Introduction

Carbon dioxide Capture and Storage (CCS) sites are considered as a valid option to 'permanently'store CO_2 from large anthropogenic point sources. Although significant leakage from CCS sites is not expected, if it did occur there could be adverse environmental consequences, that may be difficult to predict. In marine environments the potential leakage could cause local high concentrations of CO_2 and consequently a pH decrease, with possible significant impact on marine organisms. The effects of CO_2 increase on marine ecosystems as well as the consequent microbiological feedback remain largely unknown. In this context it is important to evaluate changes in microbial community structure (functional groups) and activity in response to CO_2 exposure. The response of benthic microbial assemblages to pH reduction also represents one of the main ways of assessing the impact of direct ocean storage of CO_2 on the overall functioning of the ecosystem.

In the framework of the "Research into Impacts and Safety in CO_2 Storage" (RISCS) EU Project, OGS performed a set of experiments aimed at determining short-term and long-term effects on marine communities in response to CO_2 exposure. Here we report some preliminary results from two dedicated mesocosm experiments carried out in order to evaluate the effect of CO_2 induced pH decrease on microbial benthic communities in spring-summer conditions (18 °C) and in autumn-winter conditions (10 °C).

Experimental design and analytical methods

Sediment was collected at the LTER station C1 in the Gulf of Trieste (northern Adriatic Sea). During both experiments 3 mesocosms with different pH values were set up (6.5, 7 and an aerated control ~ 8). Mesocosms each contained ~ 80 L sediment and 120 L of artificial seawater. The pH was decreased in the water, thus the CO_2 effect was studied on surface sediments). Both experiments had 3 stages: short term effect (4 samplings within the first 5 days), long term effect (4 additional samplings; from day 6 to 21), and a recovery stage (21 days during which all mesocosms were treated with air bubbling). At each sampling time surface sediment (0.5-1 cm thick) was collected using 2-3 sediment cores (5 cm Ø). All mesocosms were kept at an irradiance of ca. 100 µmol photons m^{-2} s^{-1}; a 14:10 Light:Dark cycle was used for the summer experiment whereas L:D 10:14 was applied to the winter experiment. Sampling was carried out at the end of the dark cycle.

The analyses focused on prokaryotic abundance, degradative activities, Prokaryotic Carbon Production (PCP) and other integrative parameters such as microphytobenthic community composition and benthic respiration. Prokaryote abundance was determined using the method of centrifugation of Ravenshlag et al. (2000) and a modified protocol by Lunau et al. (2005). Samples of sediment diluted to a final concentration of 1:110 were filtered on black Nuclepore polycarbonate 0.2-µm-pore-size filters. Filters were mounted on microscope slides, stained with a SYBR Green I-mounting medium Mowiol solution (1:15) and counted by epifluorescence microscopy at 1000 X magnification under a blue filter set.

Extracellular enzymatic activities were assayed using fluorogenic substrate analogues (Hoppe 1993, as modified for sediment by Fabiano and Danovaro 1998) derived from 7-amino-4-methyl-coumarin (AMC) and 4-methyl-umbelliferone (MUF). Fluorescence increase due to MUF and AMC hydrolysed from the model substrates was measured using a Shimadtzu RF-1501 spectrofluorometer (MUF = 365-nm excitation and 455-nm emission; AMC = 380-nm excitation and 440-nm emission). Standard solutions of MUF and AMC were used to produce calibration curves with 0.2 µm-filtered seawater at experimental pH. Triplicate blanks without fluorogenic substrate were used to determine the natural fluorescence increase in the samples not attributable to the tested enzymes.

Prokaryotic Carbon Production was measured following the method of ^{3}H-leucine uptake for sediment samples (van Duyl and Kop 1994, as detailed by Manini et al. 2004). Measurements of radioactivity were determined by a β-counter (Packard Tri-Carb 2900TR). For each sample, three replicates and two ethanol-treated blanks were analysed.

Preliminary results

Here we summarise some of the most evident differences or similarities occurring between CO_2 treated and untreated (control) mesocosms. At 18 °C prokaryotic abundances were lower in both treated mesocosms than in the control during the impact stage. On the other hand, values increased becoming comparable with those measured in the control during the air supply treatment (Figure 1). Slightly higher abundances in the control may suggest some kind of inhibition occurring in the more acidic surface sediments.

