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***Interactive comment on* “Reviews and synthesis: Carbon capture and storage monitoring – an integrated biological, biophysical and chemical approach” by N. Hicks et al.**

N. Hicks et al.

natalie.hicks@sams.ac.uk

Received and published: 1 October 2015

Reviewer One

We thank reviewer one for the very valuable comments. We agree that it would not be a very useful guide to a planning and monitoring system, as this is not our intended aim of this review. We have redefined our aims in our manuscript to reflect this, and reiterate that this paper reviews the current fields of science that encompass CCS monitoring, summarises the present knowledge and draws some suggestions for future monitoring using cutting edge techniques. It is important to bear in mind that HTS monitoring of CCS has not been implicated at any site yet (to our knowledge),

C6053

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

Discussion Paper



and thus there is a need for additional investigations before such monitoring programs can be implemented. However, the fields of microbial monitoring using HTS are advancing extremely fast (both within medical and environmental applications), and the rationale behind this review is to present this opportunity, including the principles and challenges, to CCS monitoring and the biogeosciences community in general. Thus, the overall paper is written to appeal to a general audience without the need for specialist knowledge in all scientific areas. We have not included recommendations, as this is outside the scope of the paper, and with such little relevant knowledge on monitoring techniques to date, we can only suggest potential solutions and an aspect of microbial technology that may be a useful tool in improving this monitoring ability.

We accept that the paper can be reordered as suggested, and to this effect we have changed the manuscript so it begins generally (what is CCS, implications of a leak, etc) and then delves into the detailed microbial work.

Specific responses to points raised:

1. We have reworded the abstract to reflect our focus on benthic systems, although this does not imply that they are more vulnerable than pelagic. Merely, the focus of our paper is angled towards benthic parameter. We agree that pH changes are an effect of elevated CO₂, but again, the pH changes are not the aspect we have focused on.
2. We understand that the use of the word ‘baseline’ is misleading, and have changed this to reflect what we actually mean i.e. “CO₂ driven changes in microbial assemblages”. We demonstrate the changes in microbial assemblages as a result of elevated CO₂ later in the manuscript (see section. . .)
3. We were missing a comma between pH and flow, this has been inserted.
4. We agree with reviewer 1, in that the microbial contribution to biogeochemical processes is significant. In fact, the sentences following this, we acknowledge and highlight the role of microbial activity in biogeochemical cycling, and place it in the context

[Full Screen / Esc](#)[Printer-friendly Version](#)[Interactive Discussion](#)[Discussion Paper](#)

of macrofaunal measurements.

5. Changed as suggested.

6. Compared to the amount of sequence data previously obtained by conventional (Sanger) sequencing, 10,000s of OTUs is indeed a lot of data – however, when we are discussing sequencing of prokaryotes, and compared to the wealth of information we can generate using Next Generation Sequencing, this figure is a small amount. We are confident that a ‘glimpse’ is the correct terminology, as microbial assemblages are dynamic in time and space, and any ‘spot’ measurement provides a small freeze frame of data collected that is only accurate at that time point.

7. As stated earlier, the use of HTS monitoring of microbial activity at CCS sites is a novel idea and has not yet been implemented. In response to the reviewer, yes, we anticipate that initially the data will be acquired to specifically test a hypothesis, such as whether there is an increase in bacteria associated with fixation of CO₂ and elevated CO₂ levels

8. The reviewer is right that the correlations do not imply causality, and that the correlations that we find may be caused by an unobserved /unmeasured variable that causes the observed correlation. However, a consistent correlation observed across the gradients in marine sediments needs an explanation and we argue that the variables that are measured in these studies are highly likely to cause these patterns.

9. By their very nature, bacterial communities are able to rapidly adjust to changes in the environment, and can switch their metabolic focus, or turn it on or off. Another feature of bacteria is that they are capable of acquiring genes from other bacteria (or from the environment) – so called Horizontal Gene Transfer (HGT). HGT events are promoted by a certain selection pressure (such as high CO₂ levels – or as well documented from the medical field, particularly in the use of antibiotics). In this case discussed in the paper, the bacteria were able to switch to take advantage of the increase in hydrocarbons as an energy source. Whether or not this was a pure selection

BGD

12, C6053–C6062, 2015

Interactive
Comment

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

Discussion Paper



process (selection for bacteria with such metabolic properties), a regulatory (switching on or off genes) or HGT we do not know. This is also accompanied by an increase in the quantity of 16S, and an abundance of such bacteria as suggested by the reviewer.

10. The increase in 16S rRNA abundance implies the bacteria grew, so in other words they generated more biomass per gram of sediment. We have adjusted our phrasing to make this clearer.

11. Ocean acidification is indeed a separate research issue to CCS, however, parallels can be drawn between the two research topics as they both examine the effects of elevated CO₂, although from different sources and on different orders of magnitude. CCS research is still in relative infancy, whilst there is a wealth of information in the ocean acidification field that gives us some indication of the effects of elevated CO₂. As reviewer 1 actually stated at the start of his/her review, ocean acidification studies show variability in responses, and we expect to see this in the case of a leak from a CCS site, as this will result in elevated CO₂ levels, similar to those often used in ocean acidification simulation experiments.

