Using prokaryotes for Carbon Capture Storage

Natalie Hicks*[1], Unni Vik*[2], Peter Taylor[1], Efthymios Ladoukakis[3], Joonsang Park[4], Frangiskos Kolisis[3], Kjetill S Jakobsen[2]

*Contributed equally to this manuscript. [1]Scottish Association for Marine Science, Scottish Marine Institute, Oban Argyll PA37 1QA, United Kingdom, [2]Centre for Ecological and Evolutionary synthesis (CEES), Department of Biosciences, University of Oslo, P.O. Box 1066 Blindern, NO-0316 Oslo, Norway, [3] National Technical University of Athens, School of Chemical Engineering, Laboratory of Biotechnology, 9 Iroon Polytechneiou street, Zografou 157 80, Athens, Greece, [4]Norwegian Geotechnical Institute (NGI), Sognsveien 72, 0855 Oslo, Norway.

Correspondence: k.s.jakobsen@ibv.uio.no (K. S. Jakobsen), Natalie.Hicks@sams.ac.uk (N. Hicks), unni.vik@ibv.uio.no (U. Vik).

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Abstract

Geological storage of CO_2 is a fast-developing technology that can mitigate rising carbon emissions. However, there are environmental concerns with long-term storage and implications of a leak from a carbon capture storage (CCS) site. Traditional monitoring lacks clear protocols and relies heavily on physical methods. Here we discuss the potential of biotechnology, focusing on microbes with a natural ability to utilize and assimilate CO_2 through different metabolic pathways. We propose the use of natural microbial communities for CCS monitoring and CO_2 utilization, and, with examples, demonstrate how synthetic biology may maximize CO_2 uptake within and above storage sites. An integrated physical and biological approach, combined with metagenomics data and biotechnological advances, will enhance CO_2 sequestration and prevent large-scale leakages.

Exploiting properties of natural occurring prokaryotes for enhanced CCS performance

Recently, an *in-situ* carbon capture and storage (**CCS**: see Glossary) leak simulation experiment showed that only a very small fraction (~15%) of incjeted CO_2 into the sub-surface sediments was accounted for across the sediment-water interface [1]. During the gas release, an increase in abundance of CO_2-fixing bacterial taxa accompanied with changes in bacterial activity was seen in the surface sediments [2]. Diverse naturally occurring prokaryotic taxa are able to utilize CO_2 through several CO_2 assimilation pathways (Figure 1), to convert CO_2 into value-added chemicals, or to induce calcium carbonate precipitation. Prokaryotes have a wide range of possible applications in CCS projects, from revealing CO_2 leakages across overlying sediments, to enhanced sequestration by biomineralization of CO_2 and converting the reservoirs to bioreactors for value-added chemicals. Here, we discuss the possibilities of exploiting natural and modified prokaryotic **assemblages** (see Glossary) in CCS projects within a multidisciplinary framework.

Carbon Capture Storage and Carbon Capture Utilization

CCS is a rapidly developing technology mitigating the impact of anthropogenic CO_2 production by capturing CO_2 from large point source emitters and storing it in sub-surface reservoirs, where it should remain for sequestration (see Box 1). A recent CCS pilot study demonstrated rapid mineralization (<2 years) of injected CO_2 into basaltic rocks [3]. However, the potential for sequestration of CO_2 in form of carbonization is limited in conventional CO_2 storage reservoirs such as deep saline aquifers and depleted oil and gas reservoirs[3]. Monitoring of CCS has largely focused on identifying potential causes and implications of a leak (see Box 2). While existing monitoring programs rely heavily on modeling predictions [4] there is a lack of clear regulatory procedures in place to ensure effective monitoring, particularly over a long term basis [5]. Offshore facilities (such as gas and oil reservoirs under the seabed) are particularly challenging, due to the difficulties of access and detection presented by the marine environment (as the reservoir itself is beneath the depth of the ocean water and below the seafloor). Whilst advances have been made on the compliance and monitoring aspects of CCS technology, there is a clear opportunity to not only enhance existing management policies and prevent leaks, but also to monitor changes within and overlying the storage area where the CO_2 is contained. Much of the activity occurring within the storage site and overlying sediments is driven by the activity of microorganisms. The microorganisms' ubiquitous presence in all environments on Earth, combined with their ability to respond rapidly to environmental changes and their various

pathways for assimilating CO_2, makes them ideal candidates for biological CCS monitoring. Targeting prokaryotic taxa or functional genes associated with CO_2 assimilation should be a feasible way of monitoring leakages from CCS. Alongside the development of CCS technology, a complementary field of research has focused on novel carbon capture and utilization (CCU) techniques [6]. CCU technology typically uses a chemical reaction to convert the carbon dioxide into fuels or chemicals for industrial use (e.g. production of urea or salicylic acid, [6]). Despite this synthetic use of CO_2 through chemical reactions, natural biological systems (e.g. microorganisms, photosynthetic organisms) are much more efficient at utilizing large amounts of CO_2 [6]. As such, biological CCU research has started to explore the potential of biotechnology and synthetic biology to enhance these biological processes, and prokaryotic microorganisms are the key to this approach.

