

**AO INSU 2015**  
**Section « Océan-Atmosphère »**

***Dossier scientifique***

**Nom du porteur du projet : Thierry Moutin & Sophie Bonnet**

**Titre du projet : OUTPACE (Oligotrophy to UlTra-oligotrophy PACific Experiment)**

La demande LEFE OUTPACE déposée à l'AO 2014 pour solliciter un financement de 2 années (81.6 k€ en 2014 et 69.7 k€ en 2015) a été évaluée positivement (voir section A). Le projet a obtenu un premier financement de 51 k€ du programme national LEFE action CyBER, une dotation « action sud » de l'IRD de 30 k€, un financement GOPS de 18.5 k€ et un soutien aux « activités Sud » du MIO de 17 k€ en 2014. La proposition ANR « OUTPACE » a été acceptée avec un financement de 400 k€; elle faisait partie des 9 projets internationaux « sans accord » retenus dans le cadre du Défi 1 "Gestion sobre des ressources et adaptation au changement climatique" après l'étape de pré-sélection. Le projet OUTPACE a reçu une lettre de soutien du programme international IMBER (Integrated Marine Biogeochemistry and Ecosystem Ressources). La mission océanographique OUTPACE, classée en priorité numéro 1, a été programmée sur le N/O L'Atalante du 14 février au 30 mars 2015.

A l'issue de l'examen du dossier par le conseil scientifique LEFE, une seule année a été financée à hauteur de 51 k€. Nous renouvelons donc notre demande dans le but d'obtenir la suite du financement sollicité, indispensable à la bonne réalisation de la campagne. Pour une gestion optimale de ce financement, nous souhaiterions également que sa ventilation soit réalisée selon un calendrier s'étalant sur 3 années (2014 : 51 k€, 2015 : 51 k€, 2016 : 50 k€, cf. tableau budget page 8 section C). Nous répondons aux critiques émises par le CS LEFE (section B) et joignons à notre demande l'annexe GMMC pour la demande de 3 flotteurs ProvBio, qui avait été mentionnée dans la demande LEFE AO 2014 mais pas officiellement réalisée. Le dossier complémentaire au dossier scientifique comprend donc l'annexe GMMC plus l'ensemble des annexes présentées dans l'AO 2014.

Le dossier de l'AO 2014 non modifiée est présenté à la suite de cette réponse à partir de la page 9..

**A) AVIS DU CS LEFE SUR LE PROJET DEPOSE EN 2014**

« Le CS aime le projet OUTPACE pour l'intérêt scientifique d'une étude multidisciplinaire centrée sur la fixation de l'azote dans le Pacifique SO, la bonne structuration du projet, les approches originales proposées, et l'excellence des équipes nationales et internationales impliquées. Le CS apprécie l'effort des investigateurs principaux d'initier ce projet structurant autour d'une campagne océanographique d'opportunité, liée à la présence du N/O L'Atalante dans le Pacifique SO en 2015.

Trois points principaux fortement liés les uns aux autres, ont été soulevés par le CS et méritent d'être clarifiés.

La stratégie en mer n'est pas encore optimale sur 2 points. 1) Conduire trois études lagrangiennes, chacune sur 6 jours, ne semble pas réaliste. Une proposition pourrait consister à réduire le temps prévu pour le transect E-O qui est assez descriptif et ainsi à donner plus de temps à une seule ou bien deux études lagrangiennes conduites sur des sites contrastés. 2) L'export de la matière organique constitue un objectif important, mais ce point est très peu développé dans le document. Les proposants mettent en avant l'utilité de l'UVP, mais les évaluateurs considèrent que cette approche doit être complétée par d'autres outils (par exemple des pièges à sédiment). D'autre part, la modélisation est dans le projet actuel très peu développé.