12. We understand that defining 'chemolithoautotroph' may seem unnecessary for a scientist that is familiar with these organisms. However, we are targeting a non-specialist audience, so we have defined terms we feel may not be understood by someone outside of that scientific area, and hence why we have also included a glossary in the supplementary material.

13. We are focusing on benthic prokaryotes that fix CO₂, and feel that the reviewer may have misunderstood this, as elevated CO₂ does not provide a risk to these fixation pathways. Our hypothesis is that metabolic pathways that fix inorganic carbon into organic material, i.e, the released CO₂ into organic material, will increase under elevated CO₂ scenarios. This can be detected using HTS of metagenomes within the sediment. The metabolic pathways represented in table 2 are all autotrophic, and have the potential to utilize released CO₂ under a leakage scenario, and are all found

[Full Screen / Esc](#)[Printer-friendly Version](#)[Interactive Discussion](#)[Discussion Paper](#)

in prokaryotes, which is the focus of this paper. This has been clarified in the table heading and in the main manuscript.

14. We have reworded this to specifically state that this means they have the potential to be exploited as a commercial applicant.

15. We have replaced 'fixate' with 'fix'

16. This is one definition of 'tautology', yes, but we use the 'tautology' definition whereby a fact is known or understood as it is logical. However, to avoid any confusion we have removed it in this instance.

17. Yes, this is a good analogy and we are pleased the reviewer agrees.

18. We have clarified this section and given some examples to make it more specific: "This results in various mechanical and biogeochemical responses at each section of the subsurface and at different time scales, e.g. from diurnal change all the way through to geological timescales. CCS projects may have a positive or negative effect on these processes, such as a change in ability to sequester carbon in the sediment, or physiological response of organisms to environmental changes, which in turn will have a knock on effect on the services they provide to society."

19. Yes, this is a first step, this is a statement of current practice, not a recommendation or a guideline.

20. We have modified this to fit within the scope of the paper "A multifaceted approach should include physical and chemical data on the sea floor sediments, the ecology and biology of the benthos as well as metagenome data that describes and characterize changes in the composition and the activity of the benthic microbial community assemblage. Such a monitoring programme may be more sensitive to small scale, or incipient leaks due to the responses of certain parameters, allowing high-resolution and early detection aspects to any such monitoring project. We suggest that incorporating next generation sequencing techniques into a monitoring program would allow quick and

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

Discussion Paper



easy identified of CO₂ driven changes in microbial assemblages”

21. Models do indeed have weakness, and we stand by our statement, as models are only as good as the data they are based on. For example, a model monitoring or predicting changes in a CCS site would require robust ground work data, and we have illustrated that this amount of data is not currently collected at CCS sites. The example that the reviewer has given on the use of the Met Office is a good example of a model supported by a huge extent of data, from a global scale for general trends to a local scale with more detail. Sadly, this sort of data is not available for CCS sites, and each site is different and distinct.

22. Mesocosm (and microcosm) studies exclude a large proportion of the background ‘noise’ / environmental complexity to allow specific testing of a theory. These kind of studies would be the first stage in an environmental hypothesis testing study, - before the theory can be tested in situ we need to have a comprehensive understanding of how the natural system may respond so we know what measurements / changes to look for. We refer the reviewer to Benton et al (2007) paper, which details the benefits and drawbacks of using mesocosm systems.

23. The mentioning of Sanger sequencing is highly relevant to this review. It is not just about costs (which are indeed highly reduced) but it is also the nature of HTS representing a massive parallel sequencing, and thus generating a “resolution” of sequence information providing an unprecedented in-depth analysis of all kinds of microbes (including also low abundant organisms).

24. This section describes the development and the subsequent potential of bioinformatic approaches for applications in CCS monitoring systems by identifying bacterial assemblages that are predominantly present in elevated CO₂ conditions. We found it trivial to include all the technical details, bioinformatic tools and methodologies as well as the corresponding solutions providing intuitive GUIs which are available for such tasks. However, a paper describing all the different solutions has already been pub-

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

Discussion Paper

lished by Ladoukakis et al. 2014 (doi: 10.3389/fcell.2014.00070) and we have included this reference in our manuscript.

25. Table 1 is not designed to be a summary table, merely to demonstrate changes in microbial assemblages along gradients. We accept that in pelagic, open ocean systems, gradients may be less important or clear cut, but in benthic systems, gradients are ubiquitous on a temporal and spatial scale. We feel this table reflects natural gradients that may be present in benthic systems (e.g. oxygen concentration from surface sediment through the first 10-15 centimetres; or a gradient of CO₂ concentration from a CCS site, with higher CO₂ levels at the point of leakage, and decreasing CO₂ levels as you move further away from this point.