Prokaryotes and marine sediments

Marine sediments play a vital role in global biogeochemical cycles, particularly in the carbon cycle [7,8]. The biogeochemical processes in these sediments are driven by physical parameters and the presence and metabolic activity of organisms that dwell in and on the sediment surface. The oceans are huge sinks for carbon, and as the carbon reaches the seabed, a large proportion is sequestered in the sediment, particularly in the deep-sea sediments where light does not penetrate to the **benthos** (see Glossary). The role of **macrofauna** (see Glossary) in benthic biogeochemical processes (e.g., nutrient flux, oxygen cycling, redox reactions) is extensively documented, and the presence and activity of macrofauna enhances this **benthic-pelagic coupling** (see Glossary) [9]. Many of the processes stimulated by macrofaunal activity are mediated by microbial activity [10,11]. Changes in environmental variables such as light, temperature, pH, flow and concentration and availability of organic matter can modify the contribution of species to ecosystem processes [12-15]. Long-term CCS leakages are likely to have several implications for benthic systems (see Box 3 for case study).

Offshore CCS sites are typically situated under extensive layers of sediment and overlying rock formations. Deeper subsurface sediment layers harbor a wide range of **chemolithoautotrophic** (see Glossary) prokaryotes that assimilate energy from inorganic substrates deposited with the sediments or that diffuse into the sediments from below or from above [16]. Due to sediment porosity, oxygen rarely penetrates more than a few mm (or cm) into marine sediments with moderate to high concentrations of organic matter [16]. As a result, the residing prokaryotic communities here rely on metabolic strategies based on

chemical redox-reactions. Prokaryotic communities are able to quickly respond to changes in biotic and abiotic environmental conditions [17], ranging from complete shifts in the species that make up the community through natural selection to changes in **metabolic pathways** (see Glossary) through gene regulation, selection of advantageous genes (and gene variants) present within a population, horizontal gene transfer between closely related (see [18]) or very distantly related microbes (see [19]) or even between kingdoms (such as bacteria and unicellular eukaryotes: see [20]). Whether this effect is due to natural selection at the species level, gene regulation, selection for genes or gene variants or horizontal gene transfer, the final result is a shift in community structure or a shift in metabolic capacity and networks within the community (see [21,22]).

CO_2 assimilating prokaryotes

Prokaryotic communities from marine sediments are linked to sediment type or geographic province, likely reflecting site-specific geochemical and physical conditions[23]. Natural assemblages of prokaryotic communities respond to elevated CO_2 levels by altered community structure, changes in their functional repertoire and shifted biomass measurements [2,17,24-27]. A phylogenetically diverse group of prokaryotes assimilate CO_2 into organic carbon, and to date, six metabolic pathways for CO_2 assimilation have been identified (Figure 1). Prokaryotes capable of assimilating CO_2 are found in a large spectrum of ecologically niches, ranging from environments with low to moderate temperatures to environments with high temperatures; they are found in niches that spans from photic to non-photic zones (Figure 1) and niches that extends to extreme environments at the thermodynamic limit (For more details see [28,29]). The **enzymes** (see Glossary) of the different pathways vary in their degree of oxygen sensitivity, and the pathways can therefore roughly be categorized as aerobic and anaerobic [28]. Assimilation of CO_2 into organic carbon requires four reducing equivalents and an input of energy [30]. Whereas anaerobic prokaryotes often use low-potential electron donors like reduced ferredoxin for CO_2 fixation, aerobes often depend on NAD(P)H as a reductant [28].

In surface sediments and in terrestrial environments, the oxygen-tolerant (Figure 1) reductive pentose phosphate cycle (the Calvin-Benson-Bassham cycle (CBB)), the hydroxypropionate bicycle, and the 3-hydroxypropionate-4-hydroxybutyrate cycle are important. The key CO_2 fixing enzyme of CBB, ribulose-1,5-bisphosphate carboxylase (Rubisco), is the quantitatively most important mechanism of fixing CO_2 in nature, and is utilized by eukaryotes (such as plants and algae) as well as microorganisms. In anaerobic marine sediments overlying

potential CCS sites, the oxygen-sensitive CO_2 fixation pathways (reductive tricarboxylic acid (rTCA) cycle (also known as the Arnon-Buchanan cycle), the reductive acetyl-CoA pathway (Wood-Ljungdahl pathway) and the dicarboxylate-4-hydroxybutyrate cycle) are of particular interest (see Figure 1). The key enzymes for the different pathways are listed in Figure 1. These pathways are only found in prokaryotes, and specific microbes displaying these pathways have been suggested as candidate species for CCS monitoring ([31], Figure 1). Furthermore, specific microbes that have these pathways are capable of converting CO_2 into compounds that can further be utilized, such as methane ([32], Figure 1) and formic acid ([33], Figure 1). Prokaryotic strains have also been shown to trap CO_2 within calcium carbonate ($CaCO_3$) structures [34].