Le CS CYBER soutiendra le projet OUTPACE pour sa première année, mais ce financement est conditionné à l'acceptation des dossiers déposés auprès de l'ANR et de la Commission Nationale Flotte Hauturière. »

## **B) REPONSE DE LA COMMUNAUTE OUTPACE**

Les 3 points principaux soulevés par le CS concernent (1) la possibilité de conduire 3 études lagrangiennes, (2) la mesure de l'export de matière organique et (3) la modélisation très peu développée. Nous allons répondre point par point.

### **1) la possibilité de conduire 3 études lagrangiennes**

Ce point a été particulièrement abordé lors de la réunion de préparation de la campagne qui s'est tenue les 2 et 3 juillet 2014 à Marseille (information disponible sur notre site web : <https://outpace.mio.univ-amu.fr/spip.php?rubrique17>). Nous comprenons l'inquiétude du CS car le terme " étude lagrangienne " a facilement pu être interprété comme consistant à suivre une structure à méso-échelle à haute fréquence spatiale et temporelle (stratégie type LATEX), structure difficile à localiser et complexe à étudier. Il s'agit en fait de repérer des zones représentant les caractéristiques biogéochimiques qui nous intéressent et au sein desquelles l'advection est faible. A l'issue de ce repérage, la stratégie lagrangienne (mise à l'eau des flotteurs) sera mise en œuvre uniquement pour vérifier que l'eau échantillonnée provient de la même masse d'eau, alors que les prélèvements seront effectués à proximité du mouillage dérivant (stratégie type BOUM) pendant le temps d'occupation des stations longues. La durée obtenue de la campagne (45 jours) nous permet de maintenir l'objectif d'étudier la production de matière organique et son devenir dans 3 stations de longue durée (7 jours) très contrastées en terme de niveau trophique et de diazotrophie.

### **2) La mesure de l'export de matière organique**

Nous reconnaissons que ce point n'a pas été suffisamment bien décrit dans le précédent document et nous nous en excusons. Il est bien entendu que l'export de matière ne sera pas déterminé uniquement à partir de l'UVP mais à partir de mesures directes à l'aide de pièges à sédiment. Trois pièges PPS5/4 seront déployés systématiquement lors de l'occupation des stations de longue durée. Les profondeurs d'immersion ont été débattues essentiellement entre physiciens et biogéochimistes lors de la réunion de pré-campagne et le compromis suivant a été adopté: 150, 250 et 500 m. De nombreuses analyses sont prévues sur la matière recueillie dans ces pièges. Les analyses C,N,P,Si " de base " seront réalisées par Nathalie Leblond de la cellule piège de l'INSU (cf annexe). D'autres analyses isotopiques sont prévues en particulier pour mesurer la part de l'azote provenant de la diazotrophie dans la matière exportée.

### **3) la modélisation très peu développée**

La modélisation qui a été présentée dans le document correspond uniquement à l'approche que nous développons au laboratoire MIO. Nous comptons utiliser cette approche de modélisation très novatrice qui permet de prendre en compte les processus de façon mécaniste uniquement au niveau des stations longues et pour la durée d'occupation de ces stations, afin de nous éclairer sur des questions concernant la production et le devenir de cette production à ces échelles de temps et d'espace. Nous avons compris que le CS LEFE pensait que la modélisation proposée ne prenait pas en compte les échelles de temps et d'espace plus grandes, à la dimension de la campagne océanographique. Il est vrai que nous avons indiqué que les données multidisciplinaires acquises au cours de la campagne OUTPACE seraient rapidement mises à la disposition de la communauté internationale (base de données LEFE CyBER) pour aider à la représentation des interactions entre climat, cycles biogéochimiques et écosystèmes, mais nous en étions restés là. Depuis, nous nous sommes rapprochés de collègues modélisateurs en France et à l'étranger, et nous sommes capables de proposer une modélisation plus large associée à notre projet, de la petite échelle proposée par S. Rabouille (LOV, Villefranche) à l'échelle régionale proposée par C. Menkes (LOCEAN, Nouméa) jusqu'à l'échelle globale proposée par A. Landolfi & A. Oschlies (GEOMAR, Allemagne). Ces trois propositions détaillées ci-après en complément de l'approche initialement proposée par M. Baklouti (MIO, Marseille) vont permettre de grandement valoriser l'ensemble des données recueillies lors de la campagne OUTPACE et sans aucun doute, de faire progresser notre connaissance du rôle de la fixation d'azote dans les bilans d'éléments biogènes à différentes échelles de temps et d'espace.