26. We agree with the reviewer in that a large proportion of CO₂ fixation is carried out by eukaryotes in the photic zone through photosynthesis. In contrast, prokaryotes fix CO₂ in the absence of sunlight as an energy source, and indeed most CCS sites are deeper than the photic zone, hence the CO₂ fixation in the sediments at this site is dominated by prokaryotes. Phosphate spelling mistake has been corrected, and we thank reviewer 1 for pointing this out to us.

27. Figure 1 – we have defined SCCS (Scottish Carbon Capture and Storage). This figure is not only to illustrate a typical CCS site, but is a useful point of reference for non-specialists to refer to in understanding the different geological terminology used to describe layers within a CCS site e.g. overburden, cap rock etc. We feel this is important since our target audience are generalists, and we found this figure useful within our author list, which reflects a range of different expertise.

28. Figure 2 describes a bioinformatics workflow which is also described in many available solutions for metagenomic data such as SmashCommunity (doi: 10.1093/bioinformatics/btq536) and MetAmos(doi:10.1186/gb-2013-14-1-r2), with the exception maybe of the taxonomic module which is essential for the microbial community characterization in a CSS monitoring project and for which there are numerous tools that can be

BGD

12, C6053–C6062, 2015

Interactive
Comment

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

Discussion Paper



integrated with the above mentioned solutions. We agree it doesn't provide a protocol, as that is not our intention, it is an illustrative suggestion of how such a monitoring procedure may work in the context of CCS monitoring.

Reviewer Two

We thank reviewer 2 for their comments, and address their points as follows:

1. We accept that it may be useful to illustrate changes in phyla shift or function gene relative abundances. To this end, we have created a simple two part figure to go in the supplementary information, which illustrates the changes that may occur (such as changes in gene abundance, loss of metabolic pathways, and turning genes on and off). For specific case studies, we direct readers to key papers (such as Haverkamp et al. 2013 (Oslofjord pockmarcks); Håvelsrud et al. 2012 (Troll oil field) and Håvelsrud et al. 2011 (Coal point)) which are already included as references within our manuscript.
2. Patterns for benthic bacteria are different to those seen in larger organisms e.g. in the event of a sudden increase in CO₂ in the benthos, e.g. from a point source leakage of a CCS site, the larger and more mobile animals will move away from this area. Bacteria, in contrast, are sessile so are unable to move as a response. Instead, they can switch on or off genes, undergo fast selection (within the communities) or even acquire additional genes (through HGT) to adapt to environmental conditions, as detailed in section 3 of our manuscript. More detail can be found in the papers referred to in our sections taken from the QICS example we used as a case study, such as Widdicombe et al, 2015 for larger organisms; and Tait et al 2015 for microbial changes.
3. We are unsure of what the reviewer means when talking about false positives and negatives. Perhaps they refer to the sensitivity of the system to elevated CO₂, or how lab measurements could provide inaccurate results e.g. incorrect priming of the PCR reactions? The reviewer mentions CaCO₃ rich sediments as providing a 'false negative', and we assume this implies that the buffering capacity of these carbonate sediments may decrease the effect of CO₂ as it moves through the sediment, causing

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

Discussion Paper



small scale dissolution. Whilst we accept in theory this is possible, it is likely that the CO₂ will diffuse through the sediment into the water at a faster rate than dissolution can buffer it, so there would still be a clear elevated CO₂ signal within the sediment (through direct measurements of the CO₂ or pH levels, or through analysis of microbial assemblage. In addition, many of these CCS sites do not contain carbonate sediments in the overlying layers, and sites at depth will be below the carbonate compensation depth (CCD), where carbonate sediments are already naturally ‘dissolved’ by the time they reach the benthos.

4. Studies from microcosm systems do indeed require careful interpretation, particularly when looking at ‘scaling up’ to natural system level. However, microcosm studies are an accepted technique for hypothesis testing experiments, allowing the exclusion of natural variation / background ‘noise’ that would interfere with experimental measurements and provide false measurements or lead to incorrect conclusions. By reducing the natural variability, the experiments can be very targeted e.g. measuring changes in microbial assemblages in response to CO₂ elevation whilst maintaining a constant temperature, salinity and light cycle. We refer the reviewer to Benton et al. (2007) paper, which details the benefits and drawbacks of using mesocosm systems.

5. We are pleased the reviewer has highlighted the different sequencing platforms available, and to this extent we have compiled a small table/figure to go in the supplementary information which illustrates the main NGS platforms, and compares these to TRFLP techniques. As of today TRFLP is an outdated way of analysing abundance of bacterial assemblages, requiring intensive procedures to yield relatively little data – NGS has the advantage of providing much more data (including gene sequences with extremely high information content) from the same sediment samples, in a faster way and with an ever decreasing additional cost. The trade-off for the wealth of information vs. cost of analysis tips the balance strongly in favour of NGS – in addition, TRFLP data could not easily be fed into the type of ‘bioinformatics pipeline used to analyse NGS data’.

[Full Screen / Esc](#)[Printer-friendly Version](#)[Interactive Discussion](#)[Discussion Paper](#)

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