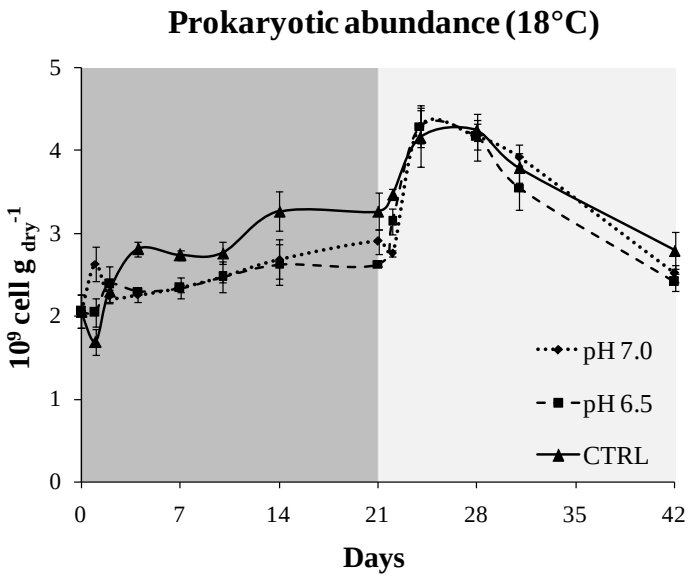

Figure 1 *Prokaryotic abundance in the summer experiment. The left side (days 0-21) is related to the impact stage, the right site to the recovery (days 21-42).*

Some prokaryotic activities displayed marked differences when subjected to higher pCO_2. Lipase activity in treated mesocosms at 18 °C seemed to be inhibited during the impact stage showing lower values than in the control. From the beginning of the recovery stage, lipid degradation rates in treated mesocosms increased reaching values comparable to those measured in the control. An inverse trend characterised the experiment at 10 °C. In this condition, in fact, lipase activity seemed to be enhanced by lowered pH, whereas during the recovery stage values were more pronounced in the control (Figure 2).

Very different trends characterised polypeptides' degradation at 18 and 10 °C. At 18 °C rates measured in the untreated mesocosm increased constantly during the entire experiment, without showing any evident difference from the CO_2-treated ones. Once again, some effect was observed during the winter experiment, when extracellular proteases were inhibited during the long-term CO_2 exposure (Figure 3).

Prokaryotic Carbon Production rates highlight an apparent lack of differences between treated mesocosms and the control both at 18 °C and at 10 °C (Figure 4).

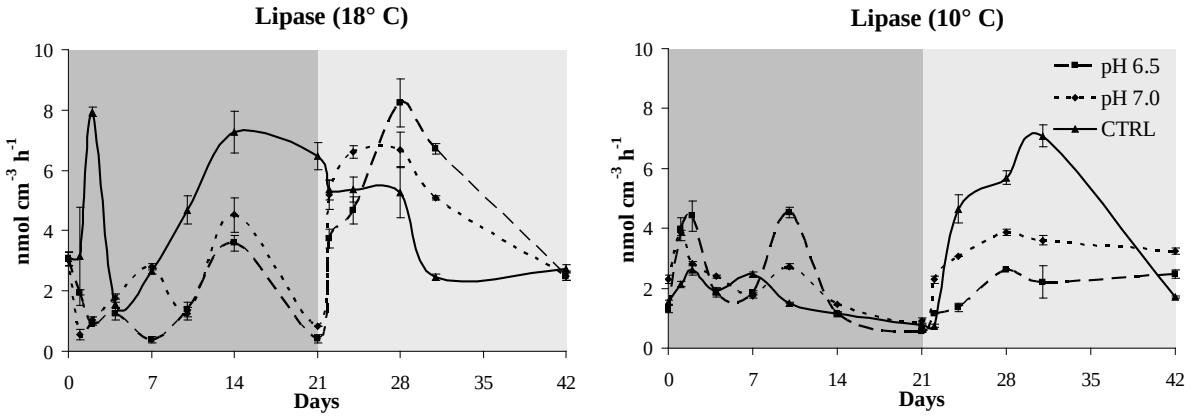

Figure 2 *Lipid degradation rates in summer (left) and in winter (right) experiment. Left sides of each graph are related to the impact stage (days 0-21), right sides to recovery (days 21-42).*

