Proposition de sujet de thèse 2020

(A remplir par les équipes d'accueil et à retourner à Isabelle HAMMAD : hammad@cerege.fr
**à renseigner obligatoirement pour la validation du sujet, (1) : A remplir lors de la campagne d'attribution des allocations, à l'issue de la session de juin des Masters*

Sujet de doctorat proposé *:
The role of diazotrophs in shaping the metabolism and composition of planktonic communities

Encadrant(s), *nom, prénom, adresse mail *:*
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Laboratoire *: Mediterranean Institute of Oceanography (MIO, UMR235)

Tableau récapitulatif du sujet

Candidat(e)(1)	
Nom - Prénom :	
Date de naissance :	
Licence (origine, années, mention) :	
Mention et classement au Master 1 année (Xème sur Y)	
Mention et classement au S3 du Master 2 (Xème sur Y)	
Mention et classement au S4 du Master 2 (Xème sur Y)	
Mention et classement au M2 (année) (Xème sur Y)	
MASTER (nom, université)	
Sujet de doctorat proposé*	**The role of diazotrophs in shaping the metabolism and composition of planktonic communities**
Encadrants (2 max, indiquer si HDR ou pas)*	BONNET Sophie, Directrice de recherche HDR https://sophiebonnet.wixsite.com/website
Laboratoire*	Mediterranean Institute of Oceanography (MIO, UMR235)
Programme finançant la recherche (indiqué si obtenu ou envisagé) (1)	• PLUME cruise (in situ experiments, Obtenue) • DOGMA (metabolite studies, obtenu) • SPIRU-Fix (co-culture devices, Obtenu) • NOTION (mass spectrometry and molecular studies, Obtenu) • TONGA (nanoSIMS, Obtenu)

The role of diazotrophs in shaping the metabolism and composition of planktonic communities

Life in the oceans is sustained by nitrogen. In >60% of the global ocean surface, nitrogen is provided by "diazotrophs", small planktonic cells capable of reducing atmospheric dinitrogen (N_2) into bioavailable forms. Diazotroph-derived nitrogen (hereafter DDN) is thought to sustain ~50-80% of new production and export (Capone et al., 2005; Caffin et al., 2018), and structures the marine food web in the oligotrophic ocean (Hunt et al., 2015; 2016; Carlotti et al., 2018). In single-cell studies, we have shown that ~10% of the DDN is quickly (within 24-48 h) assimilated by non-diazotrophic plankton, including bacteria, picophytoplankton, diatoms and zooplankton (Bonnet et al., 2016; Berthelot et al., 2016; Hunt et al., 2016; Caffin et al., 2016) and likely affect the plankton community composition at larger time scales, which has been poorly studied so far.

While the role of diazotrophs in providing nitrogen to planktonic communities has been evidenced, other potential benefits have not been explored. For instance, diazotrophs such as *Crocosphaera watsonii* produce dissolved organic matter (DOM) under the form of exopolymeric substances (EPS), often one to two orders of magnitude more than large phytoplankton (diatoms, coccolithophores) (Sohm et al. 2011). EPS contain polypeptides (Kawaguchi & Decho 2000) enriched with glycine, alanine, valine, leucine, isoleucine and phenylalanine, which could benefit to heterotrophic bacteria or mixotrophic plankton, but this has never been explored. *Crocosphaera watsonii* also produces large amounts of vitamin B_{12} (Bonnet et al., 2010), an essential growth factor for most marine eukaryotic phytoplankton (Droop 2007) that is required for the activity of several vital enzymes in central metabolism. Unlike prokaryotes, most eukaryotic phytoplankton lack the biosynthetic pathways for this nitrogen-containing B vitamin ($C_{63}H_{88}CoN_{14}O_{14}P$), and thus must depend on an exogenous pool of dissolved B_{12} produced by prokaryotes.

Other diazotrophs such as the filamentous *Trichodesmium* are themselves an holobiont system, harboring a wide diversity of epibiont heterotrophic bacteria that depend on the host (Frischkorn et al., 2017). *Trichodesmium* is known to sustain phytoplankton assemblages via DDN (Sipler et al., 2013). Moreover, the explosion of life around *Trichodesmium* blooms has been recurrently observed, such as in the form of zooplankton accumulations and even fish surrounding blooms.

