750 to 2011 (IPCC report; (Solomon *et al.* 2007)). Important natural CO_2 sinks responsible for the reduction of atmospheric CO_2 are oceans, lakes, plants and soils. On average, the terrestrial carbon sink is responsible for removing approximately one third of the CO_2 emitted from fossil fuel combustion from the atmosphere (Canadell *et al.* 2007). Several processes contribute to the terrestrial carbon sink saturation including (i) climate change, (ii) changes in the atmospheric composition (e.g. pollution) and (iii) land-use/ land management (Canadell *et al.* 2007). The consequences of rising atmospheric CO_2 concentrations for soil ecosystems, the soil carbon cycle and their role as carbon sinks must be examined. Soil microbes play an important role within the terrestrial carbon cycle. They contribute to both, the fixation of carbon into organic material and its degradation again (Gougoulias, Clark and Shaw 2014). Studies of natural CO_2 vents are useful to identify CO_2-induced alterations in soil redox-conditions and therefore microbial activity, abundance and community composition. CO_2-induced alterations in pH, soil moisture, soil organic carbon content and metal mobilisation were frequently detected at terrestrial CO_2 vents (Beaubien *et al.* 2008b; Blume and Felix-Henningsen 2009b; Rennert *et al.* 2011; Mehlhorn *et al.* 2014; Rennert and Pfanz 2015). Plant-type specific responses towards high CO_2 concentrations were also investigated revealing alterations in root respiration, plant growth, chlorophyll content, assimilation rates and different adaptation capabilities of C3 and C4 plants to high CO_2 concentrations (Vodnik *et al.* 2006; Pfanz *et al.* 2007). In contrast, little is known about CO_2-induced alterations in microbial activity and community composition. Previous studies detected various alterations in near-surface soil microbial abundance and diversity in response to natural CO_2 concentrations, however, without evidence about active microorganisms, able to utilize CO_2 (Beaubien *et al.* 2008b; Oppermann *et al.* 2010b; Frerichs *et al.* 2013a; Sáenz de Miera *et al.* 2014b; Šibanc *et al.* 2014). The first study that investigated active microorganisms using a ^{13}C-CO_2 DNA-SIP experiment revealed the predominance of *Acidobacteria* and *Methanomicrobiales* in soil samples from a terrestrial CO_2 vent in the Czech Republic (Beulig *et al.* 2015).

In the present study, we aimed to understand the geochemical and microbiological variations along a 25 m transect of a natural CO_2 vent located in the Florina Basin (Greece). For two consecutive years, 2011 and 2012, soil samples were taken from the CO_2 vent, medium and reference site. The soil samples were analysed to detect (i) geochemical alterations, (ii) alterations in the archaeal and bacterial abundance and (iii) alterations in the archaeal and bacterial community composition along the 25 m transect. In addition, a $^{13}CO_2$ incubation experiment with vent samples of 2012 was conducted to describe the active, CO_2-utilizing bacterial community. Our study provides new insights into a nearly unknown mountainous, mediterranean environment with magmatic-hydrothermal CO_2 releases. Furthermore, our

results help to understand the impacts of CO_2 on soil microorganisms and therefore to estimate the impacts of climate change and rising atmospheric CO_2 for soil ecosystems and terrestrial carbon cycle.

Materials and Methods

Site Description

The study site is located in the NNW-SSE oriented Florina-Ptolemais-Aminteo Graben system in Northern Greece. The graben is composed of metamorphic rocks and formed as a result of the Alpine orogenesis. The 800-1000 m overlying deposits consist of conglomerate, marl, Sandstone, loam, peat and limestone with clayey caprocks (Metaxas *et al.* 2007). As a result of the slow upwelling of magmatic, hydrothermal CO_2 along faults and fractures, carbonate-rich springs and CO_2-rich gas vents appear throughout the Florina Basin (Ziogou *et al.* 2013). For a detailed geological description of the Florina basin see also Koukouzas *et al.* (2015). The studied field site contains a number of gas vents of undetermined age and is used as pasture for sheep, goats and horses. The local climate is mountain Mediterranean with mild, wet winters and warm, dry summers (climate data from http://www.florina.climatemps.com).

Sampling procedure

Soil samples were taken along a 25 m transect in 2011 and 2012. At the CO_2 vent, medium and reference site in 25 m distance, soil samples were taken in 65-70 cm depth. The samples were stored at 8°C and transported to the laboratory as fast as possible. In the laboratory, the collected soil samples were homogenized and subsamples were taken for geochemical (stored at 4°C), bio molecular (stored at -20°C) and microbial activity analyses (incubated at 20°C).

Soil Geochemistry

Concentrations of CO_2, CO, H_2S, CH_4 and O_2 were determined using the portable infrared landfill gas analysers GA2000 and GA5000 (Geotech, Warwickshire, UK). Soil moisture was determined after each sampling campaign by drying 1 g of wet soil sample at 60°C to constant weight. In addition, soil carbon content (total carbon; TC) was analysed using a LECO CS 230 analyser (LECO Corporation, St. Joseph, USA). To obtain the total organic carbon (TOC), inorganic carbon was removed by adding a 10% solution of hydrochloric acid to the soil samples and analysed using a LECO CS 230 analyser. Trace elements were analysed by X-ray fluorescence spectrometry as previously described (Krishna and Govil 2008; Shibata *et al.* 2009). All samples were analysed in triplicates.

DNA Extraction

Total DNA was extracted from frozen soil samples (-20°C) in duplicates following the manufacturers manual of the FastDNA Spin Kit for soil (Bio 101; MP Biomedicals) (Webster *et al.* 2003).

Quantitative PCR analyses

Quantitative PCR was performed on a StepOnePlus Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). Each reaction contained 5 µl TaqMan 2x Universal PCR Master Mix (NoAmpEraseUNG, Applied Biosystems), 0.4 µl PCR primers and probe for *Bacteria* (2.5 µM each) and *Archaea* (20 µM primers and 5 µM probe), 2.3 µl sterile water, 0.5 µl BSA (20 mg/ml) and 1 µl

16S rDNA template added to a final volume of 10 µl. Samples were analysed for total numbers of *Bacteria* and *Archaea*. The qPCR assays were calibrated by 10-fold serial dilutions using PCR amplified pure standards. Microbial primers and description of the qPCR specifications can be found in Table 1 (Supporting information). Overall, nine replicates (3 replicates x 3 dilutions; 10-, 100- and 1000-fold) of each sample were analysed. Amplification efficiencies were calculated from the StepOne v2.1 software on the basis of threshold cycle versus gene abundance of standards according to the relationship E = 10(-1/slope). Amplification efficiencies ranged between 80 and 100%. The qPCR amplifications were analysed with the StepOne v2.1 software and the results are expressed in gene copies per gram dry weight.

Clone Library Construction and phylogenetic analyses

PCR amplicons of bacterial and archaeal 16S rDNA genes were generated using 1:10 or 1:100 dilutions of each soil DNA extract. The PCR reactions contained 25 µl DreamTaq PCR Master Mix (2x) (Thermo Scientific), 0.3 µM of each primer, 4 µl template DNA and nuclease-free water up to 50 µl. Triplicate PCRs were performed for each soil DNA extract. Microbial primers and description of the PCR specifications can be found in Table 1 (Supporting information). Purification, cloning and sequencing of the 16S rDNA PCR products was conducted at LGC Genomics GmbH (LGC Genomics GmbH, Berlin, Germany) and Microsynth AG (Microsynth AG, Balgach, Switzerland). Quality control and screen for chimeras has been made using Geneious R7 software and DECIPHER (Wright *et al.* 2012) . Sequences with mismatches or gaps were removed using MOTHUR (Schloss *et al.* 2009). For clustering the sequences into OTUs and additional analysis of community structure, MOTHUR was used (Schloss *et al.* 2009). Representatives of each OTU at the 0.03 distance level were aligned and classified with the ARB-SILVA aligner and assembled in the existing parsimony tree of the SILVA reference database SSURef 118 (Pruesse *et al.* 2007; Pruesse *et al.* 2012).

^{13}C-CO_2 labeling experiment

Microcosm incubations.

^{13}C-CO_2 incubations set up in replications with 2012 soil samples from the CO_2 vent. Therefore, 6 g soil from 65-70 cm depth were added into 56 ml serum bottles. After the headspace was flushed with sterile N_2 (100%) to remove O_2, the headspace was adjusted to 70% ^{13}C-CO_2/^{12}C-CO_2 ($^{13}CO_2$ treatment with 499 atomic percent ^{13}C; Sigma-Aldrich, St Louis, MO, USA) and 30% N_2. Microcosms were incubated at 20°C. After 50 days, the microcosms were sampled for stable carbon isotopic analysis of d^{13}C-TC and DNA-SIP.

Bacterial nucleic acid extraction and ultracentrifugation.

Total 16S rDNA after 50 days of incubation was extracted of each microcosm (^{12}C-CO_2/^{13}C-CO_2) following the manufacturers manual of the FastDNA Spin Kit for soil (Bio 101; MP Biomedicals) (Webster *et al.* 2003). DNA extracts were separated using CsCl density gradient centrifugation as previously described (Neufeld *et al.* 2007; Lueders 2010) with a VTi 65.2 rotor in a Optima L-90K ultracentrifuge (both Beckman Coulter, Krefeld, Germany). After centrifugation, 12 fractions were

collected. The fraction densities were determined using the AR200 digital refractometer (Reichert Technologies, Depew, NY, USA). The DNA was purified by PEG precipitation as described by Neufeld *et al.* (2007) and resuspended in 20 µl of TE for storage at -20°C. The bacterial abundance within the single fractions were determined using qPCR (Supplementary Figure 7).

PyroTag library preparations from SIP microcosms.

Extracted DNA from vent samples before and after 50 days of $^{13}CO_2$ incubation were analysed by amplicon pyrosequencing of bacterial 16S rRNA genes using a 454 GS FLX Titanium system (Roche, Penzberg, Germany). Barcoded amplicons for multiplexing were prepared using the primers Ba27f and Ba519r extended with the respective A or B adapters, key sequence and multiplex identifiers (MID) as recommended by Roche. The pyrotag PCR was performed in a Mastercycler ep gradient (Eppendorf, Hamburg, Germany). A description of the PCR specifications can be found in Table 1 (Supporting information). A detailed description of the pyrotag PCR cycling conditions and amplicon purification is given in Pilloni *et al.* (2011, 2012).

PyroTag data processing.

Quality filtering of the pyrosequencing reads was performed using the automatic amplicon pipeline of the GS Run Processor (Roche) with a modification of the valley filter (vfScanAll- Flows false instead of TiOnly) to extract sequences (Pilloni *et al.* 2011). PyroTag reads were quality trimmed using the TRIM function of GREENGENES with the standard settings: good-quality score 20, window size 40 bp and window threshold 90% (DeSantis *et al.* 2006). Subsequently, pyrotag reads were batched per sample based on MID-identifiers with BIOEDIT (Hall 1999) and reads with inferior read length (o250 bp) were excluded from further analysis (Pilloni *et al.* 2011). Afterwards, combined f- and r-pyrotag reads each sample were classified using the RDP classifier at a confidence threshold of 80% (Wang *et al.* 2007). Then, f- and r-pyrotag reads were assembled into contigs with the SEQMAN II software (DNAStar) using assembly thresholds of at least 98% sequence similarity over a 50-bp match window. Contigs without at least one forward and one reverse read were not considered for further analysis (Pilloni *et al.* 2011).

Statistical analyses

Analysis of variance (two-way ANOVA) was used for the detection of possible CO_2 impacts over time on soil properties and microbial abundance (qPCR). Analysis of similarity (ANOSIM) was performed to detect differences of the bacterial and archaeal communities along the 25 m transect as well as between vent samples before and after $^{13}CO_2$ incubation. Similarity percentage (SIMPER) analysis was performed to identify the taxa that contributed most to the variance along the 25 m transect and between vent samples before and after $^{13}CO_2$ incubation. Analyses of similarity and variance base on the Bray–Curtis dissimilarity index. ANOVA and multivariate statistics were performed in PAST (Hammer, Harper and Ryan 2001). For the clone libraries and 454 PyroTag libraries, the calculation of diversity (Shannon) and richness (Chao) indices was generated using MOTHUR (Schloss et al. 2009).

Comparisons of the Shannon diversities using t-tests have been conducted in PAST (Poole 1974; Hammer, Harper and Ryan 2001).

Results

Soil gas

Distinct differences in CO_2 concentrations along the 25 m transect were detected. CO_2 concentrations were the highest at the vent and decreased towards the medium and reference site. CO_2 concentrations of 5, 10 and 100 vol. % and 5, 50 and 100 vol. % were detected in 2011 and 2012, respectively (Figure 1b). CO_2 vent soil gas samples of both years 2011 and 2012 consisted of 81 ± 4 vol. % CO_2, 15 ± 3 vol. % N_2, 4 ± 1 vol. % O_2 and traces of CH_4 (0.2 vol. %) (Figure 1a). CO_2 and CH_4 gas sample analyses revealed $\delta^{13}C$ values of 0.1‰ for CO_2 and -22.4 ± 0.1‰ for CH_4 (not presented).

Soil geochemistry

Along the 25 m transect, significant alterations in soil geochemistry were detected. Soil moisture decreased from the CO_2 vent (18%, 23%) to the reference site (1% each) 2011 and 2012, respectively (Supplementary Figure 1). Significant differences in soil moisture content were detected between samples from 2011 and 2012 (two-way ANOVA, $P < 0.05$; Supplementary Table 3). A significant decrease of total and organic carbon (TC/TOC) as well as Co, Cr, Ni along the 25 m transect of both years were detected (two-way ANOVA, $P < 0.05$; Supplementary Figure 1 and Table 3). The summary of geochemical results along the 25 m transect can be found in Table 2 (Supporting Information).

Microbial abundance

Analyses of the microbial abundance along the 25 m transect showed a significant increase of bacterial 16S rDNA gene copy numbers from the CO_2 vent to the medium and reference site in 2011 and 2012 (two-way ANOVA, $P < 0.05$; Supplementary Table 3). Bacterial 16S rDNA gene copy numbers increased from $2x10^8$ to $5x10^9$ gene copy numbers gdw^{-1} (Supplementary Figure 2). In contrast, no significant alterations of archaeal 16S rDNA gene copy numbers were detected along the 25 m transect for both years (two-way ANOVA, $P > 0.05$; Supplementary Table 3). The archaeal 16S rDNA gene copy numbers ranged from $5x10^8$ to $6x10^{10}$ gene copy numbers gdw^{-1} (Supplementary Figure 2).

Archaeal clone libraries

No significant differences of the archaeal community composition between the CO_2 vent, medium and reference site were detected (ANOSIM: $F = 0.89$; $P = 0.07$). In addition, no significant differences of the archaeal community composition between 2011 and 2012 were detected (ANOSIM: $F = -0.24$; $P = 0.8$) (Figure 2). The majority of the archaeal sequences along the 25 m transect for the years 2011 and 2012 were assigned to three dominant phyla: *Thaumarchaeota* (0-88%), *Euryarchaeota* (3-58%) and *Crenarchaeota* (0-15%) (Figure 2). They represent 60-93% of the sequences within the archaeal 16S rDNA clone libraries. Alongside, 6-40% of the archaeal sequences could not be classified (Figure 2). In 2011 and 2012, the CO_2 vent was dominated by *Euryarchaeota* (49-58%) followed by *Crenarchaeota* (11-15%). *Thaumarchaeota* could only be detected in samples of 2012 (6%) (Figure 2). In contrast, medium and reference site were dominated by *Thaumarchaeota* (62-88%) followed by *Euryarchaeota*

(3-26%). *Crenarchaeota* were not detected (Figure 2). SIMPER analysis at phylum level indicated, that thaumarchaeotal sequences contributed most of the differences in the relative abundance between CO_2 vent, medium and reference site (48%) (Supplementary Table 4). Analyses at species level confirm these results. Therefore, *Nitrososphaera viennensis* affiliated sequences (*Thaumarchaeota*) contributed most of the differences (9%). They are followed by *Euryarchaeota* with 29% contribution to differences between the CO_2 vent, medium and reference site abundance (Supplementary Table 4). Euryarchaeotal representatives which contributed most were *Methanosarcina sp.* (7%) and *Methanomassiliicoccus luminyensis* (5%) (Supplementary Table 4). A summary of the archaeal clone library parameters and statistical estimation of the diversity (Shannon diversity index) and richness (Chao index) along the 25 m transect can be found in Table 5 (Supporting information).

