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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.

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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); Håvelsrrud 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 aquire 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 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'.

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