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Assessment of nitrogen removal trends in the Eastern Tropical North Pacific using ^{15}N labeling experiments

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NONTECHNICAL SUMMARY

Areas of low oxygen in the world's oceans provide a suitable habitat for bacteria involved in the nitrogen removal process. The expansion of one such area in the Eastern Tropical North Pacific (ETNP) provides a larger zone for this process to occur. Results from isotopic labeling experiments suggest that the two major pathways of nitrogen loss, denitrification and anammox, are both present in the ETNP but vary in location and intensity due to responses to biological, chemical, and physical factors.

ABSTRACT

^{15}N labeling experiments were conducted to determine the roles of both anammox and heterotrophic denitrification in the ETNP with maximum values in the region being calculated at 52.70 nmol N L^{-1} d^{-1} and 47.82 nmol N L^{-1} d^{-1} respectively. Samples were taken and incubated with the label for roughly 48 hours and were analyzed using a mass spectrometer. Throughout the transect sampled, heterotrophic denitrification is the dominant nitrogen loss mechanism which can only be expected to be enhanced by an enlarging oxygen minimum zone.

The marine nitrogen cycle is a critical process to life in the ocean because it produces and removes nutrients necessary to sustain it. For example, nutrients containing nitrogen are important to plankton populations by providing the materials necessary for tissue synthesis. Due to its use in tissue building, nitrogen can exhibit bottom up control on an entire ecosystem if it is deplete. In general, this cycle consists of four broad steps all carried out by specific bacteria. The steps include the reduction of nitrogen in its diatomic gas form to biologically active ammonium, the oxidation of ammonium to nitrite and then to nitrate, and finally the reduction of nitrate back to nitrogen gas (Cline and Richards, 1972). This final step, formally known as denitrification (Eqn. 1), has been studied extensively for quite some time.

$$NO_3^- \rightarrow NO_2^- \rightarrow NO + N_2O \rightarrow N_2\,(g) \qquad (1)$$

However, this is not the only pathway by which diatomic nitrogen gas is produced from fixed nitrogen. In the last decade anaerobic ammonium oxidation, or anammox, was discovered and research has been focused on discovering more about it. Anammox bacteria oxidize ammonium by combining it with nitrite to produce nitrogen gas as well (Kuenen, 2008) (Eqn. 2).

$$NH_4^+ + NO_2^- \rightarrow N_2 + 2\,H_2O \qquad (2)$$

Both of these pathways thrive in low oxygen areas such as oxygen minimum zones (OMZs), which are found globally (Cline and Richards, 1972). An

OMZ is defined as a region with permanent suboxic conditions with typical oxygen concentrations of about 4.5 M (Wishner et al. 2011). These zones are presently believed to account for anywhere from 30% to 50% of total nitrogen loss from the entire ocean, while only occupying about 0.1% of the ocean's volume (Kalvelage et al. 2011). Since OMZs are believed to be expanding (Stramma et al. 2008), the assumption can be made that their contribution to nitrogen losses will increase as well. The ETNP has a large OMZ and is therefore a prime location for observations of denitrification and anammox. Coastal upwelling of water along Mexico provides nutrients to the organisms at the surface, which die and sink down deeper into the water column. Once at depth the organisms decompose and release these nutrients. As the organisms decay they also deplete the oxygen levels in the water. The availability of active nitrogen is not the only factor affecting the function of both these processes. Oxygen levels play a large part as well as currents and pH.

As expected, both anammox and heterotrophic denitrification occur in the OMZ, but the two pathways differ greatly in the magnitude of their rates and geographic location. I will show that anammox is the dominant pathway near the southern tip of Baja, Mexico with a maximum value of 52.70 nmol N L^{-1} d^{-1} occurring at the very top of the OMZ. This happens to be the only area sampled that shows anammox activity of this magnitude. South of the Baja Peninsula and on the other side of the mouth of the Gulf of California the OMZ covers a much larger vertical area as it extends both shallower and deeper in the water column. This provides a large, suitable location for heterotrophic denitrification to occur. Here denitrification rates reach a maximum of 47.82 nmol N L^{-1} d^{-1} near the bottom of the OMZ.