Bacterial clone libraries

Similar to the archaeal clone libraries, no significant differences of the bacterial community along the 25 m transect in 2011 and 2012 were detected (one-way ANOSIM, $P > 0.05$; Figure 3). The majority of the bacterial sequences along the 25 m transect for the years 2011 and 2012 were assigned into five phyla: *Proteobacteria* (10-44%), *Actinobacteria* (2-40%), *Acidobacteria* (3-16%), *Firmicutes* (6-17%) and *Verrucomicrobia* (0-20%) (Figure 3). They represent 41-88% of the sequences within the bacterial 16S rRNA clone libraries. Lower abundances (2-12%) have been detected for *Bacteroidetes*, *Chloroflexi*, *Gemmatimonadetes*, *Caldiserica*, *Candidatus Saccharibacteria*, *Armatimonadetes*, *Planctomycetes* and candidate division WPS-2 (Figure 3). Unclassified *Bacteria* represented 7-50% (Figure 3). Along the 25 m transect, decreasing representatives of the phyla *Actinobacteria*, *Acidobacteria*, *Firmicutes* and *Verrucomicrobia* were detected with increasing CO_2 concentration (Figure 3). SIMPER analysis at phylum level indicated, that the number of unclassified bacterial sequences contributed most of the differences on phylum level in the relative abundance between CO_2 vent, medium and reference site (23%) (Supplementary Table 4). They are followed by the phyla Proteobacteria (21%), Actinobacteria (17%), Verrucomicrobia (9%), Acidobacteria (9%) and Firmicutes (7.5%) which contributed most to differences (Supplementary Table 4). At species level, *Bacterium SOSP1-9*-affiliated sequences *(Chloroflexi)* contributed most of the differences (4%). They are followed by *Bacillus longiquaesitum (Firmicutes)* (3%), Bacterium Ellin507 (*Verrucomicrobia*) (3%), Bacterium SOSP1-30 (*Chloroflexi*) (3%), Bacterium SOSP1-63 (*Chloroflexi*) (2%) and Bacterium Ellin5025 (*Actinobacteria*) (2%) (Supplementary Table 4). A summary of the bacterial clone library parameters and statistical estimation of the diversity (Shannon diversity index) and richness (Chao index) along the 25 m transect can be found in Table 5 (Supporting information).

Bacterial PyroTag libraries from $^{13}CO_2$ labelling experiment

No significant differences between vent samples before and after $^{13}CO_2$ incubation (t50) were detected (ANOSIM: $R = -0.25$; $P = 0.7$) (Figure 4). The majority of the $^{13}CO_2$ labelled *Bacteria* were assigned into four phyla: *Chloroflexi* (31-37%), *Proteobacteria* (17-20%), *Acidobacteria* (13-18%) and *Actinobacteria* (11-15%). Lower abundances have been detected for *Firmicutes* (5-7%), Bacteroidetes

(4%), *Candidatus Saccharibacteria* (2-3%), *Parcubacteria* (1-3%), *Armatimonadetes* (1%), *Verrucomicrobia* (1%), *Caldiserica* (0-1%), *Gemmatimonadetes* (0-1%), *Nitrospirae* (0-1%) and *Candidate Division WPS-1* (0-1%) (Figure 4). The phylum *Chloroflexi* mainly consisted of *Thermosporothrix* and *Ktedonobacter* representatives (Supplementary Figure 3). The phylum *Proteobacteria* was highly diverse with predominating *Methylocapsa* representatives (Supplementary Figure 4). Within the phylum *Acidobacteria*, representatives of the subgroup 1 dominated the samples (Supplementary Figure 5). Similar to the Proteobacteria, Actinobacteria also showed high diversity with however, the predominating genus *Conexibacter* (Supplementary Figure 6). *Conexibacter*-affiliated sequences showed at the same time the strongest growth during the 50 days of $^{13}CO_2$ incubation (Supplementary Figure 6). SIMPER analysis at phylum, order and genus level indicated, that *Chloroflexi* representatives contributed most of the differences between the vent samples before and after 50 days of $^{13}CO_2$ incubation (Supplementary Table 6). Therefore, at phylum level, *Chloroflexi*-affiliated sequences contributed most of the differences between the vent samples before and after 50 days of $^{13}CO_2$ incubation (21%). They are followed by *Actinobacteria* (18%) and *Acidobacteria* (17%) (Supplementary Table 6). At order level, *Ktedonobacterales* (13%) and *Solirubrobacterales* (10%) contributes most to differences and at genus level *Conexibacter* (7%) and *Thermosporothrix* (7%).

Discussion

Aim of the study was to detect CO_2 impacts on soil geochemical and microbiological interactions along a 25 m transect in 2011 and 2012 with focus on the detection of CO_2 resistant/adapted microorganisms. Therefore, archaeal and bacterial clone libraries were generated. In addition, *Bacteria* actively consuming geogenic CO_2 were investigated and identified using microcosm incubations with ^{13}C-labeled CO_2 and 454 pyrosequencing (PyroTag). The results of this study showed distinct ecological alterations along the CO_2 gradient.

Gas geochemistry and CO_2 concentrations along the transect

Along the 25 m transect, decreasing CO_2 concentrations from the CO_2 vent towards the medium and reference site were detected (Figure 1b). Between the two sampling years, little variations were observed with five times higher CO_2 concentrations at the medium site in 2012 (Figure 1b).

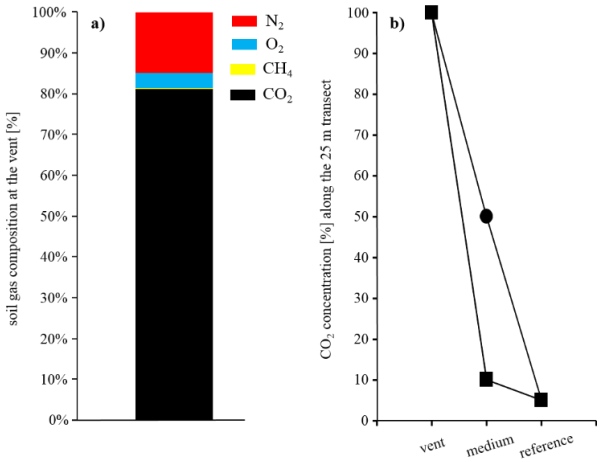

Figure 1: a) Florina vent gas composition; b) CO_2 concentration along the transect

These variations could be explained by a long dry period before the sampling campaign in 2011 in contrast to 2012 with heavy rainfall which flooded the whole area for weeks. However, similar CO_2 concentrations and seasonal effects at natural CO_2 vents were previously described with CO_2 concentrations up to >95% within the vent and <20% at the reference site (Maček *et al.* 2005; Beaubien *et al.* 2008b; Krüger *et al.* 2011). At Florina, gas sample analyses from 65 cm depth within the CO_2 vent confirm previous findings with a mixture of CO_2 (81%) followed by N_2, O_2 and traces of CH_4 (Figure 1a). In contrast to other natural CO_2 vents (Beaubien *et al.* 2008b; Blume and Felix-Henningsen 2009b; Beulig *et al.* 2015), emerging H_2S, H_2, CO or noble gases with potential toxic effects could not be detected. The composition of rising geogenic gases strongly varies from site to site (Beaubien *et al.* 2008b; Blume and Felix-Henningsen 2009b; Padrón *et al.* 2013; Beulig *et al.* 2015). At most sites, CO_2 is the main component because it is the most abundant gas species dissolved in magma. Analyses of the carbon isotopic signatures of CO_2-gas samples and soil-C confirmed the geological origin of the soil carbon within the vent (Supplementary Table 2). Similar results were detected in previous studies at natural CO_2 vents with ^{13}C-CO_2 values between 3.3‰ and -9‰ (Oppermann *et al.* 2010b; Padrón *et al.* 2013; Beulig *et al.* 2015, 2015) and soil ^{13}C values of -21.1‰ and -24‰ (Oppermann *et al.* 2010b; Frerichs *et al.* 2013a).

Effects of natural CO_2 emission on soil geochemistry

At the Florina 25 m transect, increasing soil moisture concentrations with increasing CO_2 (up to 19.5-fold) were detected (Supplementary Table 2 and Figure 1). This may be explained by the reduced plant coverage with increasing CO_2 concentration along the Florina CO_2 gradient. How CO_2 may impact the vegetation cover at natural CO_2 vents was investigated in several studies in the past with different conclusions including plant stimulation, inhibition and adaption to high CO_2 concentrations (Hättenschwiler *et al.* 1997; Maček *et al.* 2005; Vodnik *et al.* 2006; Wei, Maroto-Valer and Steven 2011; Rennert and Pfanz 2015). The CO_2-induced increase of soil moisture was previously related to e.g. an increased water use efficiency of some plants in consequence of CO_2-stimulated plant growth and increasing root biomass (Moore and Field 2006). However, the complete killing of plants within the CO_2 vent at Florina and other described terrestrial CO_2 vents (Beaubien *et al.* 2008b; Pettinelli *et al.* 2008) suggest a lack of evapotranspiration in consequence of plant death. With increasing O_2 concentrations from the medium to the reference site at Florina, the gradually less-impacted vegetation (not presented in the paper) led to decreasing soil moisture concentrations (Supplementary Table 2 and Figure 1). Analysis of variance (two-way ANOVA) has also revealed the impact of seasonal variations like rainfall or dry periods over time ($P < 0.05$; Supplementary Table 3). The high soil moisture concentration within the CO_2 vent could possibly lead to an increasing CO_2 dissolution rate and therefore to soil acidification as previously described (Altevogt and Jaffe 2005b; Harvey *et al.* 2012b). However, because of missing pH measurements along the 25 m transect, soil acidification remains speculation. Nevertheless, a CO_2-induced significant increase of metals/trace elements and TC/TOC values (up to 16 times within the vent) with increasing CO_2 concentration confirm previous findings (two-way

ANOVA, $P < 0.05$; Supplementary Table 3) (Beaubien *et al.* 2008b; Blume and Felix-Henningsen 2009b; Mehlhorn *et al.* 2014). Wei *et al.*, (2011) for example, detected an increase of several metals by up to 500% in agricultural soil due to the CO_2-induced weathering of soils during a three days CO_2 incubation time. The increasing TC/TOC values with increasing CO_2 concentration suggest restricted microbial organic matter decomposition with increasing CO_2 concentrations (Supplementary Figure 1) (Beaubien *et al.* 2008b; Rennert *et al.* 2011; Beulig *et al.* 2015; Nowak *et al.* 2015; Rennert and Pfanz 2015)

Effects of natural CO_2 emission on soil microbial abundance

Soils play an important role within the biochemical carbon cycle because they act as both carbon source and carbon sink (Shively *et al.* 2001). For the investigation of CO_2-induced alterations in the microbial abundance along the 25 m transect, archaeal and bacterial 16S rDNA gene copy numbers were determined. The results showed no significant CO_2-induced or time-dependent alterations of the archaeal abundance (10^8 to 10^{10} gene copy numbers gdw^{-1}) (Supplementary Table 3 and Figure 2b). In contrast, bacterial 16S rDNA gene copy numbers significantly increased with decreasing CO_2 concentration were detected (10^8 to $5x10^9$ gene copy numbers gdw^{-1}) (Supplementary Table 3 and Figure 2a). Similar bacterial and archaeal copy numbers were detected at several other natural CO_2 vents with 10^8 to 10^{10} bacterial and 10^6 to 10^9 archaeal 16S rDNA gene copy numbers gdw^{-1} (Beaubien *et al.* 2008b; Frerichs *et al.* 2013a; Beulig *et al.* 2015; Nowak *et al.* 2015). Similar to our study, Oppermann *et al.* (2010) detected an overall decrease of microbial gene copy numbers by up to two orders of magnitude with increasing CO_2. In addition, (Beaubien *et al.* 2008b) detected along a 50 m transect in Latera (Italy) decreasing microbial gene copy numbers with increasing CO_2 (2.5 to $7.5x10^9$ gene copy numbers gdw^{-1}). Possible CO_2 impacts on soil microbial abundance remain uncertain and seem to vary due to different microbial community compositions and climate conditions. Most previous results from natural CO_2 vents suggest a CO_2-induced decrease in microbial abundance (Fernández-Montiel *et al.* 2016; Fernández-Montiel, Pedescoll and Bécares 2016b).

Effects of natural CO_2 emission on soil *archaeal* diversity

Differences in the archaeal clone library community composition along the 25 m CO_2 transect were detected. Within the CO_2 vent, the predominating *Euryarchaeota* phylum was mainly represented by *Methanomicrobia* affiliated sequences, suggesting this class to be specific and adapted to high CO_2 concentrations. In addition, the *methanomicrobial* representatives *Methanosarcina sp.* and *Methanomassiliicoccus luminyensis* (unclassified *Methanomicrobia*) contributed most to the statistical differences between CO_2 vent, medium and reference site (Supplementary Table 4). Representatives of the genus *Methanosarcina* have a broad substrate spectrum including H_2, CO_2, acetate and methylated C-1-compounds (Thauer *et al.* 2008). These acetoclastic methanogens are predominant in many natural environments because acetate is the quantitatively more available substrate (Liu and Whitman 2008; Beckmann *et al.* 2011; Angel, Claus and Conrad 2012). Previous results of CO_2 vent sample analyses from the Czech Republic (seismic center of the Cheb Basin) showed similar results with the

predominating archaeal class *Methanomicrobiales* (50–90% of total archaeal sequence reads) (Beulig *et al.* 2015).

Besides the phylum *Euryarchaeota*, *Crenarchaeota* sequences were also detected within vent samples and were almost exclusively found in the CO_2 vent (Figure 2).

