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Interactive comment on "The influence of ocean acidification on nitrogen regeneration and nitrous oxide production in the North-West European shelf sea" *by* D. R. Clark et al.

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We thank the reviewer for providing this review. We have attempted to address all points raised as outlined below.

Response to review #2

Within the manuscript field . . . . . .well planned and neatly carried out. Response Manuscript comments requiring no action.

A direct effect of CO2 on bacterial ammonia oxidation or N2O production could not be detected. However, treatment related changes in cell abundance and community

size structure as well as DMS concentrations were observed by other cruise members working with the same bioassays. Results are presented within the same issue Richier et al. (2014) and Hopkins et al. (2014), respectively. An initial decrease in nano flagellate abundance in response to sudden acidification resulted in overall decreased production and nutrient uptake as well as a presumed "stress release "of DMS. The data are as such fairly well presented, and discussed and should thus be published.

Response In upper regions of the sunlit ocean, the rate of nitrification is extremely slow, but increases towards the base of the photic zone. Bioassay water was collected within a few meters of the surface at all stations. We therefore anticipated that measuring a treatment response for nitrification in seawater collected at this depth was going to be a challenge, given that this process is inhibited by light. N2O production, derived primarily from the first stage of nitrification, was also anticipated to be low as only a fraction of N flux through this pathway is directed towards N2O. A treatment response for N2O was also unlikely from the outset under the experimental conditions used.

The strategy to detect correlations between random combinations of variables (p3130) using multivariate statistics appears to be very thoroughly applied and objective in this manuscript. However, it makes the reader fear that important patterns or reasonable differences among locations may be overseen in the search for universal patterns. As pointed out by referee one, for most of the tested pairs educated hypothesis are lacking and pseudo correlations would be likely (e.g. [CO32-] vs. NH4+ oxidation rate).

Response The rationale behind our intension to test for broadly applicable treatment responses was that this is the assumption intrinsically made in marine ecosystem models – i.e. that it is justified to take a simple relationship describing a physiological response to environmental conditions and extrapolate across extended geographical areas. Our results suggest that this is not a robust approach. In our statistical analysis we consider relations for bioassays individually and collectively. We set the context for the number of relationships we consider within our analysis, and the number which could appear to be significant purely by chance (pseudo-relationships as stated by the re-

viewer, 3141;13-15). We will modify the text to make it clear that our analysis indicates that pCO2 explains least of the variability in the parameter set for bioassays both individually and collectively. Had this analysis indicated that there were significant and meaningful relationships between pCO2 and dataset variables to interpret (individually and/or collectively) we would have developed this line of enquiry. However, this was not the result we obtained. Consequently, we do not reject the possibility that relationships can be drawn between pCO2 and variables within this data. We state our over-arching hypothesis in the abstract (3114:24) and the discussion (3139:6) and conclude that evidence did not support the hypothesis. As noted for reviewer 1 we will supplement this over-arching hypothesis with more specific hypotheses to test. However, we have highlighted the limitations of this approach in response to reviewer 1 (i) there is very little information to base such speculation on (ii) the data set is limited. We would add that direct links between a nitrification process rate and an individual state variable (e.g. NH4+ concentration) is likely to be overly simplistic, even though it is used as a modelling term (i.e. the specific rate of nitrification, see analysis by Yool et al 2007 (Yool, Martin, Fernandez, Clark. 2007. The significance of nitrification for oceanic new production. Nature 447: 999–1002, doi:10.1038/nature05885).

Situations as the ones nicely discussed on page 3136 line 22 – 3138 line 8 are highly interesting. Obviously due to inconsistent data, such a discussion concerning the OA data is lacking

Response Yes, this was an intriguing, though limited insight captured purely by chance. Consequently, a similar discussion for OA data could not be planned as bioassay experiments were not undertaken at comparable time points.

However, I miss a discussion on the effect of whether the shock response of small flagellates to increased CO2 postulated by Richier et al. (2014) and Hopkins et al. (2014). That should have had a measurable effect on nitrogen cycling? Are there methodical reasons that the approach did not find differences? Could measurement precision be enhanced by excluding large zooplankton from bioassays (P 3134 line 16-

19)? Were bioassay bottles agitated to keep particles in suspension? Could settling of particles have affected OA results?

Response In bioassays the N-cycle processes investigated were NH4+ oxidation (the first stage of nitrification) and NH4+ regeneration. Nitrification is facilitated by both bacteria and archaea. No clear responses to environmental conditions have been reported in the literature, other than to light. We are therefore unable to suggest why any treatment response for flagellates should extend to bacterial nitrifiers or archaea. Further, as highlighted in the response to reviewer #1, the process of nitrification is sensitive to light. Bioassay seawater was collected form the upper few meters of the water column and consequently, we did not anticipate that high rates of nitrification would be measured. Given that low rates were anticipated, a clear treatment response was unlikely from the outset. In contrast, small flagellates may have contributed to the regeneration of NH4+ if, for example, there was a mixotrophic aspect to their nutrition (Mitra et al 20114, BGS 11:995-1005). In this instance, the likelihood of a treatment response for NH4+ regeneration may have been anticipated. However, results were inconclusive. There is the suggestion that NH4+ regeneration increased with PCO2 treatment for E1 and E3 for both time points, consistent with measurements of bacterial hydrolytic enzyme activity (3140; 15) in response to pCO2. However, this response was not consistent across all bioassay experiments. We have added text to highlight this specific aspect. During these experiments, particles were likely to have been colonised by microbial cells involved in N regeneration activity. We have no information on particle density at the various locations at which bioassays were performed. Bioassay bottles were not agitated during the 96 hour (total) incubations. It is unknown to what extent particle settling could have influenced these results. We have added text to the manuscript to make these points raised by the reviewer.

Technical comments Long sentences e.g. p 3114 line 22 -27 or 3119 line 21-26 should be revised. We have addressed this issue.

Explanation of Fig. 11 is missing in the results part We have included explanatory text

in the results section.

The cruise track is scaled in three ways, distance in km as well as a time scale in Julian days (Fig. 1) as well as the five OA stations named E1-5. It is difficult to directly compare observational data presented on the distance scale in Fig. 3, to nitrogen cycle data in Fig. 4 and 5. Please include days in Fig. 3 or homogenize. Please indicate data points by dots at sampling depth within Fig. 3. These points have collectively been addressed with a re-worked version of Fig 3, scaled to Julian day (linking directly with other figures) and including symbols for both observation stations and OA bioassay stations.

Carbonate chemistry is reasonably well reported, but please replace intended CO2 levels by actual measured partial pressures in bottom category bar (Fig. 8 and 9) and further legends. In this case deviations from intended values are hardly relevant but nevertheless it is misleading to title a 912μatm treatment consequently 1000μatm.

Response. We believe the best resolution to this issue is to clearly state that pCO2 values represent the initial (intended) values. We will modify text as appropriate. This is because the evidence demonstrates that pCO2 drifts and differs between bioassays. Stating that it is 912μatm may apply for 1 bioassay at 1 time point, but not for others, causing problems for combined plots (e.g. MDS plot of Fig 10).

Interactive comment on Biogeosciences Discuss., 11, 3113, 2014.