

General comments:

This article seeks to tease apart the effects of coastal biota and the settlement surface they provide on microbial nitrogen cycling. The article further aims to determine how this is influenced by factors including light vs dark and addition of glucose (mimicking provision of DOC via excretion from biota).

Understanding of the role of macrobiota communities in coastal nitrogen cycling sits well within the scope of the Biogeosciences journal and is particularly important given the widespread increase in coastal nitrogen concentrations and interest in the ability of coastal habitats to cope with or buffer against this.

The paper is not overly novel, but provides new data for this area of research and demonstrates the potential for changes in macrobiota to alter coastal N processing, which is of general interest. The authors could broaden the scope/interest of the paper by providing comment on how applicable these results are likely to be for other biota and regions, and by providing additional background information on environmental conditions, etc. It would also be helpful to see some further discussion of the possible mechanisms underlying the role of biota (vs inert substrate) in eliciting changes in microbial N transformations.

Overall, the manuscript would benefit from re-working to improve clarity, particularly relating to the methods and statistical analyses used. I found this section to be confusing, making it difficult to ascertain how reliable/robust the results are.

Specific comments

Title: The title could be improved. This seems to be the only place in the manuscript where it is specified that the biota are intertidal. Furthermore, the incubations were (apparently) fully submerged.

Abstract: The abstract seems to be missing some key information, and there appear to be some inconsistencies with the body of the manuscript, as follows:

- Why is a day/night comparison only described for mussels? Was this comparison done for other substrates? This is not specified in the methods section of the manuscript.
- DOC added to which treatments/substrates? From the abstract it sounds like DOC was added only to chambers with mussels, but then in the methods section it seems like DOC was added to algae, seawater and bioballs, but not mussels.
- No mention of mussel shells (mentioned elsewhere). Is this considered 'inert substrate', in which case the abstract should probably specify the various inert substrates that were used.
- Offshore is specified as 2-5km, whereas Ln 112 specifies 1-5km and Ln 127 specifies 2-3km.
- Abstract states day and night rates were similar, but results state that nitrate reduction was higher in the day (pg 13).

Introduction: The introduction seems lengthy and could be more concise. For example, there is much mention of N release by biota and how this might affect N cycling, but this is not tested, so could be considerably shortened or removed. However, it should be made clear why the excreted C was considered to be potentially more important than excreted N.

Pg 3: There are papers that compare different habitats characterised by different biota that also provide some insight into the potential role of macrobiota. These seem to have been overlooked. For example, B. Eyre has a number of papers comparing biogeochemistry of different benthic

habitats (with and without seagrass, with and without burrowing animals) that could provide additional context (e.g. DOI 10.1007/s10533-010-9425-6).

Ln 108: Here it states that ^{15}N -enrichment allowed for estimation of NH_4 and NO_3 fluxes, but this can be measured without ^{15}N enrichment. The reason for the ^{15}N enrichment is not made clear. It should be clearly stated how this following transfer of ^{15}N from NO_x/NH_4 to NH_4/NO_x allows identification and quantification of the pathways responsible for N fluxes.

It should be clearly stated why nitrification and nitrate reduction are important processes.

Methods:

- Two chambers were used, so incubations were run in pairs and clearly over some period. How were these paired up to avoid the possibility that temporal variations in light/temperature (for example) may have affected apparent differences between the different treatments?
- It is not immediately clear, 54 assays of 2 chambers = 54 total incubations (26 pairs) or 108 incubations (54 pairs).
- Were the chambers completely submerged within the tide pools? What was the depth of overlying water?
- What were the 'natural light and temperature conditions'? How much did they vary within an incubation and among incubations?
- What mass of substrate (mussels, algae, bioballs, etc.) were used within each chamber?
- Ln 141: Why was the cooler shaded? What depth was the water bath. What temperature was the water bath held at? What were the light conditions?
- Why was red algae used at the Tatoosh Island site and not at Second Beach. Indeed, given that the incubations were run for substrates ex situ, why were multiple sites necessary? Were the mussels and algae collected locally to each site?
- There is no mention of mussel shell incubations, yet data for these are shown in the results (e.g. fig 1).
- For ship-based incubations, what were the incubation conditions? Also, it is not clear how many incubations were done, and what was compared. It is not at all clear to me what is meant by "4 replicates of each shore and offshore chambers", particularly with $n=9$ (Ln 128).
- Ln 126: 'added an enrichment of 10000‰ of d^{15}NH_4 '. I suggest rewording, as this does not really make sense as written. Suggest '0.05M ^{15}N -labeled NH_4Cl was added to give a final approximate d^{15}N value of 10000‰. Similarly, reword statement for addition of ^{15}N - NO_3 .
- Ln 159: 'dissolved' N_2 gas.
- Ln 162: How was the chamber agitated?
- Ln 165: How were samples stored? With/without headspace? What temperature?
- Section 2.2: What treatments had DOC added? None with mussels?
- Ln 175: Clarify – 4 paired runs each for *Prionitis* and bioballs?
- Ln 176: First mention of ^{15}N -enrichment here comes 'out of the blue'. Why NH_4 and not NO_3 for the enrichment in this part of the study?
- There appears to be no mention of the day/night comparison
- There is no description of the statistical analyses used and referred to in the results.

Results:

- Ln 236: Mentions chambers during daylight hours, but what about outside of daylight hours? Are only daylight incubations shown on Fig 1?

- Ln 239-240: Nitrate and nitrite are swapped in the text or in Fig 1. Check for any impact on other sections of the text.
- Ln 241-243: Make clear that this describes changes in d15N following 15N addition. However, this section also seems to be repeat of the methods. No results are presented here.
- Ln 251: Is this an average across all bioball treatments?
- Ln 256: First mention of mussel shells. Discussion material here.
- Ln 260: 'than that for'
- Ln 257-260: Rewrite. Remineralization twice.
- Ln 278: Interaction term is not significant at $p=0.05$, in which case nitrate reduction was also lower for mussels at night.
- Ln 294: No mention in methods of DOC measurements. What about mussels? Did they increase DOC concentration too?
- Pg 14: Some discussion material in the second paragraph.

Discussion

The discussion is thorough in the most part and makes good use of previous work to support the conclusions made. However, there are a few parts of the results I would have liked to see further explained: 1) If the microbial communities associated with biota have such an effect on N processing, yet excreted DOC from biota apparently has little effect, what alternative mechanism might explain the fact that the inert substrates (also with microbial communities) had little effect on N processing? Are the communities support by inert substrates (including mussel shells) and macrobiota different? 2) DOC is made up of a variety of compounds. Glucose is one of the very labile compounds and would presumably be most likely to elicit a response from the microbial community. Is this why this specific compound was chosen? Is this representative of the DOC that would be assumed to be provided by the macrobiota? 3) So macrobiota change N processing. What is the significance of these changes? Why is this important?

I also had a few more minor notes:

- Is Prionitis common and/or a typical macroalga? Is it expected to affect N processing in a similar way to other macroalga?
- Ln 329: It is not clear exactly what data was used from Paine 2002 in calculations – just the mass estimates?
- Ln 423: Was N₂ not detected because denitrification did not occur, or because the rate was too low to be detected (or not enough label was applied)? This could possibly be determined by calculating the lowest N₂ flux that could have realistically been detected based on the amount of 15N added (and allowing for instrument error, etc.).