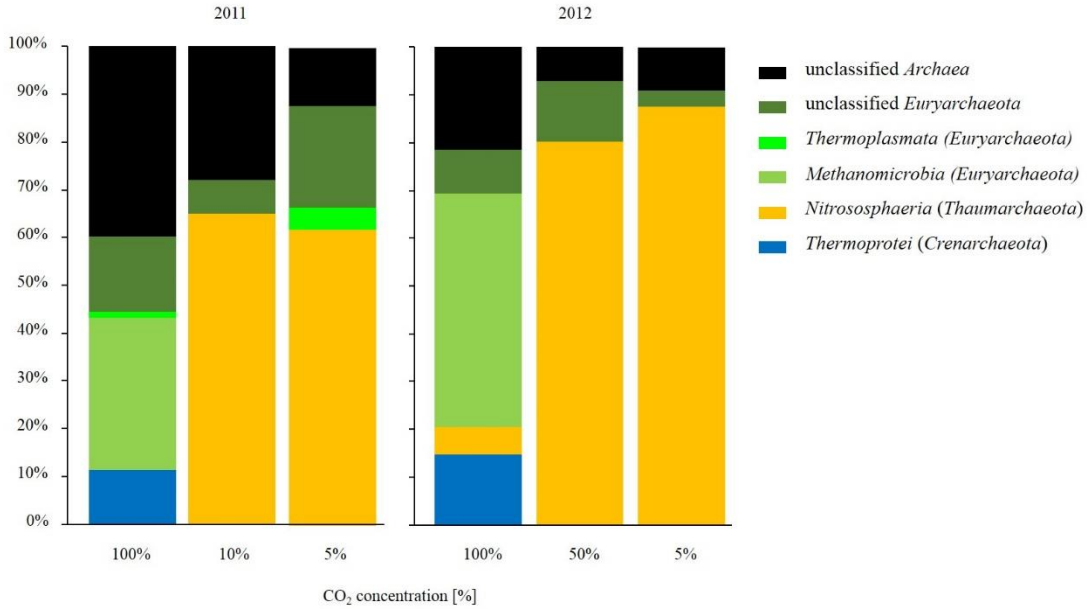

Figure 2: 16S rDNA clone libraries. Archaeal community composition along the 25 m transect for 2011 and 2012.

The ability of autotrophic CO_2 fixation (Berg *et al.* 2010; Berg 2011b; Pratscher, Dumont and Conrad 2011b) might explain the detection of unclassified crenarchaeotic *Thermoprotei* exclusively within the vent samples (Figure 2). At the Florina medium and reference site, the predominating *Thaumarchaeota* phylum was exclusively represented by the family *Nitrososphaeraceae*, especially *Nitrososphaera viennensis* affiliated sequences (Figure 2). High abundances of *Thaumarchaeota* in medium and reference soils of Florina confirms previous findings from natural CO_2 vents where representatives of the *Thaumarchaeota* were exclusively found in the reference soil (5-10%) (Beulig *et al.* 2015). *Nitrososphaera viennensis* is affiliated with group I.1b of the *Thaumarchaeota* ('soil group') and was first described by Tourna *et al.*, (2011) as an organism which oxidizes ammonia to nitrite with oxygen as electron acceptor. Stieglmeier *et al.*, (2014) showed that *N. viennensis* is a mesophilic and mixotrophic organism that requires organic acids (e.g. pyruvate, oxaloacetate, α-ketoglutarate or glyoxylate) to stimulate growth. As known so far, all characterized *Thaumarchaeota* are fixing CO_2 via the hydroxypropionate/hydroxybutyrate cycle (Stahl and de la Torre 2012; Könneke *et al.* 2014). Previous studies revealed, that the thaumarchaeotal I.1b group dominate archaeal communities in most soils (Ochsenreiter *et al.* 2003; Leininger *et al.* 2006; Bates *et al.* 2011). The Florina results for the medium and reference site are in accordance with these findings. The soils with CO_2 concentrations between 5-50% were dominated by *N. viennensis*-affiliated sequences. Their low abundance (2012) or absence (2011) within the vent samples could be explained by the CO_2 concentration of 100%, too high

for the aerobic AOA *Nitrososphaera viennensis*. However, further investigations are needed to clarify the observed variations and possible cross-effects of e.g. changing weather conditions and temporarily livestock farming on the site during the year.

Effects of natural CO_2 emission on soil *bacterial* diversity

In contrast to archaeal clone libraries, the bacterial 16S rDNA clone libraries along the 25 m transect showed higher diversity, but no significant differences between the CO_2 vent, medium and reference site (Figure 3). The major phyla including *Proteobacteria*, *Actinobacteria*, *Acidobacteria*, *Firmicutes* and *Verrucomicrobia* were detected across the 25 m transect in both years, 2011 and 2012 (Figure 3).

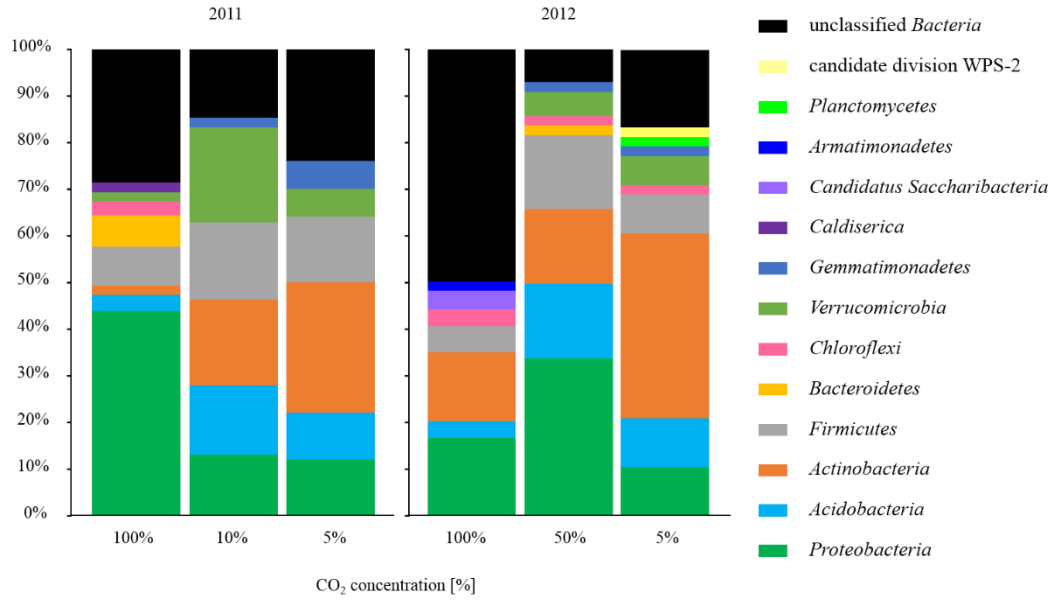

Figure 3: 16S rDNA clone libraries. Bacterial community composition along the 25 m transect.

This is not surprising, as other studies have shown that soils from different habitats roughly consisted of the same major bacterial taxa independent of the local environmental conditions (Janssen 2006; Lauber *et al.* 2009). Along the 25 m transect, no bacterial taxa increased. In contrast, the major bacterial taxa *Actinobacteria*, *Acidobacteria*, *Firmicutes* and *Verrucomicrobia* quantitatively decreased between 3-14% with increasing CO_2 concentration at Florina (Figure 3). Similar results were detected by de Miera *et al.* (2014) with an overall decrease for most major bacterial phyla with increasing CO_2 concentration along a natural CO_2 vent in Spain. They have analysed soil samples along a CO_2 gradient using high-throughput sequencing and related most abundant OTUs within vent samples to *Chloroflexi* representatives: *Ktedonobacter* and *Thermogemmatispora*. By contrast, most abundant OTUs within the medium and reference samples were *Bradyrhizobium* (α-*Proteobact.*; *Rhizobiales*), *Ktedonobacter*, *Acidobacteria* Gp 4 and *Spartobacteria* (*Verrucomicrobia*) (de Miera *et al.* 2014). Interestingly, SIMPER analyses of the Florina bacterial 16S rDNA clone libraries showed, that at species level *Chloroflexi* representatives (unclassified *Ktedonobacteria*) contributed most to differences between CO_2 vent, medium and reference site (Supplementary Table 4). Therefore, the *Chloroflexi* phylum in general and especially *Ktedonobacteria*, potentially play an important role in soils exposed to high CO_2

concentrations. Similar results were obtained within the bacterial PyroTag libraries with the predominating *Chloroflexi* representatives: *Thermosporothrix* and *Ktedonobacter* (Supplementary Table 6). A detailed discussion of the phylum *Chloroflexi* follows in the next chapter. However, due to the high amount of unclassified bacterial sequences, only a part of the bacterial community could be detected which make interpretations difficult. SIMPER analyses confirm the importance of the unclassified bacterial sequences within the clone libraries which contributed most to differences between CO_2 vent, medium and reference site (Supplementary Table 6). Presumably, the unclassified bacterial sequences also explain the differences between the bacterial 16S rDNA clone libraries and the bacterial PyroTag libraries before and after 50 days of $^{13}CO_2$ incubation (Figure 2, 3 and 4). Especially representatives of the *Chloroflexi* phylum are highly underrepresented within the bacterial 16S rDNA clone libraries presumably because of the used primer sets which might not covered the *Chloroflexi* phylum entirely. Recent reports have shown that universal primer sets do not amplify all targets why their universality has to be regarded as doubtful (Baker, Smith and Cowan 2003; Morales and Holben 2009).

^{13}C-CO_2-utilizing *Bacteria*

For the identification of active CO_2-utilizing *Bacteria*, microcosm experiments with ^{13}C-CO_2 were conducted with subsequent analyses using stable isotope probing (DNA-SIP) and pyrosequencing. No significant differences between the vent samples before and after 50 days of incubation with $^{13}CO_2$ could be detected (Table 4). Most abundant, active *Bacteria* were representatives of the phyla *Chloroflexi*, *Proteobacteria*, *Actinobacteria* and *Acidobacteria* (Figure 4).

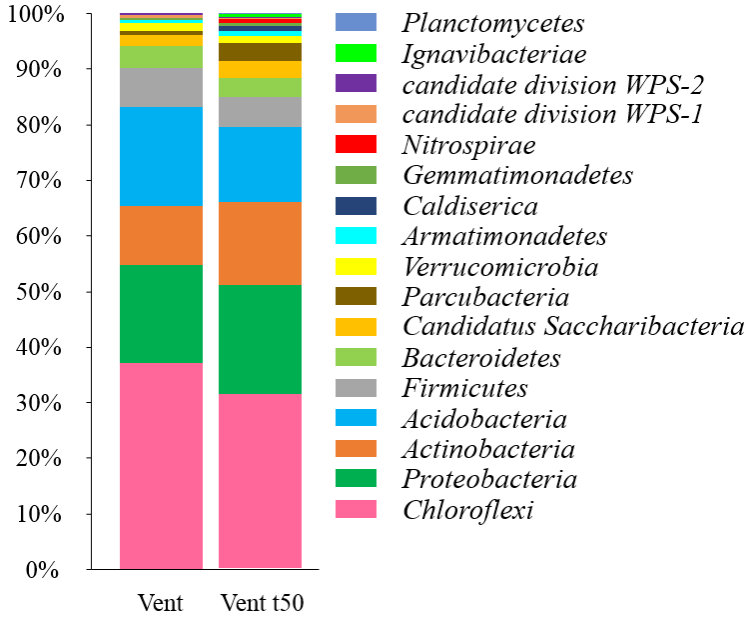

Figure 4: 16S rDNA pyrosequencing clone libraries. Bacterial community composition before and after 50 days of incubation with $^{13}CO_2$.

Thereby, *Proteobacteria* and *Actinobacteria* showed the highest diversity. SIMPER analyses on phylum level revealed that *Chloroflexi* (*Ktedonobacteria)*, *Actinobacteria* (*Conexibacter*) and *Acidobacteria*

(Subgroup 1) contributed most to differences between the vent samples before and after 50 days of $^{13}CO_2$ incubation. On deeper taxonomic levels, *Chloroflexi*- and *Actinobacteria*-affiliated sequences contributed most to differences (Supplementary Table 6). The majority of the *Chloroflexi*-affiliated sequences were assigned to the genera *Thermosporothrix* and *Ktedonobacter* of the class *Ktedonobacteria*. *Ktedonobacteria* have a complex morphology and differentiation. All are Gram-positive, aerobic, form branched mycelia with spores and all type strains sporulate via the formation of multiple exospores (Yabe *et al.* 2017). Previous studies determined sequences assigned to *Ktedonobacteria* in various environments including extreme environments like volcano deposits, volcanic caves or CO_2 vents (Weber and King 2010; Northup *et al.* 2011; de Miera *et al.* 2014; Tebo *et al.* 2015). These studies did not focus on *Ktedonobacteria* and therefore, their diversity, abundance, metabolic capabilities and ecological significance are still poorly understood. For example, Beulig *et al.* (2015) recently demonstrated during a ^{13}C-CO_2 DNA-SIP experiment with soil samples from a Czech mofette, increasing *Chloroflexi* representatives (up to 9%) with incubation time. They postulated a role of *Chloroflexi* for acetogenesis in mofette soil. However, the metabolic potential of *Chloroflexi* representatives to utilize CO_2 was recently confirmed and may explain their abundance and potential importance within CO_2 vent samples (Chan *et al.* 2013; Hug *et al.* 2013; Wasmund *et al.* 2014a). A high diversity was observed for the abundant *Actinobacteria*. The most abundant, active *Actinobacteria* were representatives of the genus *Conexibacter* with increasing abundance during incubation time (Supplementary Figure 6). The *Conexibacter* genus includes strictly aerobic and chemoorganotrophic *Bacteria* (Albuquerque and da Costa 2014). Their co-occurrence with N_2-fixing *Bacteria* like *Mesorhizobium* (*Rhizobiales*) during artificial CO_2 enrichment experiments was recently published (Tu *et al.* 2015). Recent studies also detected their widespread occurrence in soils of different pH and land use (including pasture) (Acosta-Martínez *et al.* 2008; Lauber *et al.* 2009; Rousk *et al.* 2010). In addition, Hunger *et al.* (2011) detected increasing abundances of *Conexibacter*-affiliated 16S rRNA gene sequences during $^{13}CO_2$ incubation experiments with fen soil which indicated that these phylotypes assimilated CO_2. The results from Florina also suggest that *Conexibacter* might potentially benefit directly or indirectly from elevated CO_2. However, *Actinobacteria* in general constitute one of the largest bacterial phyla, and they are ubiquitously distributed in both aquatic and terrestrial ecosystems. Furthermore, they are able to use a wide variety of nutritional sources, including various complex polysaccharides (Barka *et al.* 2016). Therefore, further investigations are needed to clarify their metabolic capabilities and potential role in soils.

Conclusions

Along the investigated 25 m transect, differences between the CO_2 vent, medium and reference site were detected. Increasing concentrations of metals as well as soil moisture and TC/TOC content with rising soil CO_2 concentrations were detected. Microbial community analyses revealed the predominance of acetoclastic methanogens and representatives of nearly all major soil bacterial phyla as $^{13}CO_2$ utilizers. Especially the potential importance of the *Chloroflexi* representatives *Thermosporothrix* and

Ktedonobacter with increasing CO_2 concentration could be demonstrated. In addition, the actinobacterial genus *Conexibacter* was identified as major actinobacterial representative and $^{13}CO_2$ utilizer during the incubation experiment. The results presented in our study were the first from a mountainous, mediterranean environment with magmatic-hydrothermal CO_2 releases. Our results help to broaden the knowledge about natural soil CO_2 vents and suggest that the bacterial phylum *Chloroflexi* may play an important role in CO_2 enriched environments. However, further investigations are needed to identify potential climate and seasonal variations and to fill the gap of information about the physiological potential of most of the described taxa. Furthermore, comprehensive analyses are needed to clarify the potential importance of the classes *Methanomicrobia* and *Ktedonobacteria* within the terrestrial carbon cycle under high CO_2 concentrations.