Metagenomics in CCS monitoring

In order to investigate microbial communities' response to environmental changes and disasters (oil or CCS leak), it has been clearly demonstrated that **high-throughput sequencing** (**HTS**: see Glossary) based methods – either by **amplicon** (see Glossary) or **metagenomic sequencing** (see Glossary) – are superior to traditional methods and can have many applications in environmental monitoring (see [31,35-37]). HTS methods range from very focused ones, unveiling the taxonomic and genetic variation in the communities via detection of specific genetic regions (amplicons, such as the 16S rRNA region), to more holistic approaches (metagenomic sequencing), engulfing all of the genomic information available in a given environmental sample. Establishing a CCS monitoring approach would require information from both amplicons and metagenomes, where genes encoding pathways for CO_2 assimilation revealed by metagenomes can be linked to CCS monitoring candidate species revealed by amplicons. By linking HTS-based data to gathered meta-data through specific hypothesis testing, the distribution of community members and their metabolic potential can be related to environmental conditions and allows for detection of small scale changes in microbial response, such as a CO_2 leak [17]. Approaches that extend beyond descriptive single site/single time point "who is there and what are they doing" studies enhance our understanding of which taxa are being selected under certain conditions [26]. The main advantage of HTS methods is the high-resolution data they provide on microbial assemblages and their subsequent response to environmental change, including the differential activation of metabolic pathways. The use of HTS has already been effective in evaluating the response of in situ bacterial populations to increased CO_2, and matching community shifts to metabolic potential [27].

A **metagenomics** (see Glossary) approach, paired with the appropriate automated bioinformatics tools, filtering out bad sequences, sequence assembly (metagenomes), clustering of similar sequences (amplicons), annotation and correlation to metadata, can be applied to an integrated CCS monitoring system, allowing collection and subsequent metagenomics analysis of environmental samples. The essential bioinformatics support for such an endeavor requires an automated solution that can tackle all analytical aspects of the complex and difficult to handle metagenomic datasets. This solution may take the form of **bioinformatics pipelines** (see Glossary) [38], comprising numerous tools that can detect and annotate any genetic markers of interest, making it possible to identify whether certain bacterial assemblages, such as those that favor elevated CO_2 conditions, are present. Such automated bioinformatics pipelines can provide a very intuitive and user-friendly environment for analytical tools for novice users, in contrast to current methods requiring informatics training. Furthermore, this modular-based tool availability provides a flexible environment that can be modified (addition of appropriate tools, customization of databases for marker detection and taxa identification, and so on) for use within a CCS monitoring program. Therefore, a sample from a CCS site can be analyzed using these HTS methods to indicate the presence of a CO_2 leak. Monitoring subsurface benthic microbial changes can directly measure prokaryotes that are able to assimilate and utilize CO_2 as a carbon source, and an increase in their abundance and presence could indicate an elevated supply of CO_2 (from a leak). In existing CCS sites, where sufficient baseline data is often lacking, use of metagenomics techniques would allow detection of a leak site based on a microbial DNA 'fingerprint', and at a smaller leakage scale than that needed to detect biological changes in larger organisms. Candidate genes/species from metagenomic and amplicon studies (Figure 1) can furthermore be used to establish a simplified monitoring approach, where target genes/species can be utilized in microbial diagnostic PCR, amplicon sequencing or targeted microarrays [17]. Such a biosensor for application in CCS leakage scenarios, through measurement of microarrays or functional gene assays, is a feasible possibility. Two examples, both PhyloChip® and GeoChip®, provide information on genes present within microbial communities in samples, and could in principle be developed into accessible tools for simply analyzing microbial changes within the environment. In order to apply a biosensor to CCS monitoring, it is essential that a clear link between specific species, or functional genes, and elevated CO_2 due to a CCS leak, is identified through metagenomics research. This approach will refine specific microbiological signals within the framework of a

biosensor. These two steps are integral in advancing the potential for development of a 'geomicrobial' sensor for use in geosequestration programs, alongside traditional CCS monitoring techniques.