### **a) Theoretical approach at the small scale (S. Rabouille)**

Long term stations are dedicated to the temporal description of the followed water mass. In particular, deck incubations will provide very precise data on growth processes in different categories of phototrophic plankton. Given the expected data on individual processes such as nitrogen fixation, net carbon acquisition or phosphorus uptake for instance, a theoretical approach at the small scale is feasible. In this perspective, LOV (S. Rabouille) will propose a formalism of the different processes related to cell growth, with a special emphasis on populations of nitrogen fixers that should be useful for the other models involved in this project.. Incorporation rates will be derived from mass budgets while the observed fluctuations in cell abundance will inform on the overall changes in growth rates. As far as possible, the proposed model(s) will be calibrated on the cruise data. In parallel, the models of diazotrophic growth already available at LOV (Rabouille et al., 2006; Grimaud et al., 2014) constitute a readily operational workbench to run simulations in the environmental conditions observed during the deck experiments or along the cruise track. Altogether, these different models will constitute a reflection tool, allowing hypotheses to be tested as for the role of the different diazotrophic populations may play in the community dynamics and in the carbon and nitrogen budgets pictured in the tracked, water body.

### **b) Implementation of N<sub>2</sub> fixation in a regional model (C. Menkes)**

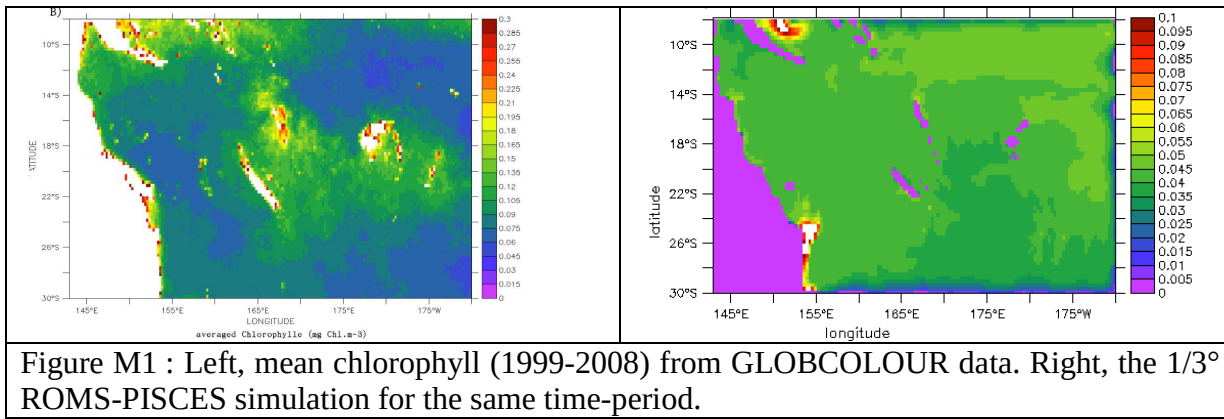
We propose here to set up a coupled dynamical-biogeochemical model intermediate between the detailed 0D-1D process model of Baklouti et al. and the low resolution global model from Landolfi et al. by coupling a high resolution 3D regional dynamical model (ROMS) to the 24 compartment model PISCES including nitrogen fixing organisms. The model configuration will be setup as realistically as possible and will be centered around the OUTPACE sea-experiments.