Acknowledgments

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Supplementary

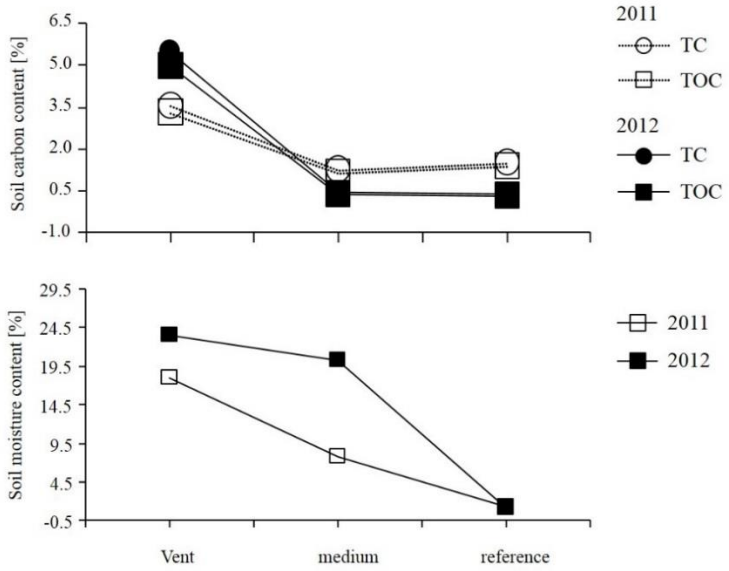
S-Table 1: Primer sets used for the qPCR, clone and PyroTag library assays along with their annealing temperature and references.

PCR assay	Primer	Sequence (5' - 3')	Annealing temp. [°C]	Reference
qPCR				
Bacteria	Bac 331F	5'-TCCTACGGGAGGCAGCAGT-3'	60	Nadkarni et al. (2002)
	Bac 797R	5'-GGACTACCAGGGTATCTAATCCTGTT-3'		
	Bac	5'-CGTATTACCGCGGCTGCTGGCAC-3'		
Archaea	Arch 349F	5'-GYGCASCAGKCGMGAAW-3'	59	Takai et al. (2000)
	Arch 806R	5'-GGACTACVSGGGTATCTAAT-3'		
	Arch 516	5'-TGYCAGCCGCCGCGGTAAHACCVGC-3'		
Clone libraries				
Bacteria	Bac 27F	5'- AGR GTT YGA TYM TGG CTC AG -3'	52	Lane et al. (1991)
	Bac 1392R	5'- ACG GGC GGT GTG TRC -3'		
Archaea	Arch 109F	5'- ACK GCT CAG TAA CAC GT -3'	52	Whitehead and Cotta (1999)
	Arch 915R	5'- GTG CTC CCC CGC CAA TTC CT -3'		Stahl et al. (1991)
PyroTag library				
Bacteria	Bac 27F	5'-AGA GTT TGA TCM TGG CTC AG -3'	52	Pilloni et al. (2011 and 2012)
	Bac 519R	5'- TAT TAC CGC GGC KGC TG -3'		

S-Table 2: Geochemical parameters along the 25 m transect for 2011 and 2012.

Sample	Moisture	CO_2	TC	TOC	d ^{13}C	Fe_2O_3	MnO	SiO_2	P_2O_5	K_2O	CaO	As	Ba	Co	Cr	Cu	Ni	Pb	Zn
	[%]	[%]	[%]	[%]	[‰]	[%]	[%]	[%]	[%]	[%]	[%]	[mg/Kg]	[mg/Kg]	[mg/Kg]	[mg/Kg]	[mg/Kg]	[mg/Kg]	[mg/Kg]	[mg/Kg]
2011																			
Vent	17.9	100	3.5	3.3	-23.6	5.7	0.1	55.7	0.2	2.0	3.2	8	674	13	94	28	43	19	65
Medium site (10.25 m)	7.8	10	1.2	1.1	-26.7	4.0	0.1	65.3	0.2	3.1	2.6	6	1131	11	64	19	24	15	46
Reference	1.3	5	1.5	1.4	-26.9	3.1	0.0	83.4	0.2	1.5	0.3	17	318	6	41	22	13	42	68
2012																			
Vent	23.4	100	5.4	5.0	-23.6	3.6	0.1	59.0	0.2	2.8	2.6	4	834	11	75	38	38	24	95
Medium site (10.25 m)	20.3	50	0.5	0.4	-26.7	4.9	0.1	64.4	0.2	2.8	3.2	3	927	9	75	38	31	14	54
Reference	1.2	5	0.4	0.3	-26.9	3.1	0.1	70.3	0.1	3.9	2.1	2	1438	7	42	23	17	12	34

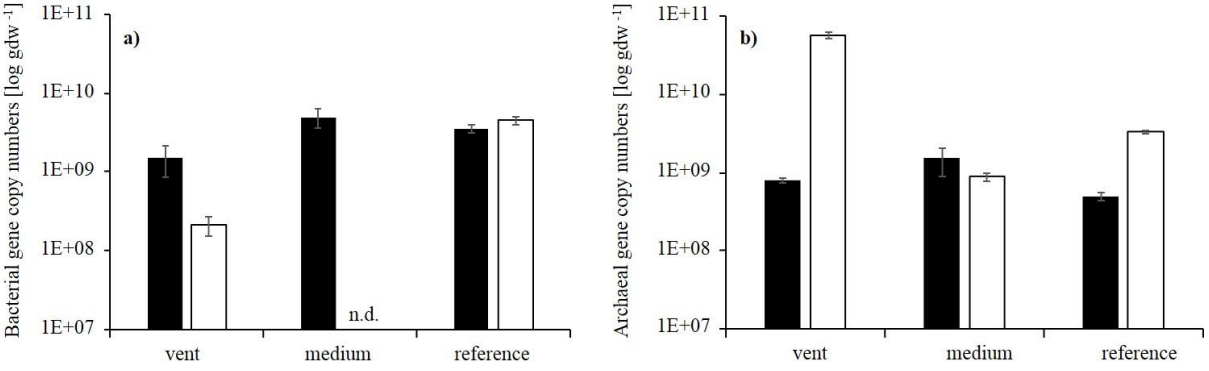
S-Figure 1: Soil carbon and moisture content along the 25 m transect for both years, 2011 and 2012.



S-Table 3: Analysis of variance (two-way ANOVA) for the detection of possible CO_2 impacts over time on soil properties and microbial abundance (qPCR). Significant differences represented in bold type ($P < 0.05$).

	CO2			Year			CO2 x Year		
	df	F	P	df	F	P	df	F	P
Moisture	2	6.26	0.08	1	6.81	**0.03**	5	0.73	0.62
TC	2	9.50	**0.05**	1	0.49	0.50	5	0.34	0.88
TOC	2	9.79	**0.05**	1	0.28	0.61	5	0.33	0.88
Fe2O3	2	1.74	0.32	1	0.18	0.69	5	0.43	0.82
MnO	2	1.61	0.33	1	0.01	0.94	5	0.98	0.48
SiO2	2	6.27	0.08	1	0.17	0.70	5	1.30	0.34
P2O5	2	6.05	0.09	1	0.05	0.84	5	0.92	0.51
K2O	2	0.17	0.85	1	2.45	0.19	5	0.76	0.60
CaO	2	2.85	0.20	1	0.43	0.55	5	0.17	0.97
As	2	0.32	0.75	1	4.57	0.10	5	1.39	0.31
Ba	2	0.17	0.85	1	1.42	0.30	5	1.00	0.47
Co	2	10.33	**0.05**	1	0.18	0.70	5	0.44	0.81
Cr	2	11.83	**0.04**	1	0.02	0.91	5	0.89	0.52
Cu	2	0.72	0.56	1	3.13	0.15	5	0.72	0.62
Ni	2	21.68	**0.02**	1	0.03	0.86	5	0.79	0.58
Pb	2	0.51	0.65	1	0.89	0.40	5	1.12	0.41
Zn	2	1.64	0.33	1	0.00	0.95	5	0.92	0.51
Bacteria	1	7.59	**0.02**	1	7.59	**0.02**	5	1.00	0.47
Archaea	1	1.33	0.28	1	1.33	0.28	5	1.00	0.47

S-Figure 2: 16S rDNA gene quantification of a) *Bacteria* and b) *Archaea* using qPCR. Black bars represent results of samples from 2011, white bars of 2012. Averages for replicates are shown with standard deviations indicated.



S-Table 4: Summary of similarity percentage (SIMPER) analysis results for the major bacterial and archaeal phyla and species within the 16S rDNA clone libraries.

Taxon	Dissim.	Cont. [%]	Cum. [%]	Query cover [1] [%]	Ident. [1] [%]
Archaea					
Phyla*					
Thaumarchaeota	25.7	48	48		
Euryarchaeota	15.7	29.3	77.3		
unclassified Archaea	7.8	14.5	91.9		
Crenarchaeota	4.4	8.2	100		
Species*					
Nitrososphaera viennensis EN76. (Thaumarchaeota)	7.2	9.1	9.1	100	95
Methanosarcina sp. strain Bavarian (Euryarchaeota)	5.1	6.5	15.6	100	99
Nitrososphaera viennensis strain EN76 (Thaumarchaeota)	4.8	6	21.6	100	96
Methanomassiliicoccus luminyensis strain B10 (Euryarchaeota)	3.9	5	26.6	100	86
Bacteria					
phyla*					
unclassified *Bacteria*	9.9	22.9	22.9		
Proteobacteria	8.9	20.7	43.6		
Actinobacteria	7.5	17.3	60.9		
Verrucomicrobia	4.1	9.4	70.3		
Acidobacteria	4	9.3	79.6		
Firmicutes	3.3	7.5	87.1		
species					
Bacterium SOSP1-9 (unclassified *Ktedonobacteria*; *Chloroflexi)*	3.6	4.1	4.1	99	89
Bacillus longiquaesitum (Firmicutes)	2.3	2.7	6.8	100	99
Bacterium Ellin507 *(Spartobacteria*; *Verrucomicrobia)*	2.3	2.6	9.4	100	93
Bacterium SOSP1-30 (unclassified *Ktedonobacteria*; *Chloroflexi)*	2.3	2.6	11.9	100	87
Bacterium SOSP1-63 (unclassified *Ktedonobacteria*; *Chloroflexi)*	1.9	2.2	14.1	100	89
Bacterium Ellin5025 *(Rubrobacteria*; *Actinobacteria)*	1.8	2	16.2	100	98

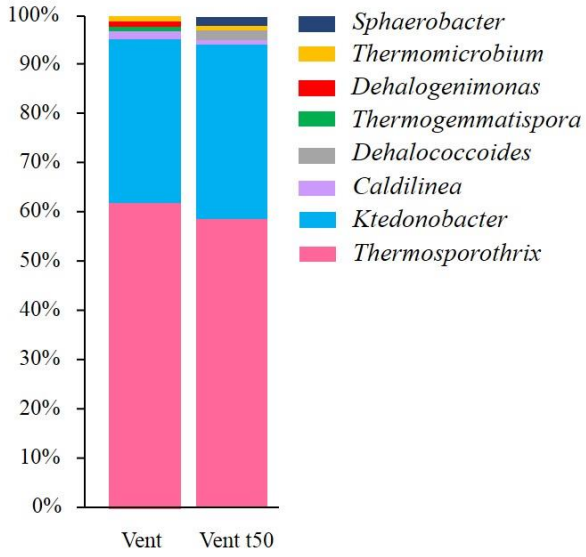
[1] Standard nucleotide blast of the the Basic Local Alignment Search Tool (BLAST)

Dissim.. Bray-Curtis Dissimilarity values; Con. %. percentage of the contributed difference; Cum. %. Cumulative percentage.
* Representatives with >5% contribution

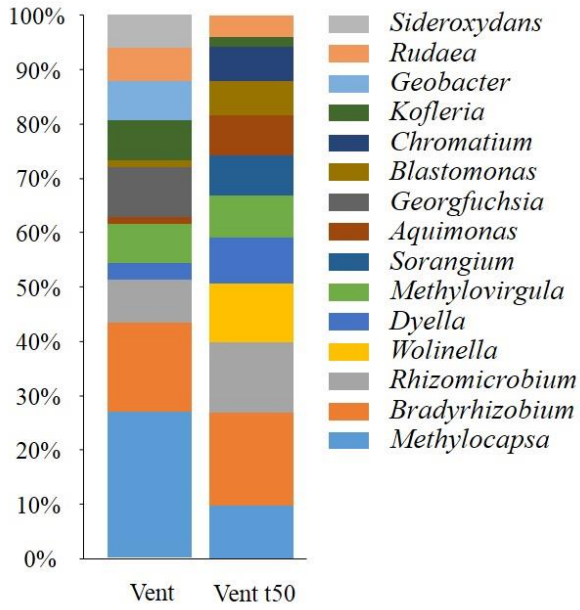
S-Table 5: Archaeal and bacterial 16S rDNA clone library parameters and statistical estimation of the diversity (Shannon diversity index) and richness (Chao index) along the 25 m transect and over time.

	Vent	Medium	Reference
Archaea			
2011			
No of sequences	88	86	89
No of OTUs	53	41	35
Shannon	3.57	3.08	3.01
Chao	204	119	104
2012			
No of sequences	88	86	89
No of OTUs	43	21	36
Shannon	3.05	2.17	3.15
Chao	192	32	82
Bacteria			
2011			
No of sequences	59	54	50
No of OTUs	49	41	45
Shannon	3.65	3.19	3.47
Chao	168	105	143
2012			
No of sequences	54	56	48
No of OTUs	29	47	46
Shannon	2.87	3.59	3.54
Chao	76	127	211

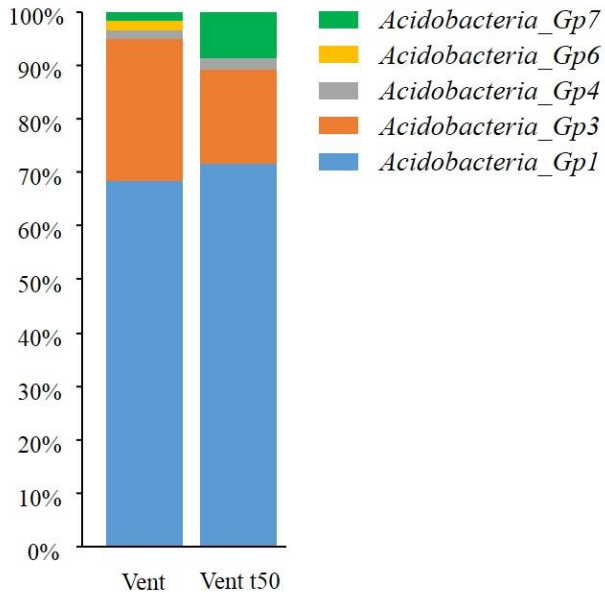
S-Figure 3: *Chloroflexi* community composition of the bacterial PyroTag libraries before $^{13}CO_2$ incubation (vent) and after 50 days of $^{13}CO_2$ incubation (t50). Results are presented for vent samples taken in 2012 without significant differences between natural vent samples and vent samples after 50 days of $^{13}CO_2$ incubation (one-way ANOSIM: R = -0.5; P = 1).



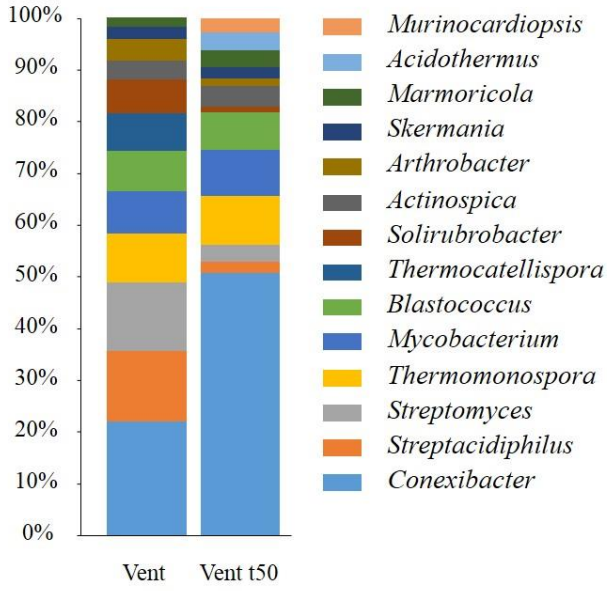
S-Figure 4: *Proteobacteria* community composition of the bacterial PyroTag libraries before $^{13}CO_2$ incubation (vent) and after 50 days of $^{13}CO_2$ incubation (t50). Results are presented for vent samples taken in 2012 without significant differences between natural vent samples and vent samples after 50 days of $^{13}CO_2$ incubation (one-way ANOSIM: R = 1; P = 0.34).