In all, diazotrophs seem to be beneficial to eukaryotic phytoplankton and bacteria, but a precise understanding of the mechanisms involved and the joint dynamics of the communities in the field remains to be explored, to better understand their role at structuring the food web and carbon export. This is particularly important as climate models predict an expansion of the oligotrophic gyres where diazotrophs represent a major component of the plankton biomass and sustain most new primary production (Polovina et al., 2008; Morán et al., 2010).

This PhD project will investigate the effect of diazotrophs on non-diazotrophic plankton metabolism and biodiversity using biogeochemical and molecular tools in both culture and *in situ* setups.

1) INTERACTIONS BETWEEN DIAZOTROPHIC AND NON-DIAZOTROPHIC PLANKTON

1.1. Co-culture experiments

The potential facilitation of non-diazotrophic plankton by diazotrophs will be firstly investigated in the lab using co-culture devices (Fig. 1A). These systems consist of two culture chambers separated by a porous hydrophilic polyvinylidene fluoride (PVDF) membrane with pores of 0.22 µm in diameter, that allows only molecules to pass between the two compartments but not cells. With this setup, we can study how substances and metabolites released from each cultured group interact with the other, without physically mixing the two cell cultures.

Cultures of diazotroph plankton will be grown on one of the chambers, while non-diazotroph plankton will be grown on the other parallel chamber. The plankton combinations investigated will include the diazotrophs *Trichodesmium erythraeum* (filamentous) and *Crocosphaera watsonii* (unicellular), while non-diazotroph plankton will include the diatom *Cylindrotheca closterium* and a natural bacterium consortium (Fig. 1B). The two diazotrophs chosen are abundant and representative of tropical ocean ecosystems, and they have been repeatedly observed to co-occur with the diatom *Cylindrotheca closterium.*

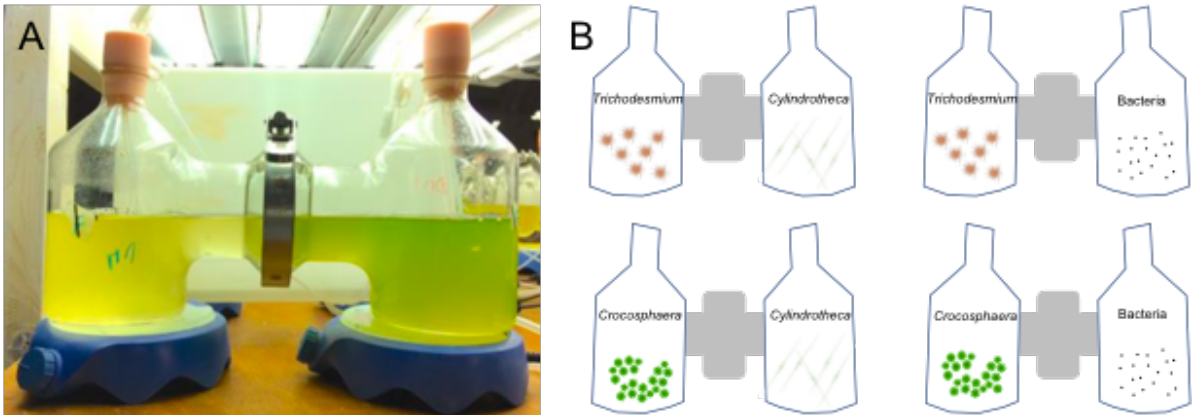

Fig. 1. A) Example of a co-culture chamber system. B) Co-culture combinations of diazotroph and non-diazotroph cells.