Biological carbon sequestration

In addition to their potential as bioindicators to detect leakages from CO_2 storage projects, prokaryotic communities may play vital roles within the geological CO_2 storage reservoir itself. Over a long timescale (tens of thousands of years), the injected and stored CO_2 may naturally precipitate onto sediment grains within the reservoir as carbonate [39] and be sequestered in a non-labile phase [40]. Several groups of prokaryotes have been reported to be involved in biomineralization processes (microbial induced calcium precipitation, MICP), including sulphate reducing bacteria, ureolytic bacteria and cyanobacteria. Natural prokaryotic communities within the storage reservoir may act as biomediators for enhanced carbon sequestration (e.g. through biomineralization) and 'speed up' the process of calcium carbonate ($CaCO_3$) precipitation at CCS injection sites (mineral trapping) [40]. A growing field of research on biological CCU has started to explore the potential to enhance these biological processes through manipulating the microbial communities. By inoculating or replacing natural prokaryotic communities within the storage reservoirs with strains able to convert CO_2 to into a solid state (e.g. $CaCO_3$), the rate of mineralization can be significantly increased. MICP can occur as a by-product of several metabolic activities, such as urea hydrolysis, photosynthesis, sulphate reduction or nitrate reduction [41]. Precipitation of carbonates by ureolytic bacteria can produce high amounts of carbonates in short periods of time [34] and provides a viable mechanism to induce subsurface $CaCO_3$ precipitation [40]. Furthermore, bacterial biofilm formation has been shown to reduce the porosity of a synthetic system, mimicking a prospective CO_2 injecting site, and thereby reduce the potential of CO_2 leakage from the reservoir to the surface [40]. Introducing prokaryotic taxa that actively convert CO_2 to another form (e.g. $CaCO_3$), as well as reduce the porosity within the CCS-site through biofilm formation, to CCS sites has huge potential for enhancing carbon sequestration of CCS projects.

CCS as bioreactors

CO_2 is regarded as a chemically stable and an unattractive raw material based on energy utilization and economic input [42]. By utilizing and maximizing the ability to bioconvert CO_2 into value-added chemicals, CCS may become economically profitable [6]. Research

and development on *in-situ* bioconversion of CO_2 in oil reservoirs by prokaryotes is currently an active area with high potential [42-44]. Injected CO_2 from CCS-projects may alter the indigenous microbial community and the metabolic pathways in deep subsurface environments that may, in turn, dictate the fate of CO_2. Several natural occurring anaerobic prokaryotes are able to convert CO_2 to a variety of different chemicals (including ethanol, acetate, acetone, lactate, butanol, 2,3-butanediol, valeroate, caproate, carpylate, closthioamide, methane and formate). Microbial activity depends on many environmental factors, including temperature, pH, concentrations of electron donors and acceptors, concentration and diffusion rates of nutrients and metabolites, so natural microbial assemblages in storage reservoirs may not be suitably adapted to the environment surrounding the injected CO_2. Studies have shown decreasing overall prokaryotic biomass with increasing amounts of CO_2 [2], but nonetheless, a few taxa apparently thrive under elevated CO_2 levels [26]. High-temperature oil reservoirs are promising bioreactors for CO_2 bioconversion and have been suggested for production of methane [42]. To successfully utilize CCS reservoirs as bioreactors, the reservoirs may be inoculated with prokaryotic taxa and/or communities that are able to withstand high concentrations of CO_2, whilst at the same time being able to convert the compounds into value-added chemicals. This can be achieved either through inoculating the reservoirs with natural prokaryotes or by introducing engineered or even synthetic prokaryotes. Recently, Yang and colleagues [42] showed that addition of formate (as a source of substrate and for low-potential electron donors, such as ferredoxin) to the production water of high-temperature oil reservoirs resulted in CO_2 conversion to methane through syntropic formate oxidation coupled with CO_2 reducing methanogenesis and formate methanogenesis. The methane production in this study was nearly equal to the formate consumed; an indication that the methane produced was by formate reduction directly or indirectly.

It is necessary to identify highly CO_2 tolerant prokaryotes to identify the genes that encode enzymes and metabolic pathways that promote withstanding elevated levels of CO_2. Enzymes and metabolic pathways with desired traits (e.g., utilizing CO_2 with maximum efficiency) can then be identified. Using these genes in synthetic and engineered biology could provide a novel way of engineering prokaryotes to alter existing carbon assimilation, fixation or conversion pathways and maximize the efficiency of CO_2 utilization. Genetic engineering of microbes such as *Escherichia coli* and *Saccharomyces cerevisiae* has allowed the conversion of these organisms into valuable chemicals, e.g. carbon-neutral biofuels and ethanol

production [6]. In terms of biological CCU, through genetic engineering, it is possible to change the properties of key proteins, such as enzymatic activity, tolerance and thermostability. This approach has, for instance, been used to increase CO_2 selectivity and efficiency through manipulation of Rubisco and carbonic anhydrase [6]. Synthetic biology approaches involving nanotechnology combined with protein engineering is an area with huge advancements and provides a useful tool for CCU applications, particularly in terms of CCS. Recently, as a strategy for artificial photosynthesis, a nanowire-bacteria hybrid was constructed for the targeted synthesis of value-added chemical products from CO_2 fixation [45]. On average, each cell yielded $(1.1 \pm 0.3) \times 10^6$ molecules of acetate per second, a rate that is comparable to conventional gas phase catalysts that require much higher temperatures. Advances like this in synthetic biology and technology illustrate the potential of these approaches for CCS projects.