S-Figure 5: *Acidobacteria* community composition of the bacterial PyroTag libraries before $^{13}CO_2$ incubation (vent) and after 50 days of $^{13}CO_2$ incubation (t50). Results are presented for vent samples taken in 2012 without significant differences between natural vent samples and vent samples after 50 days of $^{13}CO_2$ incubation (one-way ANOSIM: R = 0.5; P = 1).



S-Figure 6: *Actinobacteria* community composition of the bacterial PyroTag libraries before $^{13}CO_2$ incubation (vent) and after 50 days of $^{13}CO_2$ incubation (t50). Results are presented for vent samples taken in 2012 without significant differences between natural vent samples and vent samples after 50 days of $^{13}CO_2$ incubation (one-way ANOSIM: R = 1; P = 0.34).



S-Table 6: Summary of similarity percentage (SIMPER) analysis results for the major bacterial and archaeal taxa within the PyroTag libraries before and after 50 days of $^{13}CO_2$ incubation (t50).

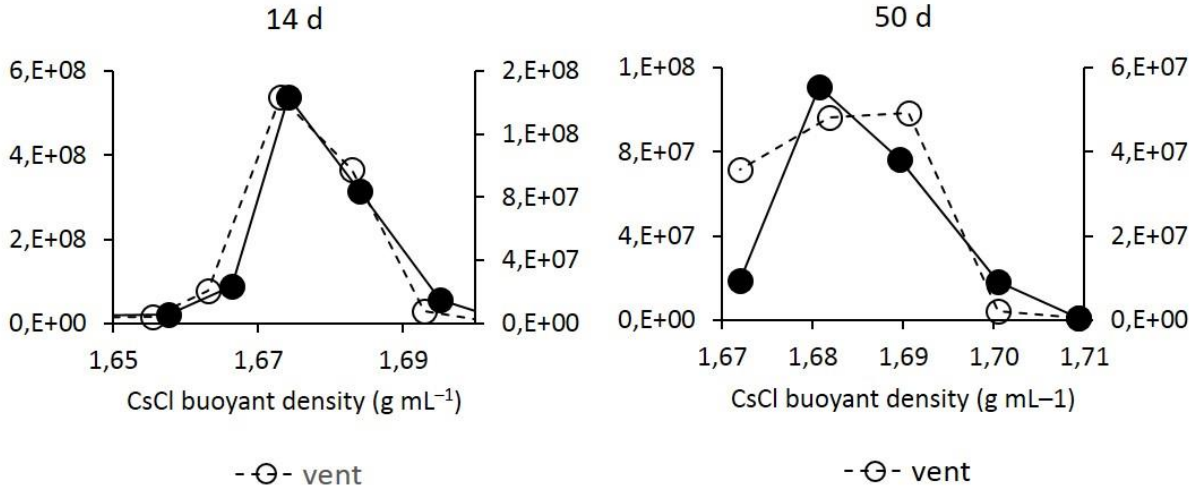
Taxon	Dissim.	Cont. [%]	Cum. [%]
Vent*			
phyla**			
Chloroflexi	2.7	21.2	21.2
Actinobacteria	2.2	17.7	38.8
Acidobacteria	2.1	16.5	55.3
Parcubacteria	1.2	9.4	64.7
Proteobacteria	1.2	9.4	74.1
Firmicutes	0.9	7.1	81.2
order**			
Ktedonobacterales (*Chloroflexi*)	3	13.3	13.3
Solirubrobacterales (*Actinobacteria*)	2.2	9.9	23.2
Bacillales (Firmicutes)	1.2	5.3	28.5
Parcubacteria	1.2	5.3	33.8
genus**			
Conexibacter (*Actinobacteria*)	2.5	7.4	7.4
Thermosporothrix (*Chloroflexi*)	2.4	6.9	14.3

Dissim.. Bray-Curtis Dissimilarity values; Con. %. percentage of the contributed difference; Cum. %. Cumulative percentage

* Comparison of vent samples before and after 50 days of $^{13}CO_2$ incubation

** Representatives with >5% contribution

S-Figure 7: 16S rDNA gene quantification (qPCR) of *Bacteria* during the ^{13}C-CO_2 incubation experiment. Results presented for fractions after 14 and 50 days of incubation (14 d; 50 d).



Manuscript 3

Vertical microbial stratification in freshwater lake sediment of the Laacher See caldera

Vertical microbial stratification in freshwater lake sediment of the Laacher See caldera

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Running title: Microbial abundance and diversity in volcanic lake sediments

Keywords: CO_2, sediment-colonizing *Archaea* and *Bacteria*, geochemistry

Abstract

Few studies have investigated volcanic lake ecosystems and potentially CO_2-adapted sediment microorganisms. In order to identify the microbial community composition and potential key microbes for the biochemical carbon cycling in Laacher See lake sediments, we took sediment cores from a reference, low and high CO_2 influenced site. Analyses of the pore water revealed a 7-fold increase and a 11.5-fold increase of CO_2 in the low and high CO_2 influenced core, respectively, compared with the reference core. Similar values were obtained for dissolved CH_4. In addition, a significant drop in pH (8-6) and decreased concentrations of Na, Ca, Mg, Fe(II) and Mn from the reference to the low and high CO_2 influenced core was detected. 16S rDNA analyses (qPCR) revealed, that *Archaea* outnumbered *Bacteria* within all sediment samples however, without significant differences in the archaeal and bacterial abundances between the three cores. The majority of the microbial sequences were assigned to six archaeal and bacterial phyla: *Crenarchaeota* (13%-97%), *Euryarchaeota* (1%-45%), *Pacearchaeota* (0%-69%), *Proteobacteria* (11%-55%), *Firmicutes* (7%-75%) and *Chloroflexi* (0%-48%). Significant differences have been observed between the bacterial community compositions of the three sediment cores (ANOSIM, P = 0.01). Simper analyses revealed, that *Thermoprotei*, *Pacearchaeota*, *Deltaproteobacteria* and unclassified *Clostridia* contributed most to differences between the reference, low and high CO_2 influenced core.

Introduction

The water filled caldera Laacher See is the largest lake of the East Eifel volcanic field, Germany. Intra-plate alkali-basaltic lavas were produced within the upper mantle (Eifel plume), where partial melting enriched the lavas in gaseous species, mainly CO_2. During their rise up to the surface, gases were partly released either in the lower crust or along faults and fractures in the upper crust (Gal *et al.* 2011). Aeschbach-Hertig *et al.* (1996) calculated an annual release of about 5000 t of CO_2. Numerous previous studies focused on gas analyses, limnology, mineralogy and fauna of lake Laacher See (Kempf and Scharf 1980; Wörner, G. and Schmincke 1984; Bahrig, B. 1985; Bahrig 1988; Giggenbach 1990; Kischnick 1992; Scharf and Björk 1992; Aeschbach-Hertig *et al.* 1996; Möller *et al.* 2011). Microbial communities play a key role in the transformation of complex organic matter and biochemical cycling in sediments (Liu *et al.* 2009; Ye *et al.* 2009; Zhang *et al.* 2015). Drivers for microbial distribution in sediments like sediment depth, salinity, nutrients, organic matter, pH or pollution were previously described (Prokofeva *et al.* 2005; Ye *et al.* 2009; Hollister *et al.* 2010; Swan *et al.* 2010; Haller *et al.* 2011; Bai *et al.* 2012; Kadnikov *et al.* 2012; Song *et al.* 2012; Raulf *et al.* 2015b). However, volcanic lakes differ remarkably from freshwater lakes and can be divided into six classes based on their activity type (Rouwet *et al.* 2014). The Laacher See lake belongs to the low activity, shallow, CO_2-dominated lakes where dissolved CO_2 can reach the surface through diffusion or bubbling degassing. High amounts of discharged and dissolved CO_2 could lead to low pH and enhanced dissolution of metals, like previously described (Harvey *et al.* 2012a). Few studies have investigated volcanic lake ecosystems including the archaeal and bacterial distribution, e.g. Bhattarai *et al.* (2012b) examined the archaeal community structure of anoxic sediments of lake Kivu (Democratic Republic of the Congo). Therefore, investigations of the unique ecosystems of volcanic lakes are needed to broaden our knowledge about the interaction of CO_2, redox conditions and microbial communities.

With this study, we aimed to identify the microbial community composition and potential key microbes for the biochemical carbon cycling in Laacher See sediments. In addition, impacts of climate change on the lake stratification and therefore its stability was observed. This first investigation of the microbial community in the Laacher See lake sediments presents results of pore water and geochemical analyses and first insights into archaeal and bacterial distribution.

Materials and Methods

Site Description

The East Eifel volcanic field, located west of the river Rhine consists of numerous CO_2 vents. Located in the centre of the volcanic field is the large water filled caldera Laacher See. The lake covers approximately 27% of the total drainage area and has a maximum depth of about 52 m (Giggenbach 1990; Aeschbach-Hertig *et al.* 1996). Because of its nutrient content, the lake is classified as mesotrophic to eutrophic (B.W. Scharf and M. Oehms 1992). At least once a year, the water layers intermix, which characterizes Laacher See as a holomictic lake (Aeschbach-Hertig *et al.* 1996). Discharge of gases are known across the Eifel region both in soils and at the bottom of the lake where rising gas bubbles can

be seen within the water column and water surface. Gas bubble analyses showed a high CO_2 content (99%) with traces of nitrogen, oxygen, argon, neon and methane (Aeschbach-Hertig *et al.* 1996; Gal *et al.* 2011). The carbon isotope signatures indicated a magmatic origin of the CO_2 (from -3.8 to -2.99 ‰ PDB) (Giggenbach 1990; Griesshaber, O'Nions and Oxburgh 1992). An annual CO_2-flux into Laacher See has been estimated of $6.4*10^{17}$ molecules m^{-2} s^{-1} or 5000 tons of CO_2 (Aeschbach-Hertig *et al.* 1996).

Location of CO_2 seeps and gas analysis

CO_2 seeps at the lake bottom and potential reference areas were located using several hydroacoustic measurements (Single beam echosounders, multi beam echosounders, side scan sonars, acoustic doupler current profilers, subbottom profilers etc.). The flux rates and composition of rising CO_2 were verified with divers and a small remotely operated vehicle (ROV). Based on the detected CO_2 concentrations and flow records, an estimation of the total amount of CO_2 released from the lake bottom was made. A detailed description of Laacher See gas analyses can be found in Möller et al. (2011).

Water column and stratification

Annual investigations of the water column and its chemical properties are carried out from the Rhineland-Palatinate Landesamt für Umwelt. The results including e.g. water temperatures, pH, O_2 concentration and DOC and are available online (http://www.geoportal-wasser.rlp.de/servlet/is/8562/). A graphical representation of water chemical data produced of the Rhineland-Palatinate Landesamt für Umwelt for the years 2004 to 2007 and 2011 can be found in the Supporting Information (Figure 01 and 02).

Sediment core sampling

Based on analyses of the lake bottom using hydroacoustic measurements, sediment cores were taken in August 2011. Sediment samples were taken at a reference site (without detectable CO_2 bubbles rising), low CO_2 site (few CO_2 bubbles) and high CO_2 site (high amount of rising CO_2 bubbles). Therefore, a coring platform was used and three cores were taken using the Niederreiter piston core technology (www.uwitec.at). The sediment cores had a length of up to 2 m and were taken in a water depth of up to 20 m.

Pore water sampling and analysis

After the retrieval of the full cores and transportation back to the shore, holes were drilled into the coring tubes (2 m length) made of PVC with an interval of 5 cm. For the retrieval of pore water, Rhizon CCS sampler (Rhizosphere Research Products, Wageningen, Netherlands) of 10 cm length and 2.5 mm diameter were inserted. The MicroRhizon sampler consists of a hydrophilic membrane (composed of a blend of polyvinylpyrrolidine and polyethersulfone) of 0.15–0.20 µm pore size. Tube connector with double luer-locks were connected to each Rhizon sampler and 10 ml syringes were connected to each tube connector to generate a vacuum. pH values of the gained pore water were determined immediately after collection with a portable pH-Meter (WTW, Germany). The concentrations of sulfate and major

ions (Na^+, K^+, Ca^{2+}, Cl^-) were determined by ion chromatography with a DX-500 system (Dionex, Germany). All samples were analysed in triplicates.

Sediment Geochemistry

After pore water collection, the sediment tubes were cut lengthwise into two halves and opened. Sediment samples were taken in intervals of 5 cm. In the laboratory, samples were filled into sterile glass bottles, sealed with butyl stoppers and flushed with N_2 to remove O_2. In addition, subsamples were taken for geochemical (stored at 4°C) and bio molecular (stored at -20°C) analyses. Sediment moisture was determined by drying 1 g of wet sediment sample at 60°C to constant weight. In addition, sediment carbon content (total carbon; TC) was analysed using a LECO CS 230 analyser (LECO Corporation, St. Joseph, USA). To obtain the total organic carbon (TOC), inorganic carbon was removed by adding a 10% solution of hydrochloric acid to the sediment samples. Trace elements were analysed by X-ray fluorescence spectrometry as previously described by Krishna and Govil (2008) and Shibata *et al.* (2009). For the stable carbon isotope analysis, dried sediment samples were treated with 10% HCl in acid washed tin capsules and stored in a desiccator until measurement. Isotope ratios were determined by a coupled system of an elemental analyzer and a MAT 252 isotope ratio mass spectrometer (Thermo Electron, USA) via a Finnigan ConFlo III open split interface. Isotopic carbon values are expressed in ‰ relative to Vienna PeeDeeBelemnite (VPDB). All samples were analysed in triplicates.

DNA Extraction

Total DNA was extracted from sediment samples (stored at -20°C) in duplicates following the manufacturers manual of the FastDNA Spin Kit for soil (Bio 101; MP Biomedicals) (Webster *et al.* 2003).

Quantitative PCR analyses

Quantitative PCR was performed on a StepOnePlus Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). Each reaction contained 5 µl TaqMan 2x Universal PCR Master Mix (NoAmpEraseUNG, Applied Biosystems), 0.4 µl PCR primers and probe for *Bacteria* (2.5 µM each) and *Archaea* (20 µM primers and 5 µM probe) , 2.3 µl sterile water, 0.5 µl BSA (20 mg/ml) and 1 µl 16S rDNA template added to a final volume of 10 µl. Samples were analysed for total numbers of *Bacteria* and *Archaea*. The qPCR assays were calibrated by 10-fold serial dilutions using PCR amplified pure standards. Microbial primers, standards and description of the qPCR specifications can be found in the Supporting Information (Table 1). Overall, nine replicates (3 replicates x 3 dilutions; 10-, 100- and 1000-fold) of each sample were analysed. Amplification efficiencies were calculated from the StepOne v2.1 software on the basis of threshold cycle versus gene abundance of standards according to the relationship E = 10(-1/slope). Amplification efficiencies ranged between 80 and 100%. The qPCR amplifications were analysed with the StepOne v2.1 software and the results are expressed in gene copies per gram dry weight of sediment.

