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Discussions

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.

We have added an additional citation for the dataset, which is now available at BODC:

Richier S.; Achterberg E.P.; Archer S.; Bretherton L.; Brown I.; Clark D.; Dumousseaud C.; Holland R.J.; Hopkins F.E.; MacGilchrist G.A.; Moore C.M.; Poulton A.; Rees A.; Shi T.; Stinchcombe M.; Suggett D.; Zubkov M.V.; Young J.; Tyrrell T. (2014). Ocean acidification impacts on Sea Surface biology and biogeochemistry in Northwest European Shelf Seas: a high-replicated shipboard approach. British Oceanographic Data

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Centre - Natural Environment Research Council, UK. doi:10/s8r.

Review #1 'This is a very interesting paper on N-cycling. . ...However, the authors add another set of incubation experiments where they amended the water with CO2 to produce scenarios of OA at 5 selected stations during the cruise and put focus on this issue throughout the manuscript. From this data set, there is no significant impact to be seen on the N-cycle processes. Although a comparison of in-situ conditions along with OA experiments is a good approach, I would, in this case advocate for a separation of both or the omission of the OA study. Moreover, the focus of the paper and the title should be shifted away from the OA issue to the field data set (4 out of 11 figures are concentrating on the OA study- the others 7 deal with the field sampling). It would add a great deal of information and a good connection of both aspects if the authors could provide pH, DIC or TA data from the various cruise station sampled.

Response. We thank the reviewer for these supportive comments. We respectfully highlight that this manuscript is a contribution to a special issue on ocean acidification. As such, omitting the OA component of this study would not be appropriate. While it could be feasible to split the study into two separate manuscripts (and we were mindful of this logical split prior to submission), this could be inconvenient for the reader who then may wish to recombine them in order to obtain the full picture. For the figures, Figs. 1-3 present regional maps and profiles which apply to both observational and OA work, Figs. 4-7 present observational data while Figs. 8-11 present OA data. Consequently, the balance between observational and OA work presented in our figures is equal. In the interest of avoiding replication of figures/discussion and to ensure that this manuscript remained focused, a full consideration of carbonate chemistry was not provided. Instead, data describing carbonate chemistry dynamics at both observational stations and during OA bioassay experiments is presented and discussed by Rérolle et al, Ribas-Ribas et al and Richier et al elsewhere in this special issue. In our revision we will consider the insights offered by these authors and how they relate to our findings.

It would certainly be helpful for the reader who wants to understand the details of the

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method to give some introduction to the paragraphs and what they are about. For non-specialists the methods approach is confusing e.g. sentences like "The concentration and isotopic enrichment of NO2- was determined by synthesizing sudan-1 in sample volumes of 100–200mL depending on ambient concentration, as described below." What exactly was done, what is sudan-1 and why not measure NO2- concentrations with standards methods and the isotope enrichment with diffusion or denitrifier method? If the new sudan-1 method is used to be able to decrease the limit of determination,please add a sentence in the method section.

Response. The methods used for this study are based on the 15N-isotope dilution techniques combined with detection by Gas Chromatography Mass Spectrometry. The methods have been developed for a range of DIN species (NH4+, NO2-, NO3-) and are capable of determining DIN concentration and isotopic enrichment in the same sample at [DIN] < 10 nmol.kg-1 (i.e. severely oligotrophic), allowing rate processes to be investigated across a wide range of marine systems with appropriate modifications (such as those described in this MS). To our knowledge there are no other methods capable of achieving this flexibility. The methods have been developed over many years and are described in detail in previous publications to which we refer (as outlined below). In order to keep the MS succinct, we present a level of methods detail which we believe is balanced by the available space and refer the reader to publications which present full details (along with the disadvantages of alternative methods for our application, such as isotopic discrimination and low sensitivity associated with the diffusion method referred to by the reviewer). Reviewer #2 commented that the 15N methodology was well elaborated. We do not propose to modify our method descriptions.

Determination of ammonium regeneration rates in the oligotrophic ocean by Gas Chromatography / Mass Spectrometry. D.R Clark, T. Fileman, I. Joint. Marine Chemistry. 2006. 98:121-130.

A method for the determination of nitrification rates in oligotrophic marine seawater by Gas Chromatography / Mass Spectrometry. D.R. Clark, A.P. Rees, I. Joint. Marine

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Chemistry. 2007. 130:84-96.

The significance of nitrification for oceanic new production. A. Yool, A.P. Martin, C. Fernández, D.R. Clark. Nature. 2007. 447:999-1002.

Ammonium regeneration and nitrification rates in the oligotrophic Atlantic Ocean: Implications for new production estimates. D.R. Clark, A.P. Rees, I. Joint. Limnology and Oceanography. 2007. 53:52-62.