METHODS

Sampling and Treatment

Water sample collection was carried out by using a CTD rosette on the *R/V Thomas G. Thompson* during the 2012 UW Senior Curise. During the downcast oxygen concentrations were measured using the oxygen sensor mounted to the CTD. The oxygen data allowed for selection of sample depths that represented the top, middle and bottom of the OMZ. In total, 4 depths were sampled throughout the OMZ for each station. Removing samples from each niskin bottle required the use of standard glass oxygen bottles. These bottles were filled completely and capped then taken back to the laboratory. Eight empty 12 mL exetainers previously purged with helium gas for 3 minutes were obtained and a syringe was used to sample from the bottom of each bottle immediately after removing the cap so as not to sample water that had come into contact with air (Jensen et al. 2011). A volume of 7.7 mL of seawater was added to each exetainer. Two exetainers were filled per each depth at every station sampled. This allowed for treatment of each sample with both ^{15}N labeled ammonium and ^{15}N labeled nitrite. One sample at each depth was injected with 0.7 mL of 120 µM $^{15}NH_4Cl$ while the other was injected with the same volume and concentration of $Na^{15}NO_2$ to measure anammox and denitrification respectively. This was done such that the final concentration of ^{15}N in each sample was at 10 µM. Each stock solution of isotope was also bubbled with helium before injection to remove any gas that may have been present. A large total volume of liquid in each sample was desired so the shorter needles used to bubble helium could reach below the headspace into the liquid. Once filled with sea water and isotope, the samples were again bubbled to remove any in situ gas dissolved in the water and then were transferred to a cold room to incubate at 10ºC for about 48 hours. After the incubation time was complete the samples were poisoned with 0.2 mL of dilute sulfuric acid to kill all bacteria thus ending the denitrification and anammox reactions.

Analysis

Samples were analyzed on a Finnigan Delta XL mass spectrometer to determine abundances of $^{29}N_2$ and $^{30}N_2$ by using single endpoint determinations (Ward et al. 2009). In order for the mass spectrometer to sample effectively, a headspace of about 6 mL was desired so that the sampling needle would come into contact with the liquid. To remove the liquid, exetainers were inverted and helium was injected into the headspace using a long needle while a shorter needle was inserted into the liquid to allow for the liquid to be drained. Calculations were carried out using previously determined equations and methods (Thamdrup and Dalsgaard, 2002).

RESULTS

Oxygen data taken from the CTD casts along the transect show an oxygen minimum zone that grows larger as you move south from Baja, across the mouth of the Gulf of California, and along the coast of mainland Mexico. At the end of the transect, the OMZ begins at about 150 m shallower and extends almost 300m deeper than at the beginning (Fig. 1). The middle of the OMZ is anoxic in quite a few instances. These oxygen conditions are are favorable to allow both anammox and denitrification to occur.

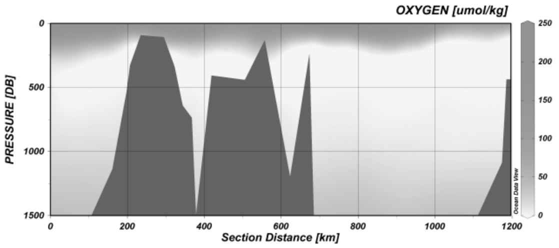

Figure 1- Transect of UW Senior Cruise showing oxygen concentrations from Baja to Manzanillo. The OMZ is the light tongue which hovers around an average depth of 500m.

Rates of anammox are only seen to be large sinks of available nitrogen at one station. This occurs at the tip of the Baja Peninsula where anammox averages a rate of 27.04 nmol L^{-1} d^{-1} throughout the water column. All other areas sampled show denitrification as the dominant process with the southern-most station sampled showing the largest average rate as 26.48 nmol L^{-1} d^{-1} (Table 1). Increased oxygen levels are shown to have a greater adverse effect on denitrification (Fig. 2) than on anammox (Fig.3) because the regression lines show a more negative slope for denitrification.