Clone Library Construction

PCR amplicons of bacterial and archaeal 16S rDNA genes were generated using 1:10 or 1:100 dilutions of each soil DNA extract. The PCR reactions contained 25 µl DreamTaq PCR Master Mix (2x) (Thermo Scientific), 0.3 µM of each primer, 4 µl template DNA and nuclease-free water up to 50 µl. Microbial primers, standards and description of the PCR specifications can be found in Supporting Information (Table 1). Triplicate PCRs were performed for each sediment DNA extract. Purification, cloning and sequencing of the 16S rDNA PCR products was conducted at LGC Genomics GmbH (LGC Genomics GmbH, Berlin, Germany) and Microsynth AG (Microsynth AG, Balgach, Switzerland). Clone library details can be found in the Supporting Information (Table 2).

Phylogenetic analyses

Quality control and screen for chimeras has been made using Geneious R7 software and DECIPHER (Wright *et al.* 2012) . Sequences with mismatches or gaps were removed using MOTHUR (Schloss *et al.* 2009). For clustering the sequences into OTUs and additional analysis of community structure, MOTHUR was used (Schloss *et al.* 2009). Representatives of each OTU at the 0.03 distance level were aligned and classified with the ARB-SILVA aligner and assembled in the existing parsimony tree of the SILVA reference database SSURef 118 (Pruesse *et al.* 2007; Pruesse *et al.* 2012).

Statistical analyses

Analysis of variance (two-way ANOVA) was used for the detection of possible CO_2 impacts on sediment properties and microbial abundance (qPCR). Non-metric multidimensional scaling (MDS) plots, based on distance matrixes, were generated for visualisation and interpretation of differences in the bacterial and archaeal community composition between the three sediment cores. Analysis of similarity (ANOSIM) was performed to detect differences within the bacterial and archaeal communities between the three sediment cores. Similarity percentage (SIMPER) analysis was performed to identify the taxa that contributed most of the variance between the sediment cores. MDS as well as analyses of similarity and variance base on the Bray–Curtis dissimilarity index. Analyses of MDS, ANOVA and multivariate statistics were performed in PAST (Hammer, Harper and Ryan 2001). The calculation of diversity (Shannon) and richness (Chao) indices was generated using MOTHUR (Schloss et al. 2009).

Results and Discussion

Freshwater sediments have been intensively investigated in the past (Schwarz, Eckert and Conrad 2007; Ye *et al.* 2009; Liu *et al.* 2010; Kadnikov *et al.* 2012; Zhang *et al.* 2015). In contrast, freshwater lakes of volcanic origin, like Laacher See, have been barely investigated. Especially information about geochemical and microbiological processes in volcanic lake sediments are scarce. With this study, we present first insights into the archaeal and bacterial populations in Laacher See sediments and the potential impact of seeping volcanic CO_2.

CO_2 flow and gas composition

The measured CO_2 flow rates showed no seasonal variation. Analyses of the gas composition showed an average of 93.47 (±3.04) vol% CO_2, 3.9 vol% N_2, 2.5 vol% O_2 and Ar and 373.9 ppm CH_4 (average;

not presented in the paper). Based on the detected CO_2 concentrations and flow records, an estimation of the annual amount of CO_2 released from the lake bottom was calculated with about 5000 (±1212) tons of CO_2, similar to previous findings (Aeschbach-Hertig *et al.* 1996). A detailed description of Laacher See gas analyses can be found in (Möller *et al.* 2011).

Water column and stratification

The Laacher See lake was previously described as a holomictic lake with a full circulation of the water layers (destratification) at least once a year (Aeschbach-Hertig *et al.* 1996). Rising ambient temperatures (because of climate change) could compromise the full circulation of the water by warming up the Epilimnion. In consequence, the Laacher See lake would face the possibility to become meromictic with a constant anoxic Hypolimnion. For this reason, the continuous monitoring of the Laacher See lake is of crucial importance. So far, investigations of the Rhineland-Palatinate Landesamt für Umwelt determined no impacts of climate change and the annual destratification appears to be stable (Supplementary Figure 1 and 2).

Pore water and sediment chemistry

Pore water analyses of the reference, low and high CO_2 influenced core revealed significant differences. Dissolved CH_4 and CO_2 significantly increased from reference to low and high CO_2 influenced cores (Figure 1; Supplementary Table 3).

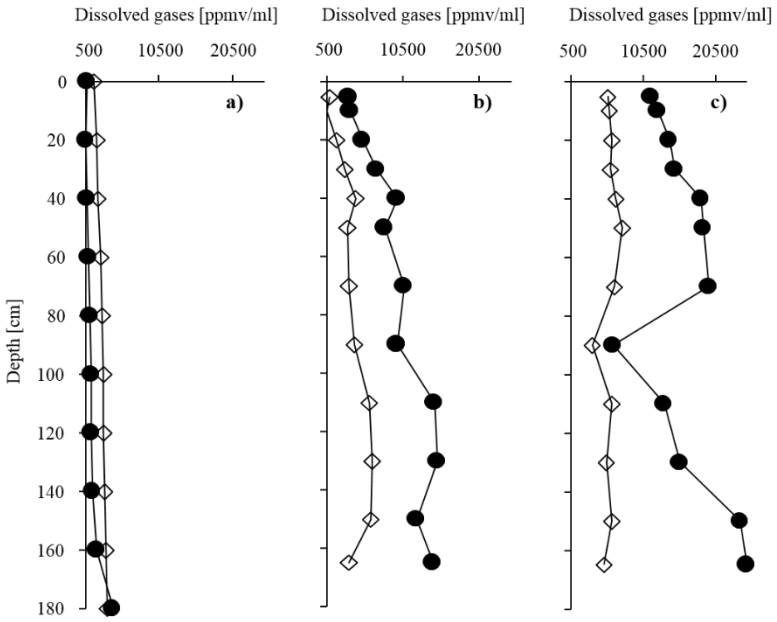

Figure 1: Dissolved CO_2 and CH_4 concentrations in the pore water of the reference, low and high CO_2 influenced sediment core.

The dissolved CO_2 rose from 1414 ppmv/ml (reference core) to 9551 ppmv/ml (low CO_2 influenced core) and 16227 ppmv/ml (high CO_2 influenced core) (mean values over depth; not presented). A similar trend was detected for the CH_4 concentrations. The CH_4 concentrations rose from 2656 ppmv/ml (reference core) to 3651 ppmv/ml (low CO_2 influenced core) and 5870 ppmv]/ml (high CO_2 influenced core) (mean values over depth). The increase of the CH_4 concentration in parallel to increasing CO_2 indicates increasing anaerobic microbial activity i.e. methanogenesis. Similar processes were previously described for anaerobic lake sediments (Bhattarai *et al.* 2012b). In contrast to rising dissolved CO_2 and CH_4, a significant drop in pH and contents of Na, Ca, Mg, Fe(II) and Mn from the reference to low and high CO_2 influenced core was detected (Supplementary Table 3 and 4). The pH of the reference core was 6.9 to 7.7 (Figure 2), in contrast to pH of 6.3 to 6.6 in the low CO_2 influenced core and 6.1 to 7 in the high CO_2 influenced core (Figure 2).

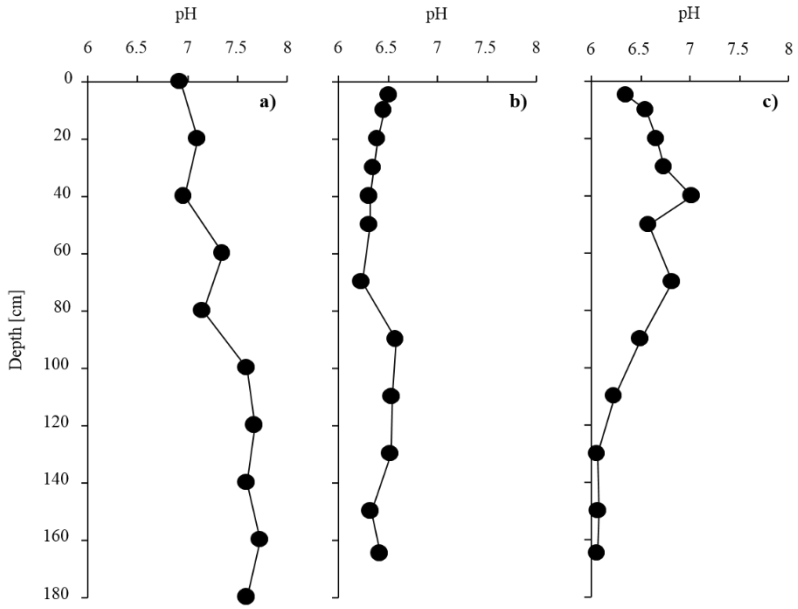

Figure 2: pH values detected for the pore waters of the reference, low and high CO_2 influenced sediment core.

A previous investigation of Inagaki *et al.* (2006) detected a similar pH range for sediment cores of a hydrothermal field and reference site. The cause of decreasing pH with increasing CO_2 concentrations is based on the dissolution of the rising CO_2 in the pore water and its formation of carbonic acid as previously described (Harvey *et al.* 2012a). Previous studies described that decreasing pH leads to the dissolution of minerals and increasing amounts of metals and subsequently cations/anions concentration (Kharaka *et al.* 2010a; Lu *et al.* 2010; Harvey *et al.* 2012a). We cannot confirm these findings. In contrast, we detected a significant decrease of cations/anions in the pore water and no significant changes in the sediment chemistry (Supplementary Table 5). This might be explained by an accelerated removal of dissolved cations/anions as well as other trace chemicals by a gas bubble driven transport. In contrast to soils for example, lake sediments are continuously penetrated by pore water. In

combination with the rising gas bubbles from the lake bottom, a continuous exchange between sediment and lake water body seems most likely.

Archaeal and bacterial abundance

The archaeal abundance varied with 10^7-10^{10} 16S rDNA gene copies gdw^{-1} sediment (Figure 3). A similar trend was detected for bacterial abundance with 10^6-10^{10} 16S rDNA gene copies gdw^{-1} sediment (Figure 3).

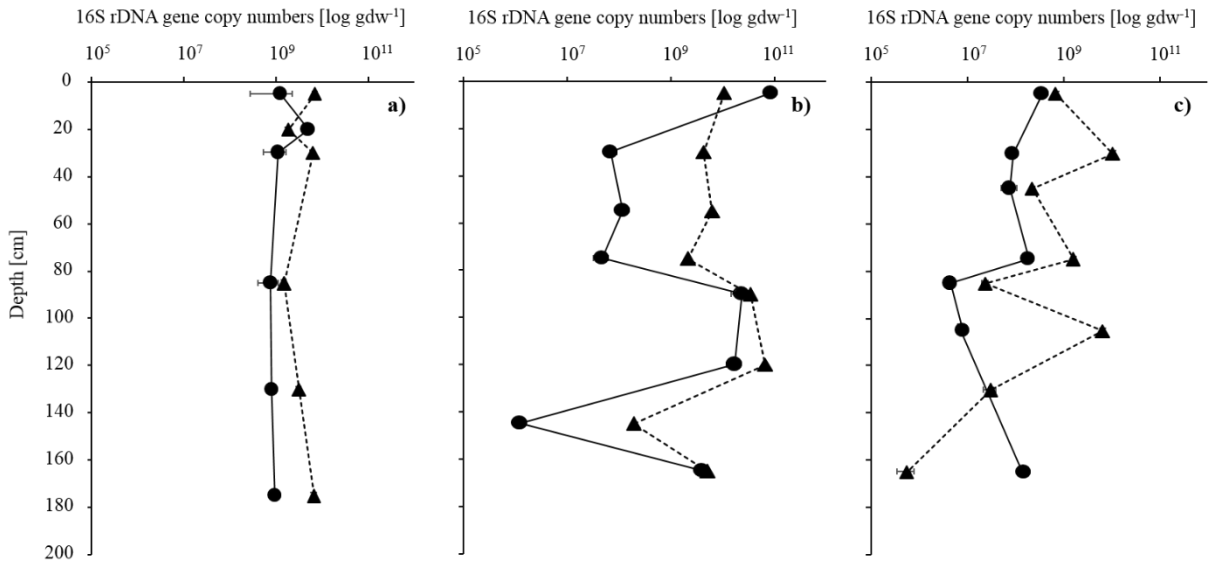

Figure 3: 16S rDNA gene copy numbers for *Archaea* (triangles) and *Bacteria* (circles) detected in the sediment of the reference, low and high CO_2 influenced sediment core.

For both, archaeal and bacterial abundance, no significant differences between the reference, low and high CO_2 influenced core could be detected (Supplementary Table 5). However, the 16S rDNA gene copy numbers in the present study demonstrated that *Archaea* were about 10-100 times more abundant than *Bacteria* within all Laacher See sediment samples (Figure 3). Several previous studies presented similar 16S rDNA copy numbers as detected for Laacher See sediments with however, the predominance of bacterial 16S rDNA gene copy numbers (Schwarz, Eckert and Conrad 2007; Ye *et al.* 2009; Zhang *et al.* 2015). Increasing dissolved CO_2 concentrations from the reference and low CO_2 influenced sediment core towards the high CO_2 influenced core suggest increasing anaerobic microbial activity due to hypoxia and therefore, increasing archaeal 16S rDNA gene copy numbers. Potential drivers for both, microbial abundance and distribution are various environmental parameters. In the Laacher See lake sediment oxygen availability, pH and nutrient availability can be particularly important. Further investigations are needed to clarify the main drivers within the Laacher See sediment.

Microbial community composition

Archaea

The archaeal community composition in the sediments of Laacher See varied among samples (Figure 4). For the three sediment cores, the majority of the archaeal sequences were assigned to the phylum *Crenarchaeota* (13%-97%) (Figure 4). 1%-45% of the sequences were assigned to the phylum

Euryarchaeota followed by 0%-69% *Pacearchaeota* representatives (Figure 4). They represent 49%–93% of the sequences within all 16S rRNA clone libraries. Lower abundances were detected for *Thaumarchaeota* (0%-2%), *Woesearchaeota* (0%-1%), *Aenigmarchaeota* (0%-1%) and unclassified *Archaea* (0%-30%) (Figure 4).

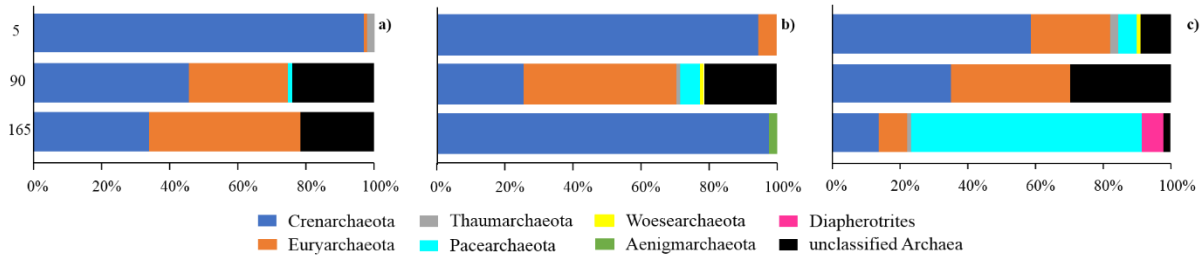

Figure 4: 16S rDNA clone libraries. Archaeal community composition of the reference, low and high CO_2 influenced sediment core.

No significant differences were observed between archaeal community compositions from the reference, low and high CO_2 influenced sediment samples (ANOSIM, R = –0.14, P = 0.18; Figure 5a).