Inorganic nitrogen assimilation and regeneration in the coastal upwelling region of the Iberian Peninsula. D. R. Clark, P.I. Miller, E.M.S. Woodward, A.P. Rees. Limnology and Oceanography 2011. 56:1689-1702.

Also, why was the SPE method choosen - due to the low concentrations? And if the concentrations where too low for standard methods how can you be sure to have everything captured on the columns/resin (?) and how did you double-check? Please add a rate of rediscovery for the pre-concentration using the resin.

Response. While the GCMS does have a limit of detection (as with all analytical instrumentation), this does not directly relate to the concentration of DIN in seawater. If the ambient DIN concentration (i.e. the amount of N per unit volume) is very low, then in order to 'collect' sufficient nitrogen for the analysis you simply process a larger volume of seawater for your sample. Consequently, the question becomes one of introducing an optimum amount of nitrogen to the GCMS system (i.e. within its linear range) by making appropriate modifications during sample processing. In all but oligotrophic systems, the challenge with this method is not to collect too much material – ambient DIN concentration is rarely an issue. Sudan-1, and indophenol for NH_4+ analysis, is synthesised with these methods as they are stable compounds under the conditions used and they are amenable to collection by SPE. Recovery efficiencies and other aspects which influence accuracy and precision are represented in the 'Marine Chemistry' papers cited above. We will add a quotation of the SPE recovery efficiency in a revised version of the MS.

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The statistic applied is all fine and suitable to detect patterns in large data sets. What is lacking here is clear hypothesis concerning the variables which are supposed to correlate with others for a specific reason. "an attempt to identify links between nitrogen cycle process rates and environmental variables" is not specific enough. Response. We state our over-arching hypothesis in the discussion (3139; 4-10); 'our hypothesis was that the underlying mechanism through which pelagic biota responded to OA conditions would be independent of location, and that a mechanistic understanding of how N-regeneration (nitrification, NH4+ regeneration) and N2O production responded to OA simulations could be developed.' At the start of the 'Results' section we will add new text to outline specific hypothesis to be tested. It is worth noting that with the exception of light intensity, there is very little information about links between nitrification rates and environmental variables to guide hypothesis formation. The limited number of stations and the use of a single depth further constrain our ability to develop and test our hypotheses.

Would it be possible that the low estimates of N2O saturation are a result of a steady state in the water column with production and consumption at an approximate balance? (page 3136 lines 11-21).

Response. N2O consumption is associated with denitrification and is restricted to low oxygen environments (or possibly the micro-environment of free-floating particle aggregates) and will not represent a significant sink for N2O at the locations investigated. In an oxygenated water column the major source of N2O production is nitrification, the highest rates of which are associated with the base of the photic zone and below. Consequently, there is a separation between N2O source and the atmosphere. In the absence of a strong N2O source in close proximity to the atmosphere, we found that surface waters were approximately at equilibrium with atmospheric concentrations. While this is subtly different from a production-consumption balance, the result is the same. We have added text to the MS to make it clear that we do not expect N2O consumption to represent a significant sink for this study.

Can you say anything about the quantitative effect of the storm event – was more PON produced and was that related to the nutrient uptake. Moreover, which area was affected by the storm event? (page 3137)

Response. We noted that throughout the study PON variability was rather low (3132-25) and have added text specifically comparing the two station visits discussed in this section. Essentially, although [PON] increased, this was not significant. Evidence for increased N cycle activity, presumably due to mixing, was presented although the temporal separation between the storm passage, taken from the cruise report to be Julian day X, and the station re-visit on Julian day 175 may not have been sufficient for a significant PON response to be evident. We may have detected the initial up-shock phase, with a subsequent modification to phytoplankton community structure evident over the following days (which were not sampled of course). Geographically, we know the storm influenced a large area of the southwest coastal region as evidenced by the cruise report, although we are unable to give precise details. Within the context of this study the only stations influenced (and sampled) are those discussed.

The lack in statistically significant relationship between processes and the environment could also be due to missing variables? E.g. the light regime which may have been important was not measured at all stations. . .Please discuss this issue.