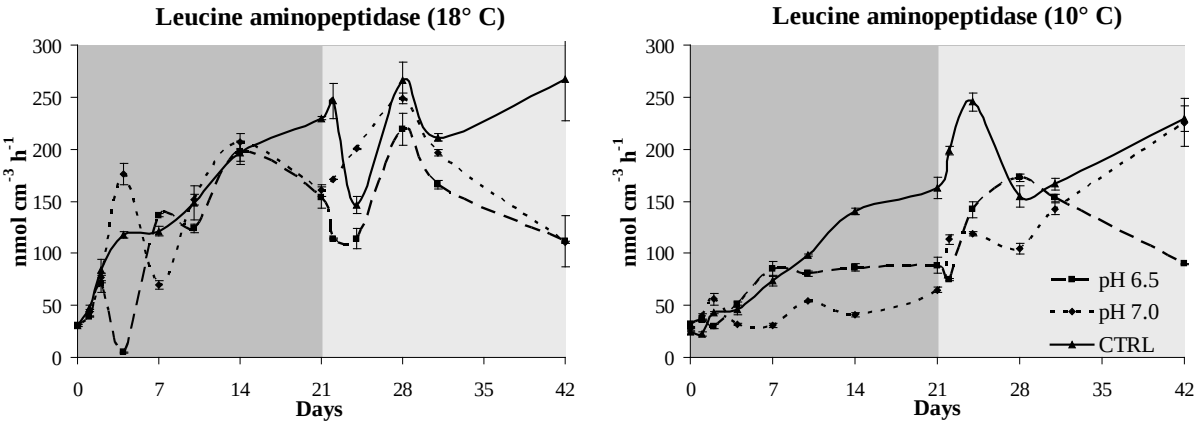

Figure 3 *Polypeptides' degradation rates in summer (left) and in winter (right) experiment. Left sides of each graph are related to the impact stage (days 0-21), right sides to recovery (days 21-42).*

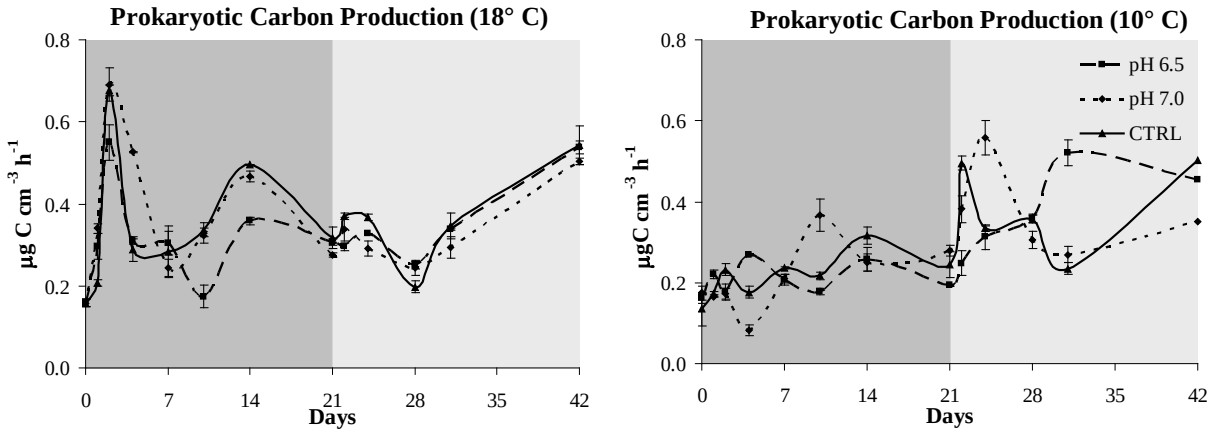

Figure 4 *Prokaryotic Carbon Production rates in summer (left) and in winter (right) experiment. Left sides of each graph are related to the impact stage (days 0-21), right sides to recovery (days 21-42)*

Conclusions

Preliminary results indicate some differences in metabolic response to elevated CO_2 concentration on surface sediment. Some of the tested degradative activities showed a combined effect of pH and temperature on hydrolysis rates, meaning that organic matter reworking and thus its release to the overlaying water and/or burial, could be affected by CO_2 leakage from shallow CCS. The integration of these functional parameters with others, both functional and structural, as benthic respiration, community composition (microphytobenthos, meiobenthos, prokaryotes), and sediment chemical features would enhance the current knowledge on this ecosystem functioning when subjected to elevated CO_2 concentration.

Acknowledgments

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