Condition 1: Obligate diazotrophy

In the first set of experiments, the different combinations shown on Fig. 1 will be performed under obligate diazotrophy conditions, i.e. with diazotrophs growing on N_2 as the sole source of nitrogen. This will allow studying under which form (NH_4^+, DON) and how fast (from hours to days) the DDN is translocated from the diazotrophs to the non-diazotrophs. As a control, the experiments will also be carried out by replacing the PVDF membrane with an impermeable polytetrafluoroethylene (PTFE) membrane. This will allow culturing diazotroph and non-diazotroph cells separately and compare with when substance interchange is allowed. $^{15}N_2$ isotopic labelling in the diazotroph compartment will allow to trace kinetic transfer of DDN from the atmosphere, to the diazotrophs, then to the dissolved compartment and eventually to the non-diazotrophic plankton (Fig. 2). We will use both standard mass spectrometry (IRMS coupled to elemental analyzer) and nanoSIMS (single-cell isotopic analyses) as described in Bonnet et al., (2016) and Benavides et al., (2017).

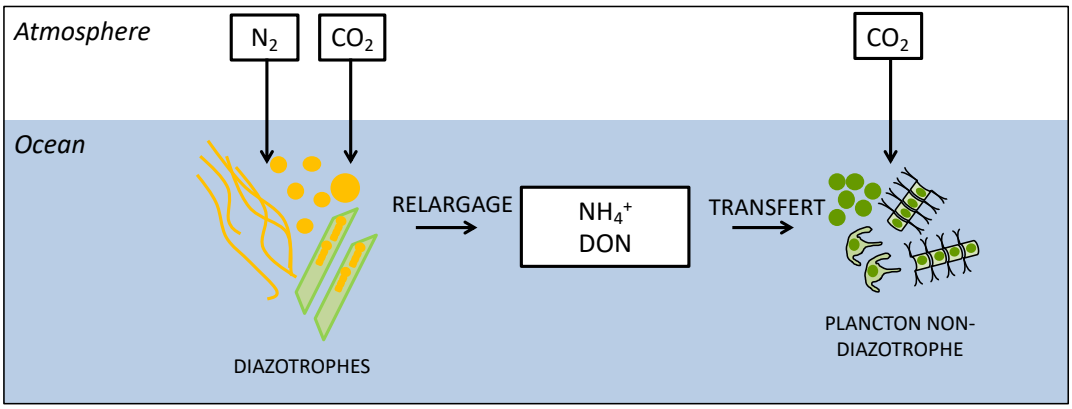

Fig. 2. Translocation of DDN from diazotrophs, to the dissolved compartment, and to non-diazotrophic plankton

Condition 2: Facultative diazotrophy

In this second set of experiments, diazotrophs will be grown on nitrate (NO_3^-) as the sole source of nitrogen to suppress N_2 fixation. This will allow testing whether diazotrophs provide any benefit to non-diazotrophs besides DDN. As in condition 1, the cultures will be run both with permeable and

impermeable membranes. We will produce ^{13}C-labeled EPS by incubating diazotrophs with ^{13}C-bicarbonate. This will allow us to trace the incorporation of diazotroph-derived EPS into non-diazotroph biomass.

Cultures of diazotrophs will be provided by the Tropical Culture Collection of Cyanobacteria (TCCC) hosted by MIO. In each condition above, the cultures will be maintained at 26ºC under a 12h:12h light:dark cycle. A range of metabolic variables will be measured to quantify the potential facilitation effect, including: cell counts to monitor growth rates, organic and inorganic nutrient stocks, particulate organic matter stocks, N_2 and C fixation rates, bacterial production, and respiration rates. Chemical substances exchanged between the two species through the membrane will be sought by an untargeted metabolomics approach by HPLC-HRMS. The annotation of the metabolites of interest will be attempted using their fragmentation pattern and open-access databases (Collaboration Eva Ternon, SCRIPPS Institution of Oceanography, San Diego, MIO Partner).

1.2. Diazotroph effects on natural plankton communities

To expand on the in vitro knowledge gained above, the effects of diazotrophs on the activity and structure of non-diazotroph communities will also be tested in natural marine communities. Cultures of diazotrophs (*Trichodesmium* and *Crocosphaera*) will be added to natural planktonic assemblages during the PLUME cruise (Vietnam). On a time-series setup, the evolving effect of diazotrophs on the metabolism and diversity of the natural planktonic community will be studied. Metabolism measurements will include primary production and respiration rates, measured with stable isotope tracers and oxygen microsensors, respectively. The planktonic community composition will be studied by a combination of optical and molecular techniques. Among optical techniques flow cytometry and microscopy will be employed. To characterize the community composition of prokaryotic and eukaryotic plankton 16S and 18S metabarcoding methods will be used, respectively.