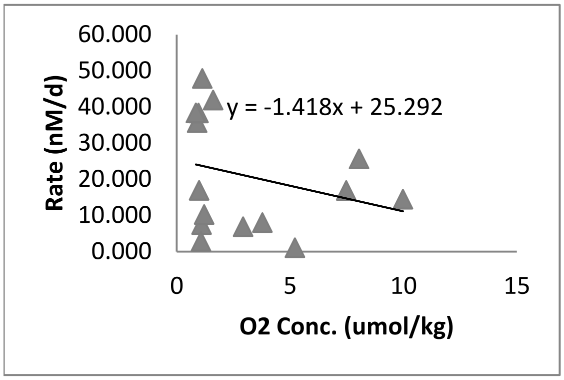

Figure 2- Denitrifaction rates against oxygen concentrations

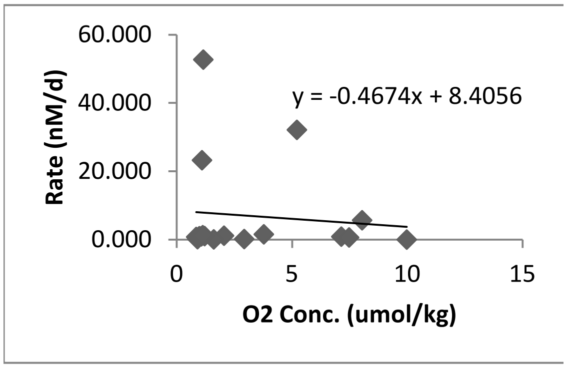

Figure 2- Anammox rates against oxygen concentrations

DISCUSSION

The fact that the ETNP has a large OMZ that promotes large scale nitrogen losses has already been discussed at length. Focusing on reasons why nitrogen losses due to anammox in this region are lower than expected produces many hypotheses. The absence of ammonium in this region suggests that it is being depleted by some process, possibly anammox. However, the presence of large amounts of nitrate shows that there is a potential for dissimilatory nitrate reduction to ammonium (DNRA) to occur. Since anammox rates are so high at station 19 DNRA could be producing quite a bit of ammonium here

Table 1-Each rate with its corresponding station, depth, oxygen concentration and treatment.

Station	Depth (m)	Treatment	O2 Conc. (umol/kg)	Rate (nM/d)
3	400	Ammonium	7.14	0.88
3	800	Nitrite	8.04	25.63
3	800	Ammonium	8.04	5.68
6	160	Nitrite	9.98	14.47
6	160	Ammonium	9.98	0.02
6	200	Nitrite	3.78	8.01
6	200	Ammonium	3.78	1.56
6	300	Nitrite	2.93	6.87
6	300	Ammonium	2.93	0.18
6	540	Ammonium	2.05	1.16
19	150	Nitrite	5.21	1.06
19	150	Ammonium	5.21	32.14
19	300	Ammonium	1.15	52.70
19	450	Nitrite	1.10	7.46
19	450	Ammonium	1.10	23.25
19	600	Nitrite	1.61	41.89
19	600	Ammonium	1.61	0.08
24	150	Nitrite	7.48	16.89
24	150	Ammonium	7.48	0.64
24	300	Nitrite	0.90	35.65
24	300	Ammonium	0.90	0.14
24	450	Nitrite	0.96	38.33
24	450	Ammonium	0.96	0.88
24	600	Nitrite	1.21	10.24
24	600	Ammonium	1.21	1.04
32	150	Nitrite	0.85	38.35
32	150	Ammonium	0.85	0.78
32	300	Nitrite	0.99	16.92
32	300	Ammonium	0.99	1.00
32	450	Nitrite	1.07	2.80
32	450	Ammonium	1.07	0.97
32	600	Nitrite	1.13	47.86
32	600	Ammonium	1.13	1.31

which is then all used up by the anammox bacteria. The station with the largest denitrification rates, station 32 which lies at the southern end of the transect shows plenty of other evidence for intense denitrification at that site. By comparing the rate to nitrite levels at the same station, it is observed that these larger rates are coupled with larger nitrite concentrations (Fig. 4). This is because nitrite accumulation is a well-known proxy for the occurrence of denitrification (Groffman et al. 2006). This brings the other large denitrification rates observed into question since this about the only zone where there nitrite is concentrated. These values could possibly be erroneous because of a couple possible reasons. First, it is plausible that when labeled nitrite was introduced to samples that previously had no nitrite in them, the bacteria rapidly converted it to nitrogen gas after being nitrite starved. Second, the low pH water from the OMZ could have converted the nitrite label straight to nitrogen gas (Granger and Sigman, 2009). This could be a large possibility since my samples had a low pH during storage and transit back to the lab as they had been poisoned with acid.

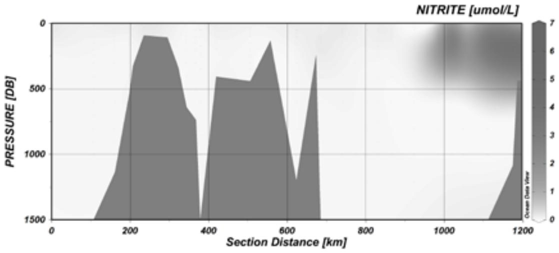

Fig. 2- A large tongue of nitrite rich water can be seen at the top right corner of the plot. This intermediate in the denitrification process is a well known sign of denitrification.

CONCLUSIONS

In conclusion, research of anammox and denitrification rates in the ETNP has yielded many trends consistent with previously believed aspects of the two processes. It is surprising that denitrification is the larger nitrogen sink in the region, but because of decreasing oxygen levels there may be a shift towards heterotrophic denitrification as conditions become more suitable for them.

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