Response. We identify a number of factors which may have contributed towards the lack of significant relationships for both observational and bioassay data including the issue of missing variables (although light is removed as a variable in both instances since N-cycle rates at observational stations were all measured at the same sPAR depth (55%) while for OA bioassay stations a fixed light intensity was used in a controlled environment). For observational statistical analysis we state that 'Factors which may have influenced this outcome [a lack of statistically significant relationships] included the limited number of observations, and the possibility that microbial rates reflected conditions associated with the recent past rather than those at the point of sampling, which are not necessarily the same in a dynamic environment (3138; 16-19). We

will add text to state that additional biogeochemical variables may have been excluded from the parameter set which could have explained observed variability in N-cycle parameters. We will provide limited speculation as to what these variables may be. For the statistical analysis of bioassay data we state that 'After location, factors excluded from the parameter set explained the greatest variability in bioassay data, potentially indicating that the most significant biological processes responsible for this observation [in the response between PCO2 treatment and nutrient concentration] were not measured'(3142; 9-11). And we conclude that our manuscript 'raises the question about which combination of biogeochemical characteristics drives the short-term biological response to ocean acidification.' (3142; 23-25). We speculate as to the identity of these variables which could include nutrient assimilation by both phytoplankton and bacteria, and virally induced cell lysis (3142; 13-14). We believe that the text already in place covers the concern raised by the reviewer.

I am confused by the statement about the spectral light quality and that it may have differed so much in the field – usually bottles are neutral to light spectra. (page 3139 lines 19-269).

Response. There are two aspects to this question – (i) the light environment in the field and (ii) the light environment of an incubation bottle. (i) Our hypotheses is that coccolithophorids modify the characteristics of their light environment which in turn increases the coupling strength between the two processes of nitrification, effectively providing the coccolithophorid cells with regenerated nitrate in an otherwise nitrate deplete environment. It is important to note that while observational samples were taken at the 55% sPAR depth, there is a crucial distinction between light intensity (i.e. μmol photonsâĂćm-2âĂćs-1 in the broadband 400-700nM) and light quality (the composition of light in terms of specific wavelength contribution to light intensity). Consequently, although both samples were from the 55% sPAR, light quality was not the same between the two 'bloom' stations (discussed in this section) due to the presence of coccoliths, which both scatter light (i.e. provide a diffuse field) and modify its quality. Therefore, we

hypothesise that this difference in light between the two sampling stations contributed to the observed coupling strength of nitrification. As these rate processes are slow, we anticipated that this coupling continued once samples were contained within incubation bottles despite the inevitable change in light field within bottles. (ii) Incubation bottles inevitably modify the light environment; they remove UVa/b and can also influence light quality by selectively diminishing the contribution of specific wavelengths to the light field within the bottle. Neutral density bottles influence quality to lesser extent, but do diminish light intensity across all wavelengths (and obviously exclude UVa/b). During short incubations in which a relatively slow process is measured, this may be less of an issue. However, it is feasible that its significance will increase as incubation time increases (i.e. this may have influenced the bioassay results).

The authors used short term incubations (max. 96 hours) to investigate CO2 effects on nitrogen cycling. Please discuss the lack of pre-incubation time and the possible disadvantages of short term experiments which might explain the lack of significant effects (e.g. lag growth phase).

Response. The point being made here is slightly unclear. In terms of bottle incubation, 96 hours (adopted by the consortium) is a long time. For N assimilation studies we use short incubations which can be as little as 4 hours. The reason for this is that bottle confinement represents a significant perturbation to the system (mixing and turbulence, exchange processes, particle settling, trophic interactions, light field (UV) all modified from natural conditions by bottle confinement). By minimising incubation time you minimise the risk of measuring artefacts. For longer term studies mesocosms must be used, but these were not an option for this study. It is unclear what a 'pre-incubation' time is or the relevance of a 'lag growth phase'. During batch culture of phytoplankton cell lines a lag phase may occur prior to a phase of exponential growth following subculture into fresh (i.e. nutrient replete) media. Only the carbonate system of bioassay experiments described here was amended, no other nutrients were added and so a response to replenishment would not be expected. Possibly the reviewer is suggesting

that the time frame of the bioassay was insufficient to observe a treatment response? We presented rationale for those parameters which we anticipated may respond to carbonate system manipulations (3139; 10-18), and treatment responses consistent with this rationale has been reported by other contributions to this special issue (Richier et al, Hopkins and Archer). We believe that our results primary relate to the fact that the process of nitrification is inhibited in the light. We have added this comment to our text.

To link field data and the OA experiment a set of carbonate parameter from the field stations would be helpful (e.g. pH, DIC). Response As noted above, this information is presented and fully discussed elsewhere (Rérolle et al, Ribas-Ribas et al and Richier et al). In our revision we will consider the insights offered by these authors and how they relate to our findings.

Minor details Typo line: Riebesell P3113; 27 The correct spelling has been inserted.

Figure 2 lacks readable color bar We have updated the figure with a new scale bar which hopefully is clearer.

Figure 3 the x axis may have some indication of where the ship was and dates not just km. We have re-constructed the profile plots for Fig 3 to provide time (Julian day) as the x-axis and by indicating the exact position of observational stations and bioassay stations on these plots.

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