Concluding Remarks and Future Perspectives

The potential of utilizing microorganisms for CO_2 binding and monitoring in CCS projects through an integrated multidisciplinary approach involving all disciplines of physical sciences is enormous, and it could be implemented in marine and terrestrial subsurface CCS projects worldwide. We emphasize that similar approaches, where prokaryotes can be applied to detect environmental changes, and to convert little-valued compounds into value-added compounds, show vast potential. Such uses include a wide array of other environmental monitoring and applications, such as hydrocarbon utilization and detection (and effects of oil spills) and monitoring of various polluting agents through their microbial environmental effects, both in terrestrial and marine environments.

There are several metagenomics issues that need to be addressed before such approaches can be a reality (see Outstanding Questions). These include a cautious optimization and standardization of molecular methods, excluding as many as possible of the known biases (including contaminations) associated with **nucleic acid** (see Glossary) extraction, **PCR amplification** (see Glossary) and sequencing. Furthermore, studies to identify key target species and/or genes among the CO_2 fixing prokaryotes (Figure 1) and thorough testing of prototype monitoring instruments should be performed before these methods can be applied in an automated user-friendly single instrument. Such an instrument may be designed to include all of the steps from sampling to analysis of the samples and would solve the difficulties of collecting sediment through specialized equipment (e.g. U-tube sampling).

299 Enhanced $CaCO_3$ precipitation by prokaryotic communities may ensure carbon sequestration
300 in the CCS-reservoirs and minimize the risk of leakages through undetected or reactivated
301 fractures and faults, and is an area of research that should further be explored, particularly in
302 the light of a recent study demonstrating that mineralization of CO_2 can occur in much less
303 time than previously assumed [3]. Utilization of engineered or synthetic
304 prokaryotes/nanotechnology approaches coupled with addition of electron donors to convert
305 CCS-reservoirs to bioreactors, is an exciting possibility. Currently, we do not know whether
306 this is possible in a single organism, multiple organisms (community) or in a
307 nanotech/synthetic biology setting – nor how efficient such approaches will be. Little is
308 furthermore known on how production of biomolecules within a reservoir affects the
309 dynamics of the indigenous microbial communities, and subsequently how the indigenous
310 microbes influence the performance and fate of bioconversion. There is obviously a lot of
311 fundamental research needed here including evaluating the suitability of prokaryotic
312 species/communities to be inoculated into reservoirs. Additionally, approaches like these
313 require thorough risk assessment. For instance, *ex-situ* testing and modeling microbial
314 dynamics within potential bioreactors to avoid unwanted effects should be carried out and
315 evaluated before any pilot testing is done. Furthermore, scenarios where value-added
316 chemicals, such as methane, a gas with 25 times more potential than CO_2 in global warning,
317 or genetically modified prokaryotes leaks from potential bioreactors should in all cases be
318 avoided. However, we cannot afford not to look into this potential for generation of value-
319 added chemicals from CO_2, both from an economic and environmental viewpoint, and this
320 research area that will highly profit from focused and extensive multidisciplinary studies.

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324

Box 1: Carbon capture and storage

Capture

There are three main methods to capture CO_2 generated from fossil fuels (as detailed in the IPCC 2005 report):

a) Post-combustion (capture of CO_2 from flue gases of fuel combustion, normally using a liquid solvent)
b) Pre-combustion (production of a saturated synthesis gas in air or oxygen, and separation of CO_2 and hydrogen)
c) Oxyfuel combustion (pure oxygen used to produce a flue of CO_2 and water)

Storage

After capture, the CO_2 is compressed, often into a '**supercritical** fluid' (see Glossary), and transported to a reservoir where it is stored for geological time scales [46]. Storage reservoirs typically consist of deeply buried porous and permeable rock, blanketed by at least one layer of physically impermeable rock, commonly known as the '**cap rock**' (see Glossary). The cap-rock usually consists of shale and or clay, and sits above the storage formation (see Glossary) [46]. On the most basic level, the reservoir, the cap rock and the "**overburden**" (see Glossary) are shaped in one of a range of possible geometries, each of which means that any fluid injected into the reservoir and which tends to migrate upwards due to buoyancy will be trapped inside the structure. Over time, it is probable that injected CO_2 will firstly dissolve in pre-extant pore fluids within the reservoir (whether this is saline water or hydrocarbons) and may eventually, over tens of thousands of years, precipitate onto sediment grains within the reservoir as carbonate [39].

