

Proposition de stage

Parcours Master 2 « Microbiologie, Environnement, Santé »

1. Laboratoire / Entreprise d'accueil :

Intitulé : Etude du rôle de la diversité phytoplantonique dans la production d'ammonium via dégradation de matière organique dissoute.

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Perspectives de poursuite de thèse :

X oui
o non

avec une bourse spécifique

o oui
X non

2. Titre, description du sujet, approches utilisées, références (2 pages maximum) :

The nitrogen cycle is involved in key aspects of the biogeochemistry of marine organic matter and therefore intervenes in the chemical composition of any planktonic microbial community. At the center of this cycle, ammonium (NH_4^+) is produced by microbial activity during the degradation of dissolved organic matter in a process called ammonification (Bronk 2002). Tightly linked to this process, a diverse nitrifying community composed by archaea and bacteria ensures the production of nitrate by oxidizing ammonium in the euphotic and deep layers of the ocean. Although a seemingly linear relationship, this link between organic matter degradation and inorganic nitrogen regeneration holds several unanswered questions for oceanography. The subject of this M2 training will tackle one of the most important: *How does dissolved organic matter originated by specific functional groups influence the potential for ammonium release via ammonification?*

The study area is located in central Chile (36°S) and the approach will be largely based on laboratory experiments.

The objective will be to evaluate the production rates of NH_4^+ via biotic ammonification of dissolved organic matter from diatom and diazotrophic marine groups that represent different trophic levels of primary production (eutrophic vs oligotrophic). A large part of the experiments relies on the use of stable isotopes (non radioactive variants of a particular chemical element which differ in neutron number but not in the number of protons in each atom). The known proportion of heavy (^{15}N) and

light (^{14}N) forms of nitrogen in marine environments allows using them as tracers for flux and activity determination in the nitrogen cycle.

Experiments will be performed monthly at the French International Associated Laboratory Marine Biogeochemistry and functional Ecology (LIA MORFUN CNRS; UdeC) at University of Concepcion in CHILE. Students will be able to use the experimental platform of the Time Series Station (E18) of the COPAS center at UdeC and will be supported by FONDECYT project (1150891, Fernandez PI).

The experiments will combine isotopically labeled and natural POM and DOM obtained from cultures and ultimately the estimation of the production of $^{15}\text{N-NH}_4$ (determined by mass spectrometry). We will combine this technique with standard enrichment techniques and cell focused approaches.

STEP 1) DOM sources for this experimental setup include the following:

-Diatom axenic cultures: We will use natural and isotopically labeled (^{15}N) NO_3^- and will consequently obtain labeled and non enriched POM and DOM. We will then evaluate ammonification as standard fluorimetric ammonium determination (Holmes, Aminot et al. 1999) and via the detection of labeled $^{15}\text{N-NH}_4^+$ (Pujo-Pay and Raimbault 1994, Fernández, Farias et al. 2009, Fernandez and Farias 2012). Representative species from the study area include *Chaetoceros muelleri*, *Thalassiosira weissflogii* and *Thalassiosira minuscula*, which have already been used to estimating ammonification potential (Rain-Franco, Muñoz et al. 2014). Cultures are available through collaboration with Dr Marcela Cornejo (Catholic University of Valparaiso, Chile).
-Diazotroph cyanobacteria axenic cultures: Diazotrophic cyanobacteria maintained in cultures will receive, as a unique source of N, N_2 (^{15}N labeled and also non-labeled). This will allow studying natural ammonification via ammonium production (fluorimetric method) and compare with $^{15}\text{N-NH}_4$ production from ^{15}N enriched POM and DOM.

STEP 2) A second step will include the evaluation of changes in the natural microbial community structure (archaea and bacteria from E18) as a response of NH_4^+ generation via the characterization of 16S rRNA genes phylogenetic markers (Molina, Ulloa et al. 2007, Molina and Farias 2009, Molina, Belmar et al. 2010). For this we will use fingerprinting techniques (Terminal restriction fragment polymorphism, TRFLP) and next-generation sequencing of barcode 16S rRNA genes (initial and final incubation times). This will be done in collaboration with Dr Veronica MOLINA (University of Playa Ancha, Valparaiso Chile).

By the end of the training the student is expected to be introduced to work with isotope data, microbial diversity techniques (e.g. phylogenetic analyses), work with sequence data, culture techniques for diatoms and cyanobacteria and standard ammonium determination (fluorimetric method).

References

- Bronk, D. (2002). Dynamics of DON. Biogeochemistry of Marine Dissolved organic matter. D. A. Hansell and C. A. Carlson. San Diego, USA, Academic Press: 186-207.
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- Fernández, C., et al. (2009). "Primary production and nitrogen regeneration processes in surface waters of the Peruvian upwelling system." Progress In Oceanography **83**: 159-168.
- Holmes, R. H., et al. (1999). "A simple and precise method for measuring ammonium in marine and freshwater ecosystems." Canadian Fisheries and Aquatic Sciences **56**: 1801-1808.
- Molina, V., et al. (2010). "High diversity of ammonia-oxidizing archaea in permanent and seasonal oxygen-deficient waters of the eastern South Pacific." Environmental Microbiology.
- Molina, V. and L. Farias (2009). "Aerobic ammonium oxidation in the oxycline and oxygen minimum zone of the eastern tropical South Pacific off northern Chile (~20°S)." Deep Sea Research Part II: Topical Studies in Oceanography **56**(16): 1032-1041.

Molina, V., et al. (2007). "Ammonia oxidizing β -proteobacteria from the oxygen minimum zone off Northern Chile." *Applied and environmental microbiology* **73**(11): 3547-3555.

Pujo-Pay, M. and P. Rimbault (1994). "Improvement of the wet-oxidation procedure for simultaneous determination of particulate organic nitrogen and phosphorus collected on filters." *Marine Ecology Progress Series* **105**: 203-207.

Rain-Franco, A., et al. (2014). "Ammonium production off central Chile (36°S) by photodegradation of phytoplankton-derived and marine dissolved organic matter " *PLOS One* **9**(6): e100224. doi:10.1371/journal.pone.0100224.