Technical corrections

- Ln 16-17: Sentence needs re-writing. Role for microbial activity? Or inert substrate for microbial activity? Clarify please.
- Ln 17: 'only seawater' as a control (all the chambers had seawater).
- Ln 19: Change 'of seawater' to 'in seawater'
- Ln 19-20: Remove "effect of simply an"
- Ln 28: Change 'elevating the concentration of DOC' to 'DOC addition'
- Ln 28 and 30: 'indicate' used twice close together. Find an alternative?
- Ln 54: 'enhance' should be 'enhances'

- Ln 60: manipulated DOC 'concentration'
- Ln 74: Remove full stop after 'animals'
- Ln 81: Full stop missing from 'e.g.'
- Pg 5 (check for same elsewhere): NH_4 should be NH_4^+
- Ln 101: "The effects macrobiota have on both nitrogen excretion and DOC release are poorly understood." But this was not tested?
- Ln 112: Reword – e.g., does the microbial activity differ for seawater collected from nearshore and offshore. Also, not inconsistency in distance offshore vs other locations in the text.
- Ln 114: Remove 'experimental'.
- Ln 120: 'both retain and lose' replace with 'contribute to loss and retention'
- Ln 126: Remove extra 'W' from co-ordinates.
- Ln 127: 2-3km, vs 2-5 or 1-5 elsewhere
- Ln 130: Remove extra bracket before 'Pfister'
- Ln 160: 'is' should be 'are'. Space missing in $\mu\text{mol/L}$
- Ln 165: injected 'this'
- Ln 214: Reword to 'estimated ammonium oxidation by monitoring ^{15}N enrichment in nitrate following $^{15}\text{NH}_4$ addition. Similarly for nitrate reduction sentence that follows.
- Ln 225: model 'to the' decline...
- Ln 230: mean concentration of ammonium = mean of what? Start and end? A number of replicates?
- Ln 274: Figure reference.
- Ln 278: Full stop missing from e.g.
- Switching between 'ammonia oxidation' and 'nitrification' could be confusing.
- Ln 288: Remove 'either'
- Ln 293: Space missing before 1000
- Ln 321: replace 'on' with 'for'
- Ln 329: mm should be mmol
- Ln 331: what about nitrate reduction?
- Ln 335: remove 'via'
- Ln 338: change to 'metabolism to offshore seawater'
- Ln 345: Any chance nearshore enrichment could reflect wastewater input?
- Ln 368: Change to 'glucose additions have resulted in decreased nitrification' (delete 'with DOC')
- Ln 369: decreased oxygen 'concentration'
- Ln 374: comma after microbes
- Ln 378: 'explanation to explain' Reword.
- Ln 391: remove comma before Joo
- Ln 655: 'mussel' should be 'mussels'
- Ln 666: 2-5km offshore
- Ln 667: Refer to parts b and c of the figure upon first mention of DIN and silica. Re-write this sentence (doesn't make sense as currently written).
- Ln 711: Replace 'in an' with 'following'
- Figure 1: 1) Mussel shells come as a surprise. No mention of these incubations in the methods. 2) Why is data for *Prionitis* and mussels shown together and not in separate bars? 3) Difficult to distinguish the two sets of data in this format. 4) Show units for d, e, f on the figure (and on all other figures in the manuscript). 5) What are the lines connecting some data points? Error bars..?
- When referring to enriched ammonium and nitrate, better to say " ^{15}N -enriched" to avoid confusion, as many papers use the term enriched to refer to higher concentration (which you also have in here, albeit for DOC).

- Numerous places where ref formatting has problems such as extra brackets, first names included, names within brackets that should be part of the text, etc. (e.g. ln 37, 51, 81, 201, 328, 369, 380, 397)
- Check for instances where u should be μ . E.g. ln 172, 287, 292, 294, 654, Figure 1 axis labels.
- Check reference list for capitalization within some refs and not others: e.g. Azam, Capone, Croll, Diner, de Goeij, McIlvin, Offre, Paine, Worm, Zehr, Zhang.
- Reference Beman (ln 470) has first names in full.
- Ln 481: Space missing 'ina'
- Ln 466: <i>Mytilus</i>
- Ln 477: subscript
- Italics for scientific names in references Flombaum, Joo, Jacobs, Bayne