There is a financial incentive to using CCS in oil drilling operations: enhanced oil recovery (EOR). When producing oil from a reservoir, it is common practice to inject a fluid into the reservoir [46]. This fluid (commonly sea water or brine from a saline aquifer) serves two purposes: it replaces a volume of oil that has been extracted and serves to maintain reservoir pressure and aid production, but it can also be used to "sweep" oil from distant areas of the reservoir towards production wells. Injecting CO_2 as a substitute for the fluid will fulfil both of these purposes, but it will also dissolve in the oil. This process reduces the viscosity of the oil and its surface tension, allowing greater production of the reservoir, with retrieval estimates as high as approximately 25% more oil [46].

356 **Box 2: Environmental impacts of CCS**

357 Much of the environmental concerns around CCS sites center on the potential implications of
358 a CO_2 leak. Despite the many precautions that may be taken prior to implementation of CCS,
359 there remains a possibility of a leak from an injection facility. The two most likely leak
360 scenarios are abrupt leakages (through injection well failure or abandoned well leakage) or
361 gradual leakages (through undetected or reactivated fractures and faults) [46-48]. The leaking
362 CO_2 will migrate upwards and eventually reach the surface sediment layers and overlying
363 water. Large-scale leakage of CO_2 from the storage site into the overlying water and sediment
364 layers will cause the seawater to acidify, resulting in a range of effects on the organisms
365 present [49,50], and directly impact processes such as nutrient cycling. Although marine
366 ecosystems are adapted to cope with temporal and spatial changes in pH, rapid and extreme
367 changes to environmental pH and seawater chemistry outside of this range are likely to be
368 detrimental to organisms, directly impacting health, activity and survival, resulting in high
369 mortality across many species in large scale leakages [51].

370 A growing body of research on the effects of lowered pH in the ocean, as a consequence of
371 ocean acidification driven by elevated atmospheric CO_2, has demonstrated predominantly
372 negative effects on marine organisms [49,50], and ecosystem processes such as primary
373 production and nutrient cycling [52,53]. To date, most research has focused on 'open ocean'
374 species and ecosystems [54]. The effects of elevated CO_2 on benthic systems and their
375 contribution to biogeochemical cycling remain less understood, with the exception of a few
376 studies which have focused on macrofaunal impacts [9,55,56]. Many benthic processes are
377 driven by the activity and metabolism of microbial communities (often dominated by
378 prokaryotes at depth), but little detailed attention has been given to their role in benthic
379 processes under changing environmental conditions. Research has shown that microbial
380 communities respond to changes in CO_2 [2,17,24,25] through altered community structure
381 and biomass changes. A CO_2 leak from a CCS site will have ecosystem-wide consequences
382 from microbial scale to higher trophic levels, particularly as the concentration of CO_2 will be
383 much higher than that used in manipulative experiments simulating ocean acidification.

384

385 **Box 3: A case study: environmental impacts of CCS**

386 Different approaches have been used to quantify the impacts of a CCS leak, from modelling
387 techniques [57,58], to manipulative **mesocosm** (see Glossary) studies with elevated CO_2 [59-
388 61] and studies around natural CO_2 seeps [62,63]. However, these approaches are not ideal,
389 as they either lack understanding of ecological or biological responses (modelling) [64]; lack
390 natural variability (mesocosms) [65] or provide no opportunity to establish a baseline or
391 measure recovery (natural CO_2 seeps).

392 To address these concerns, a field scale experiment was conducted that simulated the impact
393 of CO_2 leaking from a sub-seabed reservoir [66], whilst providing a baseline and monitor the
394 recovery after release.

395 The experiment took place on the west coast of Scotland in 2012 [1,66,67]. A pipeline drilled
396 into the seabed through which a total 4200 kg of CO_2 was released into the sediments over 37
397 days [66]. Changes in benthic processes and characteristics [68,69]; macrofauna species [70];
398 and microbial response [2] were examined. Monitoring was carried out through geophysical
399 monitoring of gas propagation [71] and modelling of CO_2 bubble dynamics [72,73].

400 During gas release, changes within the pore-water chemistry (lowered pH; increased
401 dissolved inorganic carbon, DIC) [68,69] were measured, although these parameters returned
402 to normal within a month of stopping gas release. Benthic macrofaunal abundance and
403 diversity was negatively affected (i.e., abundance and biodiversity declined) during the gas
404 release phase, but both also recovered quickly once leakage had stopped [74]. Changes in the
405 microbial community were much more rapid than macrofaunal effects [2], and corresponded
406 with the sediment porewater properties. Microbial abundance (as measured as16S rRNA)
407 increased after 14 days of gas release, both at the gas release point and up to 25m away, and
408 showed clear changes in microbial diversity [2]. However, a decrease in the microbial
409 abundance (16S rRNA genes) was measured during the initial recovery phase, and this
410 corresponded to the highest measured levels of pore-water DIC [2], and the potential increase
411 of toxic metals.

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Figure 1.