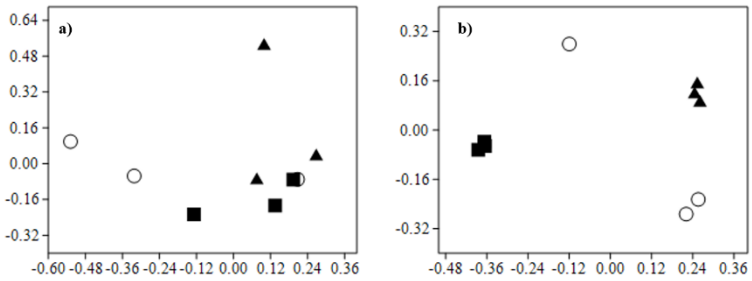

Figure 5: Non-metric multidimensional scaling (MDS) plots. A) Archaeal distance matrix; b) Bacterial distance matrix.

Similar results were detected for the Shannon diversity index and Chao1 richness index without significant CO_2-induced alterations (t-test, P > 0.05) (Supplementary Table 2, Figure 3). SIMPER analysis at phylum level indicated that *Crenarchaeota* contributed most of the differences in the relative abundance between the three sediment cores (33%-47%) (Supplementary Table 6). *Pacearchaeota* (26%-27%) and *Euryarchaeota* (18%-31%) are following (Supplementary Table 6). On class level, unclassified *Thermoprotei* (32%-47%), *Pacearchaeota* (25%) and *Thermoplasmata* (12%-20%) contributed most to the differences between the cores (Supplementary Table 6).

The predominance of *Crenarchaeota* representatives in lake sediments has been described previously (Liu *et al.* 2010; Zhang *et al.* 2015). Bhattarai *et al.* (2012b) for example, affiliated archaeal sequences in lake Kivu sediments mainly with the uncultured environmental C2 cluster of *Crenarchaeota* and *Candidatus Nitrososphaera* (*Thaumarchaeota*). In the Laacher See lake sediments, the obligate anaerobic *Thermoprotei* (*Crenarchaeota*) representatives previously described as moderate acidophilic

to acidophilic with a pH optimum between 2.5 to 7 and best growth at 70-107°C were most abundant (Garrity 2012; Itoh and Iino 2013; Itoh 2014). The thermophilic microbes have also been detected by Raulf *et al.* (2015b) along natural CO_2 gradients at a volcanic vent. They detected a significant increase of *Thermoprotei* with decreasing pH. Our results from Laacher See sediment samples do not confirm the correlation between pH and *Thermoprotei* occurrence. Within the high CO_2 influenced core, the abundance of *Thermoprotei* decreased compared with the two other sediment cores (Figure 4). In addition, previous studies reported the ability of *Thermoprotei* and *Pacearchaeota* representatives for CO_2-fixation (Sato, Atomi and Imanaka 2007; Ramos-Vera *et al.* 2011; Castelle *et al.* 2015b; Jay *et al.* 2016). Especially *Pacearchaeota* are of interest because of their high abundance within the high CO_2 influenced core. *Pacearchaeota* were detected in various environments including sediments, hydrothermal vents, hot springs, freshwater and soil (Ortiz-Alvarez and Casamayor 2016). So far, less information about their metabolic capabilities and their potential environmental importance is available. However, Castelle *et al.* (2015a) investigated e.g. the diversity and metabolic capacities of *Archaea* in terrestrial subsurface biogeochemical cycles using genome-resolved metagenomic analyses. Their results suggested that *Pacearchaeota* have a saccharolytic and fermentative lifestyle with the ability to degrade and utilize complex carbon compounds. Furthermore, they found evidence for CO_2-fixation. Still, more information about the metabolic capabilities and the potential environmental importance of *Pacearchaeota* are needed.

Our results revealed that in the Laacher See sediments unclassified *Thermoprotei* and *Pacearchaeota* with the ability of CO_2-fixation were widespread. However, the metabolic capabilities of the dominant archaeal representatives detected in Laacher See sediments are still poorly understood. Therefore, further investigations are needed to understand the importance of the detected archaeal groups within this unique environment.

Bacterial community composition

Similar to the archaeal community composition, the bacterial communities in the sediments of Laacher See varied among samples with however, high bacterial diversity (Figure 6). The majority of the bacterial sequences were assigned to three phyla: *Proteobacteria* (11%–55%), *Firmicutes* (7%–75%) and *Chloroflexi* (0%–48%) (Figure 6). They represent 67%–86% of the sequences within all 16S rRNA clone libraries. Lower abundances (14%–33%) were detected for *Acidobacteria*, *Actinobacteria*, *Aminicenantes*, *Armatimonadetes*, *Bacteroidetes*, *Candidate* division WPS-1, *Candidatus Saccharibacteria*, *Hydrogenedentes*, *Ignavibacteriae*, *Microgenomates*, *Nitrospirae*, *Omnitrophica*, *Planctomycetes*, *Spirochaetes*, *Verrucomicrobia* and unclassified *Bacteria* (Figure 6).

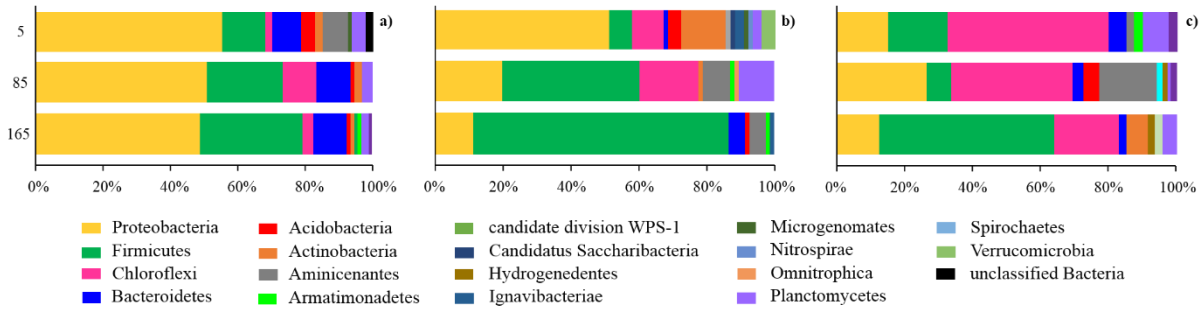

Figure 6: 16S rDNA clone libraries. Bacterial community composition of the reference, low and high CO_2 influenced sediment core.

Significant differences were observed between the bacterial community compositions of the three sediment cores (ANOSIM, R = –0.73, P = 0.01) (Figure 5b). Therefore, for each sediment core, one of the three dominant phyla were most abundant: Within the reference core, *Proteobacteria* with sequences assigned to the predominant class *Deltaproteobacteria* were most abundant (Figure 6). Within the low CO_2 influenced core, *Firmicutes* with sequences assigned to the predominant class *Clostridia* were most abundant (Figure 6). Within the high CO_2 influenced core, *Chloroflexi* with sequences assigned to the predominant class *Dehalococcoidia* were most abundant (Figure 6). SIMPER analysis confirmed these findings. Thus, *Proteobacteria* contributed most of the differences in the relative abundance between the three cores (20%-46%) followed by *Firmicutes* (14%-35%) and *Chloroflexi* (7%-16%) (Supplementary Table 7). Similar results were detected on class level where *Deltaproteobacteria* (31%-42%) and unclassified *Clostridia* (19%) contributed most to differences between the sediment cores (Supplementary Table 7).

Previous studies have shown the predominance of *Proteobacteria* in lake sediments, with a large shift in the composition and proportion of bacterial taxa (Kadnikov *et al.* 2012; Dai *et al.* 2013; Zhang *et al.* 2015). Similar to our results for the reference core, *Deltaproteobacteria* has been previously found to be the predominant group in various freshwater lake sediments (Schwarz, Eckert and Conrad 2007; Ye *et al.* 2009). In the present study, deltaproteobacterial sequences were mainly affiliated to representatives of the genus *Geobacter*. *Geobacter* spp. were detected in a diverse range of soils and aquatic sediments (Mahadevan, Palsson and Lovley 2011). It was demonstrated, that *Geobacter* spp. showed diverse metabolic functions, including the potential for the degradation of aromatics, dehalogenation, psychrotolerance and the reduction of insoluble metal oxides coupled with the oxidation of acetate, a primary product of fermentative metabolism in anaerobic environments like Laacher See sediment (Lovley, Holmes and Nevin 2004). Mahadevan, Palsson and Lovley (2011) summarized, that the primary metabolic niche of *Geobacter* spp. in anoxic submerged soils, aquatic sediments and aquifers seems to be the oxidation of acetate coupled with the reduction of Fe(III) oxides.

The results of the pore water analyses (Supplementary Table 3) and the *Geobacter* distribution (Figure 6) revealed a correlation between Fe II concentration in the pore water (especially of the reference core) and the *Geobacter* distribution. It seems likely, that Fe(III) oxide minerals might be reduced by the

Fe(III) -reducing *Geobacter* resulting in high concentrations of Fe II particularly in the reference core (Supplementary Table 3) (Weber *et al.* 2006).

Firmicutes representatives, most abundant within the low CO_2 influenced core, are common *Bacteria* in aquatic ecosystems with broad metabolic capabilities (Zhang *et al.* 2015). For example, Song *et al.* (2012) described the predominance of Firmicutes representatives for sediments of the shallow lake Dongping (China). Beckmann and Manefield (2015) additionally detected the potential importance of e.g. *Clostridia* and *Firmicutes* representatives in anaerobic hydrocarbon-degrading processes in river sediments. In the Laacher See lake sediment, representatives of the genus *Thermanaeromonas* were most abundant, especially within the low CO_2 influenced core. Mori *et al.* (2002) described the type species of the genus, *Thermanaeromonas toyohensis* sp. nov., as a spore forming, strictly anaerobic thermophilic microorganism with optimal growth at neutral pH. In addition, they described the ability to use saccharides for fermentation or thiosulfate respiration and the reduction of nitrate and nitrite. Our results suggest that *Chlostridia* affiliated sequences play an important role in the fermentation of organic matter particularly within the low CO_2 influenced sediment core. Still, the drivers for the distribution of *Firmicutes* representatives in sediments are poorly understood. Further analyses are needed to clarify the importance of *Firmicutes* in Laacher See sediments.

Chloroflexi, most abundant in the high CO_2 influenced core, have been frequently identified in marine and freshwater sediments (Kadnikov *et al.* 2012; Hug *et al.* 2013; Wasmund *et al.* 2014a). The phylum contains many organisms with diverse metabolic lifestyles, including photoautotrophs, fermenters, organohalide respiring and aerobic thermophiles (Gupta, Chander and George 2013). Most of the Laacher See sediment *Bacteria* assigned to *Chloroflexi* were affiliated with the class *Dehalococcoidetes*. Recently, Hug *et al.* (2013) revealed the potential role of *Chloroflexi* in anaerobic sediment carbon cycling beyond organohalide respiration including respiration of sugars, fermentation, CO_2 fixation, and acetogenesis. They detected evidence for both anaerobic and aerobic mechanisms of energy generation suggesting the ability of *Chloroflexi* to adapt to changing sediment redox conditions. The findings of Wasmund *et al.* (2014a) confirm these results. They have sequenced the genome of a marine subsurface *Dehalococcoidia* and found evidence for a broad metabolic versatility e.g. the ability to use the reductive acetyl-CoA pathway for the oxidation of organic compounds or CO_2 fixation. The results of Hug *et al.* (2013) and Wasmund *et al.* (2014b) suggest that *Chloroflexi* representatives can play an important role in organic matter degradation particularly because of their adaption capabilities due to their broad metabolic versatility. However, further studies will be required to reveal the properties of other *Dehalococcoidia* genotypes particularly of freshwater sediment and extreme ecosystems like Laacher See volcanic lake sediment.

Overall, the results from the reference, low and high CO_2 influenced sediment core showed the predominance of bacterial taxa with a broad metabolic versatility e.g. metal oxide reduction coupled with the oxidation of organic and inorganic compounds as well as fermentation and CO_2-fixation.

However, further investigations are needed to clarify the drivers for the bacterial distribution in the volcanic lake sediment of Laacher See.

Summary and Conclusions

Overall, significant differences of pore water and geochemical parameters between the reference, low and high CO_2 influenced sediment core were determined e.g. pH, dissolved CO_2 and CH_4 concentrations as well as trace elements. In addition, *Thermoprotei* (*Crenarchaeota*), *Pacearchaeota*, *Geobacter* (*Deltaproteobacteria*), *Thermanaeromonas* (*Firmicutes*) and *Dehalococcoidetes* (*Chloroflexi*) dominated the sediment cores and contributed most to differences between them. Therefore, archaeal and bacterial representatives with a broad metabolic versality e.g. fermentation, acetogenesis or metal reduction were dominating the sediment cores. Interestingly, with *Thermoprotei*, *Pacearchaeota* and *Dehalococcoidetes*, at least three of the five most abundant taxa are presumably capable of CO_2-fixation. Still, this can only be the first investigation of Laacher See sediments and microbial abundance and distribution. Comprehensive microbiological analyses are needed to gain a broader knowledge about the metabolic potential of abundant microbial groups and to clarify what microbial groups are active and which dormant. Furthermore, potential future impacts of climate change like rising ambient temperatures and their possible implications to the stability of the Laacher See lake stratification requires continued monitoring.

Acknowledgements

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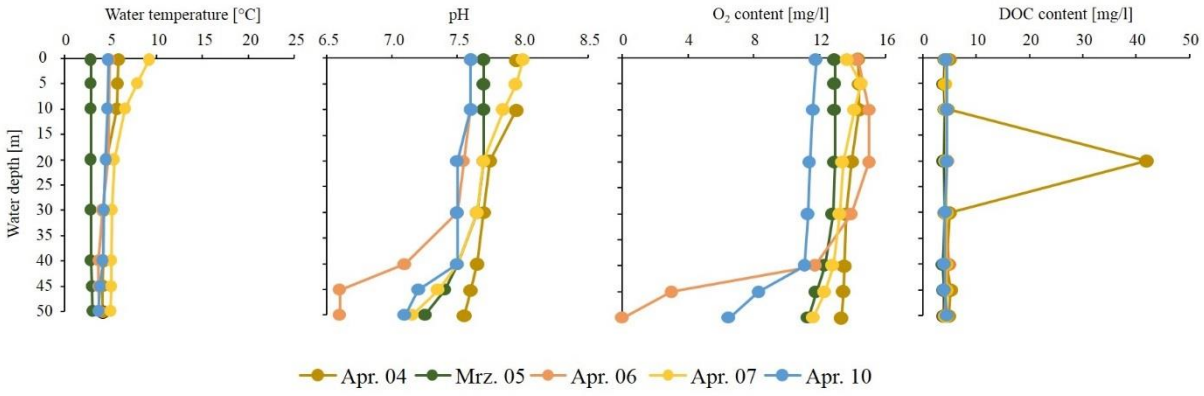
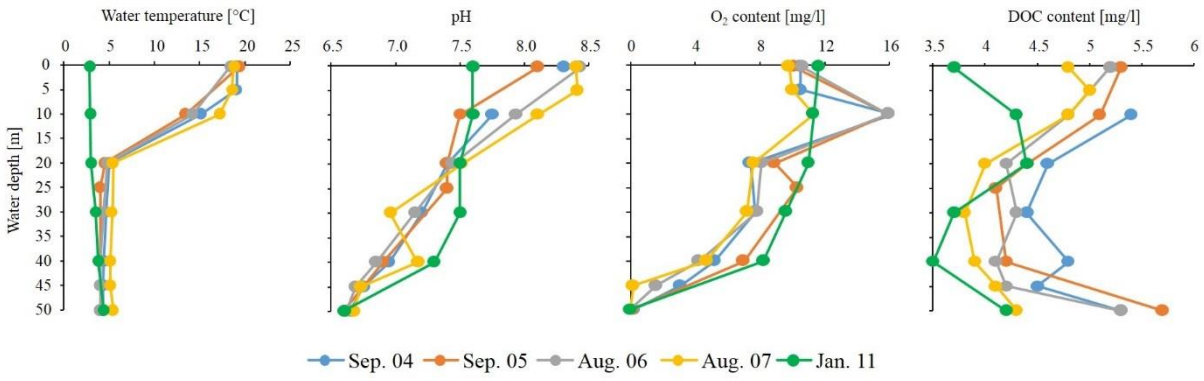
Supplementary

S-Figure 1: Depth profiles of water temperature, pH, dissolved organic carbon (DOC) and O_2 content in the water column of the Laacher See lake during (March/April). For the graphical representation, data generated by the Landesamt für Umwelt Rheinland-Pfalz (Germany). For more information, please see also: http://www.geoportal-wasser.rlp.de/servlet/is/8562/.



