

DISTRIBUTION AND ENVIRONMENTAL CONTROLS ON MARINE NITROGEN BIOGEOCHEMICAL FUNCTIONS

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Abstract

Nitrogen is one of the basic elements of life, and microbial communities play crucial roles in all transformation pathways of the nitrogen biogeochemical cycle. In the Oceans, fixed nitrogen is one of the most important growth-limiting nutrients for photosynthetic organisms and serves as an important energy source or as an oxidant for marine bacteria and archaea. The nitrogen cycle includes a multitude of complex processes which occurs through the coordinated functioning of several genes from polyphyletic group of microorganisms (prokaryotes and fungi). In this study we used the metadata and metagenomic data generated from the 2014 OSD initiative to determine global patterns regarding distribution, interrelationships and environmental controls on key Nitrogen pathways in a large spatial scale covering different marine ecosystems.

Keywords: *Nutrients, Bacteria, Mediterranean Sea, Open sea*

Prokaryotes dominate most global biogeochemical cycles and for the last decade, microbial metagenomics has provided invaluable insights into prokaryotic systems, allowing us to go one step forward towards the linkage between biodiversity and ecosystem function. The marine nitrogen (N) cycle controls the productivity of the oceans and these new methodologies have uncovered new processes and microorganisms involved, complicating the already complex picture of oceanic N biogeochemistry (Zehr et al. 2011). Global oceanic sampling campaigns (OSD and Tara Oceans) covering a wide range of marine environments using standardized methods not only enabled the comparison of results, but also the discovery of global trends. In this study, we leveraged publicly available datasets from the OSD 2014 initiative and applied state of the art bioinformatics and statistical tools to perceive worldwide N-related trends with a view to better understand the drivers and dynamics of the global marine N cycle and the microorganisms that mediate it. We compared the abundance of genes associated with the main nitrogen cycling pathways (IPR) identified within the 150 metagenomes of the global 2014 OSD database, to identify interrelationships between them and relate their distribution of those genes with the contextualized environmental data available. We performed all analyses using R (R Development Core Team, 2008) and a series of R specific packages. From the lower taxonomic level OTU table generated from SILVAngs we selected OTUs representing individual ammonia-oxidizing bacterial (AOB) and archaea (AOA) and bacterial nitrite-oxidizing.

Among the 150 global OSD sites, a high differentiation was found between the levels of occurrence of specific genes related to the enzymes involved in the different key N biochemical pathways (Figure 1).

While specific genes related to ammonia oxidation (the first step of nitrification mediated by AOA and AOB) were found to be less abundant in the IPR data set, genes involved in N-fixation (nitrogenase enzyme), and in nitrate, nitrite and nitrous oxide reduction pathways (denitrification) were found to be widely distributed along the OSD data set. Results consistently showed that sites with similar frequency of the genes involved in N processes (included in the same cluster) are distributed across the OSD map; thus the distribution of the genes involved in the marine N machinery was found to be latitudinal and longitudinal independent. Results showed a high degree of interrelationships between the genes involved in the different pathways (e.g between the different steps of the denitrification pathway; nitrate reduction - nitrite reduction - nitrous oxide reduction) and within the same pathway (e.g. ammonia oxidation; nitrate reduction).

Few strong relationships were observed between the abundance of the different genes related to N enzymes and the metadata available. However, fluorometer values were clearly and positively related with the majority of the genes involved in the denitrification step and a tendency for negative relations was observed with the genes involved in ammonia oxidation and N fixation. The same trend was observed for nitrate and ammonia

concentrations in the water, suggesting that the occurrence of some N genes are connected with high abundance of primary producers and fixed N availability, whereas other N genes are associated with low N and Chl_a levels. We believe that the addition of the 2015 data, with an anticipated richer metadata set, will add the temporal scale to our analyses and will allow us to draw more robust conclusions regarding the environmental controls on N related pathways in the ocean. Genes involved in ammonia oxidation (a widely distributed pathway in marine environments) were surprisingly under-represented among the OSD sites. Interestingly, this finding seems to be confirmed when we cross IPR dataset with the 16S rRNA gene dataset, as OTUs affiliated with archaeal and bacterial ammonia oxidizers showed relatively low frequencies of occurrence and a generally confined distribution. In contrast, nitrite oxidizing bacteria (NOB) were the most abundant and widely distributed nitrifiers within the 16s rRNA gene OSD dataset. The recent discovery of the presence of both ammonia and nitrite oxidation steps in NOB strains (*Nitrospira* genus) (Daims et al. 2015, van Kessel et al. 2015) may explain this paradox, supporting the hypothesis that NOB are the main players in driving complete nitrification in the ocean (ammonia oxidation plus nitrite oxidation) with a new type of amoA gene as yet undetected in our analysis. This study expanded our knowledge regarding the N biogeochemical cycle across multiple marine ecosystems, and provided an enriched perspective on how marine N biogeochemistry and the microbial communities involved respond to environmental changes.

References

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