Phylogenetic representation of the diversity of CO_2 assimilating prokaryotes (bacteria; darker grey, Archaea; lighter grey) using either of the 6 CO_2 assimilation pathways; the reductive pentose phosphate cycle (Calvin-Benson-Bassham cycle; cyan), the reductive tricarboxylic acid cycle (Arnon Buchanan cycle; yellow), the reductive acetyl-CoA pathway (Wood-Ljungdahl pathway; grey), the 3-Hydroxypropionate bicycle (light blue), the 3-Hydroxypropionate-4hydroxybutyrate cycle (pink) and the Dicarboxylate-4-hydroxybutyrate cycle (green). The figure legend denotes [1]chemolithotrophic taxa and [2] photosynthetic taxa. The key enzyme(s) of each pathway is(are) listed along with their enzymatic reaction(s).

Glossary

Amplicon – a section of nucleic acid (DNA or RNA) that is the source or the result of amplification or replication (whether an artificial or natural process)

Assemblage - the identity (presence/absence) and/or relative abundance of species that make up a community (e.g. bacterial assemblage refers to the bacterial species within that community)

Benthic-pelagic coupling - processes that occur over the sediment-water interface e.g. biogeochemical cycling

Benthos - seabed / seafloor (can refer to substrate or habitat)

Bioinformatics - A sub-discipline of biology and computer science concerned with the acquisition, storage, analysis, and dissemination of biological data, most often DNA and amino acid sequences

Bioinformatic pipeline - A set of bioinformatic tasks that are configured to run consecutively in an automated way

Cap rock - an impermeable formation located above a storage formation that prevents injected CO2 from escaping or leaking

CCS – Carbone Dioxide Capture and Storage or Sequestration

Chemolithoautothrophs - organisms that utilize chemicals (chemo) from the bedrock (litho) as an energy source for making their own (auto) food (troph)

Enzyme - A biological catalyst that is almost always a protein. It speeds up the rate of a specific chemical reaction in the cell. A cell contains thousands of different types of enzyme molecules, each specific to a particular chemical reaction.

High throughput sequencing (HTS) – Nucleotide sequencing where more than one sample can be processed in parallel, generating a high number of sequences, often applied for sequencing platforms such as Illumina, 454 and PacBio

Macrofauna - invertebrates that live within or on the sediment or hard substrate; often classified by size (often defined as organisms greater than 250 or 500µm)

Mesocosm - container/tank used as an experimental tool to manipulate and control the natural environment

Metabolic pathway - series of biochemical reactions occurring within a cell

Metagenomics - The study of genetic material from mixed templates, such as from environmental samples

Meta-processing - Data processing that involves handling and filtering of large datasets, advanced search queries and statistical analysis

Nucleic acid - DNA and RNA

Overburden - Denotes all formations above a storage formation up to the top surface or seabed/seafloor

Polymerase chain reaction (PCR) amplification - a laboratory technique used to amplify DNA sequences by using short DNA sequences (primers) to select the portion of the genome to be amplified.

Storage formation - a reservoir that is used to store any kind of fluids or waste (e.g. cutting injection, captured CO2, etc.)

Supercritical CO_2 - A fluid state of carbon dioxide where it is held at or above its critical temperature (304.25 K) and critical pressure (72.9 atm or 7.39 MPa)

Underburden - Denotes all formations below a reservoir storage formation

Outstanding Questions box

Can microbial use of CO_2 form the principle of a viable approach of monitoring Carbon Capture Storage (CCS) by measuring the genes that drive this process? If so, what are the best genetic indicators and the most efficient molecular approach for a semi- or fully automated system for measuring this activity?

Can natural microbial communities bind CO_2 in a sufficiently efficient way? Is it feasible to use natural assemblages of microbes capable of utilizing the needed pathways for autotrophic CO_2 fixation (including energy input) as an ecologically adapted system?

What are the implications of methane (CH_4) production as a side effect of CO_2 fixation? Methane is a greenhouse gas that contributes to global warming, and is 20 times more efficient in retaining heat than CO_2. Many microbes use carbonic anhydrase to convert CO_2 to CH_4 in anoxic conditions. Methane is currently the focus of many bioenergy studies, so this could be harnessed to provide energy in the form of natural gas. Would large levels of CO_2 fixing bacteria/communities exposed to CO_2 produce large volumes of CH_4, and how would this be captured in a marine environment? Would we be swapping the solution of one problem (elevated CO_2) for another environmental issue (elevated CH_4)? Can we use additional microbes (i.e. methanotrophs) to utilize the CH_4?

Can genetically modified micro-organisms be introduced at a CCS monitoring site? Release of genetically engineered microbes represents a substantial ecological and environmental risk. Thorough investigations of the biology and ecology of the modified microbes will aid in risk assessment. It should be feasible to engineer the microbes in such a way that they will only survive within a CCS compartment. Risk assessment will have to take into account the benefits of CO_2 capture vs. potential ecological negative effects or risks. A strong research and environmental focus is needed here, while the decision will remain a political issue of international character.