2) DATA MINING

The second part of the PhD will consist in data mining into several databases to explore the potential statistical links between the biogeographical distribution of diazotrophic and non-diazotrophic plankton in the ocean.

2.1. At the regional scale: Western Tropical South Pacific Ocean

This work will be based on 4 cruises performed from 2012 to 2019, during which 5 to 7 groups of diazotrophs (UCYN-A1, UCYN-A2, UCYN-B, UCYN-C, *Trichodesmium*, Het-1, Gamma-A) were quantified in parallel with the quantification of i) pico- and nano-plankton (heterotrophs and autotrophs), ii) siliceous plankton (diatoms, radiolarians, silicoflagellates), iii) pigments (biomarkers of phytoplankton). The objective of this work will be to determine if specific groups of diazotrophs are associated with specific groups of non-diazotrophic plankton groups in the natural ocean. Environmental data (CTD, nutrient stocks) together with primary production and N_2 fixation rates data will also be available for the analyses. The 4 cruises/databases are the following:

- OUTPACE-LEFE-CYBER database: 18 stations, 6 depths, sampled across trophic and nutrient availability gradients
- TONGA-LEFE-CYBER database: 12 stations, 6 depths sampled across iron availability gradients
- PANDORA-LEFE-CYBER database: 18 stations, 4 depths sampled across coast-large (and nutrient) gradients
- VAHINE-MIO-hosted database: Contrary to the OUTPACE and TONGA that explore the spatial variability of plankton communities across gradient, VAHINE explore the temporal variability of the planktonic communities inside closed mesocosms: 23 days, 3 mesocosms, 3 depths

2.2. At the global scale

Potential links between diazotrophic and non-diazotrophic plankton will also be explored at the global scale thanks to the world atlas of MARine Ecosystem DATa (MAREDAT, Buitenhuis et al., 2013). The following global databases will be used for the analyses (note that contrary to the regional databases mentioned above, sampling of all plankton groups was not synchronous in the global databases, but statistical relationships can still be investigated):

- Luo et al., (2012): Diazotrophs
- Peloquin et al., (20113): Pigments
- Buitenhuis et al., (2012): Pico-heterotrophs
- Buitenhuis et al., (2012): Pico-phytoplankton
- Leblanc et al., (2012): Diatoms

Détail du Programme finançant la recherche* :

- SPIRU-Fix (co-culture devices)
- DOGMA (metabolite studies)
- TONGA (nanoSIMS)
- NOTION (mass spectrometry and molecular studies)
- PLUME cruise (in situ experiments)

Intégration au sein du M.I.O et collaborations extérieures

AT dont relève le sujet

AT End-to-End (confirmation des coordonnatrices D. Banaru & C. Chevalier après présentation du sujet en réunion AT)
AT Pompe Bio (confirmation du coordinateur C. Tamburini)

Plateformes concernées :

Cultures expérimentales
PACEM
OMICS
PRECYM

Collaborations extérieures

SCRIPPS San Diego (partenaire du M.I.O)

Directeur(s) de thèse proposé(s)*

Directeur HDR proposé*

Nom - Prénom : BONNET Sophie
Corps : DR2 IRD
Laboratoire : M.I.O - Équipe CYBELE
Adresse mail : sophie.bonnet@mio.osupytheas.fr

Choix de cinq publications récentes (souligner éventuellement les étudiants dirigés co-signataires) :

Meunier, V., **Bonnet, S**., Pernice, M., Benavides, M., Lorrain, A., Grosso, O., Lambert, C. & Houlbrèque, F. Bleaching forces coral's heterotrophy on diazotrophs and *Synechococcus*. *The ISME journal*, 13(11), 2882-2886, 2019.

Saulia, E., Benavides, M., Henke, B., Turk-Kubo, K., Cooperguard, H., Grosso, O., Desnues, A., Rodier, M., Dupouy, C., Riemann, L. and **Bonnet. S**. Seasonal Shifts in Diazotrophs Players: Patterns Observed Over a Two-Year Time Series in the New Caledonian Lagoon (Western

Tropical South Pacific Ocean). *Frontiers in Marine Science*. 7:581755. doi:10.3389/fmars.2020.581755, 2020.