S-Figure 2: Depth profiles of water temperature, pH, dissolved organic carbon (DOC) and O_2 content in the water column of the Laacher See lake during stratification (August to January). For the graphical representation, data generated by the Landesamt für Umwelt Rheinland-Pfalz (Germany). For more information, please see also: http://www.geoportal-wasser.rlp.de/servlet/is/8562/.



S-Table 1: Primer sets used for the qPCR and clone library assays.

PCR assay	Primer	Sequence (5' - 3')	Annealing temp. [°C]	Reference
qPCR				
Bacteria	Bac 331F	5'-TCCTACGGGAGGCAGCAGT-3'	60	Nadkarni et al. (2002)
	Bac 797R	5'-GGACTACCAGGGTATCTAATCCTGTT-3'		
	Bac	5'-CGTATTACCGCGGCTGCTGGCAC-3'		
Archaea	Arch 349F	5'-GYGCASCAGKCGMGAAW-3'	59	Takai et al. (2000)
	Arch 806R	5'-GGACTACVSGGGTATCTAAT-3'		
	Arch 516	5'-TGYCAGCCGCCGCGGTAAHACCVGC-3'		
Clone libraries				
Bacteria	Bac 27F	5'- AGR GTT YGA TYM TGG CTC AG -3'	52	Lane et al. (1991)
	Bac 1392R	5'- ACG GGC GGT GTG TRC -3'		
Archaea	Arch 109F	5'- ACK GCT CAG TAA CAC GT -3'	52	Whitehead and Cotta (1999)
	Arch 915R	5'- GTG CTC CCC CGC CAA TTC CT -3'		Stahl et al. (1991)

S-Table 2: Archaeal and bacterial 16S rRNA clone library parameters of the three sediment cores: reference, low and high CO_2 influenced. Data are presented for three depths: 5cm, 90cm and 165cm within each sediment core.

	Reference			**Low**			**High**		
Depth [cm]	5	90	165	5	90	165	5	90	165
Archaea									
No of sequences	95	85	79	90	89	50	89	94	91
Shannon	1,49	3,08	2,77	3,22	2,94	1,12	3,29	2,72	2,33
Chao1	32	136	40	110	225	13	158	49	59
Bacteria									
No of sequences	94	89	89	83	83	81	90	91	91
Shannon	1,99	1,71	1,65	2,59	1,92	1,06	2,36	2,25	2,02
Chao1	30	16	59	28	16	25	16	20	12

S-Table 3: Chemical parameters of the porewater analyses for the three sediment cores: reference, low and high CO_2 influenced. Results are presented for three depths: 5cm, 90cm and 165cm within each sediment core.

Core	Depth [cm]	pH	CH_4 [ppmv]/ml	CO_2 [ppmv]/ml	Na [mg/L]	Ca [mg/L]	Mg [mg/L]	SO4 [mg/L]	Fe(II) [mg/L]	Mn [mg/L]
Reference	5	6.9	1667.7	654.8	57.0	54.0	34.7	0.8	7.1	4.2
	90	7.2	2657.2	1140.1	96.0	58.0	38.0	0.2	22.0	2.6
	165	7.7	3318.7	2005.1	124.0	54.0	33.4	0.3	7.0	1.8
Low	5	6.5	824.5	3480.7	24.3	27.2	15.3	2.9	1.1	0.9
	90	6.6	4145.0	9811.2	23.2	27.5	14.3	0.2	3.4	1.2
	165	6.4	3474.2	14602.1	24.7	40.8	14.6	0.1	2.8	1.1
High	5	6.4	5512.8	11686.8	24.0	30.7	15.5	0.1	5.6	2.0
	90	6.5	3435.5	6313.8	11.1	21.1	7.6	0.0	2.3	0.6
	165	6.1	4989.2	24997.4	26.6	55.9	17.2	0.1	8.4	1.3

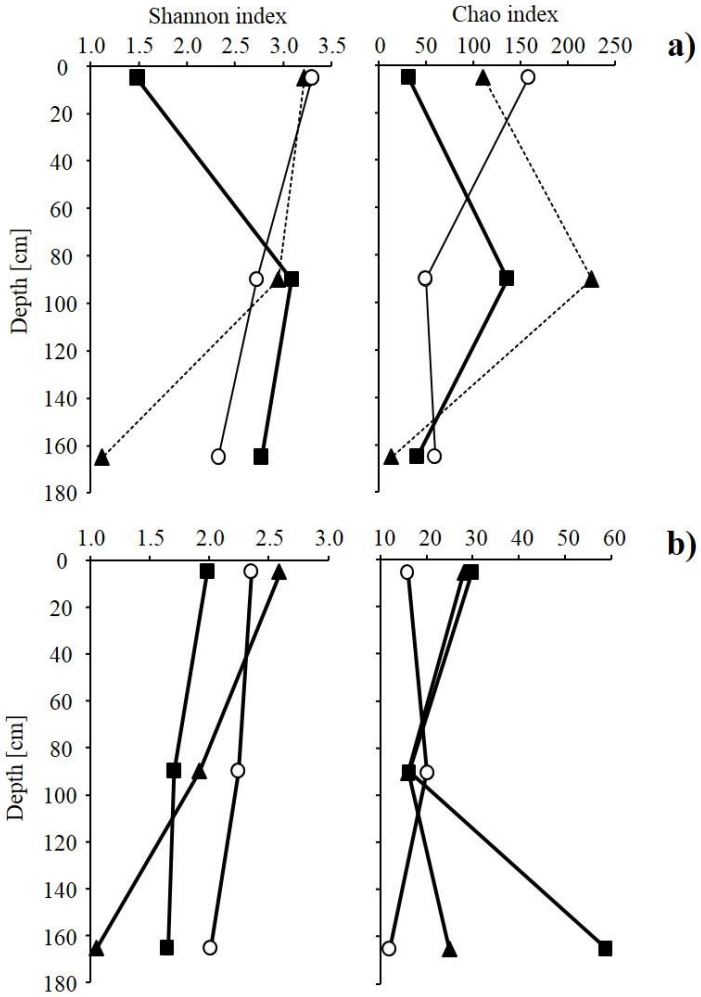
S-Table 4: Geochemical parameters for the three sediment cores: reference, low and high CO_2 influenced. Results are presented for three depths: 5 cm, 90 cm and 165 cm within each sediment core.

Core	Depth	C gesamt	$d^{13}C$	MnO	Fe_2O_3	SO_3	P_2O_5	K_2O	CaO	SiO_2
	cm	%	‰	%	%	%	%	%	%	%
Reference	5	6.8	-25.8	0.2	7.1	0.2	0.4	2.7	1.5	48.9
	90	15.9	-25.4	0.1	5.3	0.1	0.2	1.8	1.3	41.3
	165	13.9	-27.5	0.1	4.2	0.1	0.2	2.2	1.0	44.1
Low	5	12.4	-20.5	0.4	6.7	3.4	0.2	0.9	8.1	39.7
	90	7.3	-25.3	0.1	4.8	0.1	0.2	2.7	1.4	49.7
	165	11.9	-1.5	0.4	2.6	4.0	0.1	0.2	30.9	23.3
High	5	7.2	-25.1	0.1	5.1	0.1	0.2	2.7	1.4	49.4
	90	17.3	-23.9	0.7	7.4	0.3	0.2	1.8	2.4	32.2
	165	0.8	-11.7	0.3	6.1	0.1	0.2	4.2	1.7	56.6

S-Table 5: Analysis of variance (two-way ANOVA) for the detection of differences between the three sediment cores based on CO_2 and depth. Analyses of pore water, sediment properties and microbial abundance (qPCR). Significant differences represented in bold type ($P < 0.05$).

	CO_2			depth [cm]			CO_2 x depth		
	df	F	P	df	F	P	df	F	P
Pore water									
pH	2	39.43	**0.00**	9	0.05	1.00	29	0.92	0.59
CH_4	2	14.89	**0.00**	9	0.54	0.83	29	1.00	0.48
CO_2	2	36.19	**0.00**	9	0.38	0.93	29	1.00	0.48
Na	2	63.19	**0.00**	9	0.17	0.99	29	1.01	0.48
Ca	2	5.45	**0.01**	9	1.04	0.44	29	1.15	0.32
Mg	2	48.20	**0.00**	9	0.24	0.98	29	0.87	0.65
SO_4	2	1.29	0.29	9	1.62	0.18	29	0.81	0.73
Fe(II)	2	14.23	**0.00**	9	0.44	0.89	29	0.72	0.83
Mn	2	7.07	**0.00**	9	0.82	0.61	29	0.75	0.80
Sediment geochemistry									
C total	2	1.54	0.25	4	0.41	0.80	14	0.79	0.67
Soil C isotopy	2	1.16	0.35	4	1.13	0.40	14	1.39	0.22
MnO	2	0.18	0.84	4	0.74	0.59	14	1.10	0.40
Fe_2O_3	2	1.96	0.18	4	0.77	0.57	14	0.56	0.87
SO_3	2	4.16	**0.04**	4	0.29	0.87	14	1.04	0.44
P_2O_5	2	1.85	0.20	4	2.31	0.13	14	0.90	0.56
K_2O	2	3.89	**0.05**	4	0.02	1.00	14	1.35	0.24
CaO	2	1.63	0.24	4	0.67	0.63	14	1.21	0.32
SiO_2	2	0.39	0.68	4	0.16	0.96	14	0.97	0.51
qPCR									
Bacteria	2	1.87	0.20	4	0.87	0.52	14	1.00	0.48
Archaea	2	1.35	0.30	4	0.57	0.69	14	1.00	0.48

S-Figure 3: Microbial diversity index (Shannon) and richness index (Chao) for a) *Archaea* and b) *Bacteria*. Results for the reference (squares), low (triangles) and high CO_2 (circles) influenced core.



S-Table 6: Summary of similarity percentage (SIMPER) analysis. Listed are archaeal phyla and class representatives contributes with > 5% most to differences between the sediment cores.

Taxon	Dissim.	Cont. [%]	Cum. [%]
phyla*			
Reference vs. Low			
Crenarchaeota	18.6	46.9	46.9
Euryarchaeota	12.3	30.9	77.9
unclassified *Archaea*	6.8	17.3	95.1
Reference vs. High			
Crenarchaeota	16.6	35.8	35.8
Pacearchaeota	12.8	27.5	63.3
Euryarchaeota	8.2	17.7	81.0
unclassified *Archaea*	6.9	14.9	95.9
Low vs. High			
Crenarchaeota	17.2	33.2	33.2
Pacearchaeota	13.4	26.0	59.2
Euryarchaeota	11.2	21.8	81.0
unclassified *Archaea*	7.6	14.6	95.6
class*			
Reference vs. Low			
Thermoprotei (*Crenarchaeota*)	18.6	46.9	46.9
Thermoplasmata (*Euryarchaeota*)	8.1	20.4	67.3
unclassified *Archaea*	6.8	17.3	84.6
Methanomicrobia (*Euryarchaeota*)	4.2	10.5	95.1
Reference vs. High			
Thermoprotei (*Crenarchaeota*)	16.6	32.8	32.8
Pacearchaeota	12.8	25.2	58.0
unclassified *Archaea*	6.9	13.7	71.7
Thermoplasmata (*Euryarchaeota*)	6.7	13.3	85.0
Methanomicrobia (*Euryarchaeota*)	4.2	8.3	93.3
Low vs. High			
Thermoprotei (Crenarchaeota)	17.2	32.2	32.2
Pacearchaeota	13.4	25.2	57.5
unclassified *Archaea*	7.6	14.2	71.7
Thermoplasmata (*Euryarchaeota*)	6.2	11.6	83.2
Methanomicrobia (*Euryarchaeota*)	4.9	9.3	92.5

Dissim., Bray-Curtis Dissimilarity values; Con. %, percentage of the contributed difference; Cum. %, Cumulative percentage.
* representatives with >5% contribution

S-Table 7: Summary of similarity percentage (SIMPER) analysis. Listed are bacterial phyla and class representatives contributes with > 5% most to differences between the sediment cores.

Taxon	Dissim.	Cont. [%]	Cum. [%]
phyla*			
Reference vs. Low			
Proteobacteria	15.2	33.6	33.6
Firmicutes	12.9	28.4	62.0
Bacteroidetes	4.2	9.2	71.2
Chloroflexi	3.3	7.3	78.5
Actinobacteria	2.1	4.7	83.2
Reference vs. High			
Proteobacteria	26.2	46.4	46.4
Chloroflexi	8.8	15.5	61.9
Firmicutes	8.1	14.2	76.1
Bacteroidetes	5.0	8.8	84.9
Aminicenantes	3.0	5.3	90.2
Low vs. High			
Firmicutes	18.9	34.9	34.9
Proteobacteria	10.9	20.2	55.1
Chloroflexi	8.8	16.3	71.4
Aminicenantes	3.4	6.4	77.8
Actinobacteria	3.0	5.5	83.3
class*			
Reference vs. Low			
Deltaproteobacteria (Proteobacteria)	19.1	30.8	30.8
Clostridia (Firmicutes)	11.9	19.3	50.1
Bacteroidia (Bacteroidetes)	4.4	7.1	57.2
Reference vs. High			
Deltaproteobacteria (Proteobacteria)	26.1	41.8	41.8
Bacteroidia (Bacteroidetes)	5.0	8.0	49.8
Clostridia (Firmicutes)	4.6	7.4	57.2
Dehalococcoidia (Chloroflexi)	4.4	7.1	64.3
Negativicutes (Firmicutes)	3.6	5.7	70.0
Low vs. High			
Clostridia (Firmicutes)	17.1	26.7	26.7
Dehalococcoidia (Chloroflexi)	5.8	9.1	35.8
Deltaproteobacteria (Proteobacteria)	5.8	9.1	44.9
Betaproteobacteria (Proteobacteria)	4.0	6.3	51.2
Negativicutes (Firmicutes)	4.0	6.2	57.4
Anaerolineae (Chloroflexi)	3.6	5.7	63.1
Aminicenantes	3.4	5.4	68.4

Dissim., Bray-Curtis Dissimilarity values; Con. %, percentage of the contributed difference; Cum. %, Cumulative percentage.
* representatives with >5% contribution

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7. Curriculum Vitae

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8. Publications and Presentations

Publications

Gwosdz, S., Gemeni, V., Ziogou, F., West, J. M., Jones, D., Rakoczy, J., Green, K., Blöthe, M., Barlow, T., Koukouzas, N. and Krüger, M. *Euryarchaeota* and *Chloroflexi* representatives dominate terrestrial CO_2 vent. FEMS Microbiology Ecology, submitted.

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