Key Figure

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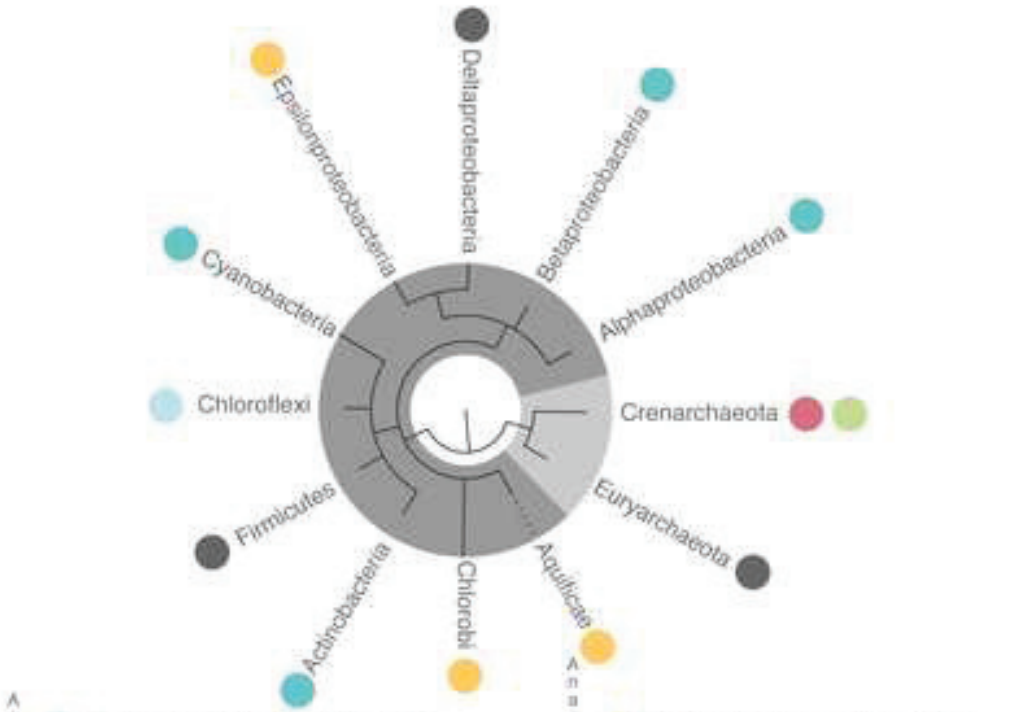

Key enzyme	Enzymatic reaction
Reductive pentose phosphate cycle (Calvin-Benson-Bassham cycle)	
Rubisco	D-ribulose 1,5-bisphosphate + CO_2 + H_2O<=>2 3-phospho-D-glycerate
Phosphoribulokinase	ATP + D-ribulose 5-phosphate<=>ADP + D-ribulose 1,5-bisphosphate
3-Hydroxypropionate bicycle	
Malonyl-CoA reductase	malonate semialdehyde + CoA + $NADP^+$<=>malonyl-CoA + NADPH + H^+
3-hydroxypropionyl-CoA synthase	3-hydroxypropanoyl-CoA + diphosphate + AMP<=>3-hydroxypropanoate + CoA + ATP
Malyl-CoA lyase	(S)-malyl-CoA<=>acetyl-CoA + glyoxylate (2R,3S)-2-methylmalyl-CoA<=>propanoyl-CoA + glyoxylate
3-Hydroxypropionate 4-hydroxybutyrate cycle	
Acetyl-CoA-propionyl/CoA carboxylase	ATP + propionyl-CoA + HCO_3^-<=>ADP + phosphate + (S)-methylmalonyl-CoA
Methylmalonyl-CoA mutase	(R)-methylmalonyl-CoA<=>succinyl-CoA
4-hydroxybutyryl-CoA dehydratase	4-hydroxybutanoic acid + ATP + CoA<=>4-hydroxybutyryl-CoA + AMP + diphosphate
Reductive tricarboxylic acid cycle (Arnon-Buchanan cycle)	
2-Oxoglutarate synthase	2 reduced ferredoxin + succinyl-CoA + CO_2 + 2 H^+<=>2 oxidized ferredoxin + 2-oxoglutarate + CoA
ATP-citrate lyase	ADP + phosphate + acetyl-CoA + oxaloacetate<=>ATP + citrate + CoA
Reductive acetyl-CoA pathway (Wood-Ljungdahl pathway)	
Acetyl-CoA-synthase/ CO dehyderogenase	CO + CH_3-CFeSP + CoA<=>Acetyl-CoA + CFeSP CO_2 + 2H^+ + 2e<=>CO + H_2O
Dicarboxylate-4-hydroxybutyrate cycle	
4-Hydroxybutyryl-CoA dehydratase	4-hydroxybutanoic acid + ATP + CoA<=>4-hydroxybutyryl-CoA + AMP + diphosphate