Caffin, M., Moutin, T., Foster, R. A., Bouruet-Aubertot, P., Doglioli, A. M., Berthelot, H., Guieu, C., Grosso, O., Helias-Nunige, S., Leblond, N., Gimenez, A., Petrenko, A. A., de Verneil, A., and **Bonnet, S**. N2 fixation as a dominant new N source in the western tropical South Pacific Ocean (OUTPACE cruise), *Biogeosciences*, 15, 2565–2585, https://doi.org/10.5194/bg-15-2565-2018, 2018.

Caffin, M., Berthelot, H., Cornet-Barthaux, V., Barani, A., and **Bonnet, S**. Transfer of diazotroph-derived nitrogen to the planktonic food web across gradients of N2 fixation activity and diversity in the western tropical South Pacific Ocean, *Biogeosciences*, 15, 3795–3810, 2018.

Bonnet, S., Caffin, M., Berthelot, H., Moutin, T. A hot spot of N2 fixation in the western tropical South Pacific pleads for a spatial decoupling between N2 fixation and denitrification. *PNAS*, doi: 10.1073/pnas.1619514114, 2017.

Thèses encadrées ou co-encadrées au cours des quatre dernières années*
NB : n'a pas obtenu de bourse ED depuis 2012

Nom : CAFFIN Mathieu (soutenue)
Intitulé : Devenir de la fixation d'azote et export de carbone dans l'océan Pacifique tropical sud-ouest
Type d'allocation : ANR OUTPACE
Date de début de l'allocation de doctorat : Septembre 2015
Date de soutenance (si la thèse est soutenue) : Décembre 2018
Programme finançant la recherche :

- OUTPACE (ANR, INSU, CNES, IRD): Interactions between planktonic organisms and biogeochemical cycles across trophic and N2 fixation gradients in the western tropical South Pacific Ocean: a multidisciplinary approach (OUTPACE experiment)

Situation actuelle du docteur (si la thèse est soutenue) : Post-Doc CMore, Univ. Hawaii
Pourcentage de participation du directeur à l'encadrement en cas de co-direction : 50%

Nom : SAULIA Emmrick (soutenue)
Intitulé : Cyanobactéries diazotrophes du Pacifique Sud : variabilité saisonnière, caractérisation morpho-génétique/chimique et potentiel de valorisation
Type d'allocation : Province Sud Nouvelle Calédonie
Date de début de l'allocation de doctorat : Février 2016
Date de soutenance (si la thèse est soutenue) : Avril 2019
Programme finançant la recherche:

- MICRO-ALGUES et MACROMOLECULES (Fonds Pacifique, MAEDI)
- TCCC (IRD, M.I.O): Tropical Culture Collection of Cyanobacteria

Situation actuelle du docteur (si la thèse est soutenue) : Post-Doc M.I.O - IRD Nouméa, Création de start-up en cours
Pourcentage de participation du directeur à l'encadrement en cas de co-direction : 70%

Nom : MEUNIER Valentine (soutenue dans un mois)
Intitulé : Interactions entre coraux scléractiniaires et diazotrophes planctoniques dans le contexte du changement climatique
Type d'allocation : LABEX CORAIL
Date de début de l'allocation de doctorat : Février 2018
Date de soutenance (si la thèse est soutenue) : Avril 2021
Programme finançant la recherche:

- TOUCAN (LABEX CORAIL): Trophic links between corals and diazotrophs

Situation actuelle du docteur (si la thèse est soutenue) : Soutenance dans 1 mois
Pourcentage de participation du directeur à l'encadrement en cas de co-direction : 50%

Nom : LORY Caroline
Intitulé: Rôle des métaux traces dans le contrôle de la fixation d'azote atmosphérique dans l'Océan Pacifique tropical Sud-ouest (Projet TONGA)
Type d'allocation : ANR TONGA
Date de début de l'allocation de doctorat : Décembre 2018
Date de soutenance (si la thèse est soutenue) :
Programme finançant la recherche:

- TONGA (ANR, AMIDEX, INSU, IRD): Shallow hydroThermal sOurces of trace elemeNts: potential impacts on biological productivity and the bioloGicAl carbon pump

Pourcentage de participation du directeur à l'encadrement en cas de co-direction : 50%

Nom : ABABOU Fatima-Ezzahra
Intitulé : Devenir de la diazotrophie dans l'Océan tropical : étude de nouvelles espèces récemment isolées au sein de la souchothèque IRD-MIO Tropical Culture Collection of Cyanobacteria (TCCC).
Type d'allocation : IRD ARTS
Date de début de l'allocation de doctorat : Mars 2020
Date de soutenance (si la thèse est soutenue) :
Programme finançant la recherche:

- TONGA (ANR, AMIDEX, INSU, IRD): Shallow hydroThermal sOurces of trace elemeNts: potential impacts on biological productivity and the bioloGicAl carbon pump
- TCCC (IRD, M.I.O): Tropical Culture Collection of Cyanobacteria

Pourcentage de participation du directeur à l'encadrement en cas de co-direction : 50%

Nom : CHOWDHURY Subhadeep
Intitulé : Dinitrogen Fixation in the Indian Ocean: an interbasin and seasonal comparison
Type d'allocation : Bourse Campus France
Date de début de l'allocation de doctorat : Janvier 2021
Date de soutenance (si la thèse est soutenue) :
Programme finançant la recherche:

- DINDE: "Dinitrogen Fixation in the Indian Ocean: an interbasin and seasonal comparison", Indo French Centre for the Promotion of Advanced Research, France-India
- IDEFIX: "Dinitrogen Fixation in the Indian Ocean: an interbasin and seasonal comparison", Pure Ocean Fund, France:

Pourcentage de participation du directeur à l'encadrement en cas de co-direction : 50%

Références citées

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Berthelot, H., Bonnet, S., Grosso, O., Cornet, V., and Barani, A. Transfer of diazotroph derived nitrogen towards non-diazotrophic planktonic communities: a comparative study between Trichodesmium erythraeum, Crocosphaera watsonii and Cyanothece sp., *Biogeosciences* 13, 4005-4021, 2016.

Bonnet, S., Webb, E., Panzeca, C., Karl, D. M., Capone, D. G., and Sanudo-Wilhelmy, S. Vitamin B12 excretion by cultures of the marine cyanobacteria Crocosphaera and Synechococcus, *Limnology and Oceanography*, 55, 1959-1964, 2010.

Bonnet, S., Berthelot, H., Turk-Kubo, K., Cornet-Bartaux, V., Fawcett, S. E., Berman-Frank, I., Barani, A., Dekaezemacker, J., Benavides, M., Charriere, B., and Capone, D. G. Diazotroph derived nitrogen supports diatoms growth in the South West Pacific: a quantitative study using nanoSIMS, *Limnology and Oceanography*, doi: doi:10.1002/lno.10300, 2016b.

Caffin, M., Moutin, T., Foster, R. A., Bouruet-Aubertot, P., Doglioli, A. M., Berthelot, H., Guieu, C., Grosso, O., Helias-Nunige, S., Leblond, N., Gimenez, A., Petrenko, A. A., de Verneil, A., and Bonnet, S. N2 fixation as a dominant new N source in the western tropical South Pacific Ocean (OUTPACE cruise), *Biogeosciences*, 15, 2565–2585, 2018.

Caffin, M., Berthelot, H., Cornet-Barthaux, V., Barani, A., and Bonnet, S. Transfer of diazotroph-derived nitrogen to the planktonic food web across gradients of N2 fixation activity and diversity in the western tropical South Pacific Ocean, *Biogeosciences*, 15, 3795–3810, 2018.

Carlotti, F., Pagano, M., Guilloux, L., Donoso, K., Valdés, V., Grosso, O., and Hunt, B. P. V.: Meso-zooplankton structure and functioning in the western tropical South Pacific along the 20th parallel south during the OUTPACE survey (February–April 2015), Biogeosciences, 15, 7273–7297, https://doi.org/10.5194/bg-15-7273-2018, 2018.

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