There are two major objective to that work:

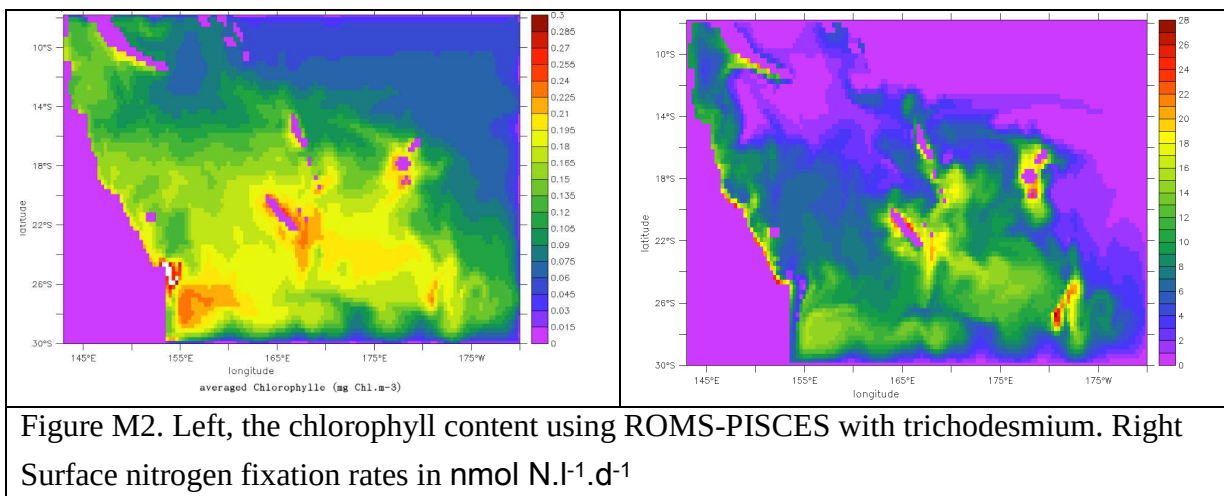
- To be able to improve the ROMS-PISCES South Pacific configuration whose simulated chlorophyll is unrealistic despite a correct dynamic.
- To provide a 3D dynamical –biogeochemical environment at relatively high resolution ( $\sim 1/20^\circ$  degree) to possibly interpret some features of the OUTPACE cruise.

We start with the fact that global models of the South Pacific do not reproduce the detailed features of the chlorophyll patterns. At least two reasons may be invoked for this. First, simulating the South Pacific circulation west of French Polynesia requires to at least resolve scales associated with the numerous islands which separate the SEC into series of jets as it flows westward (Figure 2a). Couvelard et al., (2008) has shown that the model resolution needed to resolve the main SEC features should be lower than  $1/3^\circ$  which is not the case for global models. Second and perhaps more importantly, nitrogen fixing organisms must be included in the biogeochemical models as the western Pacific is a region of high nitrogen fixation.

Typically, a  $1/3^\circ$  degree simulation of ROMS (Menkes et al., 2014) which gives a reasonable dynamics in the region, coupled to PISCES without nitrogen fixing organisms raises a uniform and non-realistic chlorophyll pattern (Figure M1) with an unrealistic north-south gradient in particular.



In an attempt to improve the current state of the model and in collaboration with O. Aumont (LOCEAN), a “Trichodesmium like” compartment has been recently added to a non redfieldian version of the PISCES model. Compared to the other species, the modelled Trichodesmium growth is dependent on temperature following Ye et al. (2012), and nitrogen fixation is limited by both iron and phosphate with a limitation functional form which depends on the main limiting nutrient. Other parametrisations have been added such as the high requirement of iron per unit carbon compared to other species (e.g Kustka et al., 2003). Overall, the simulation of ROMS-PISCES with this new compartment seems to give a more reasonable representation of the chlorophyll with realistic north-south gradient, and structured patterns with enhanced chlorophyll in the New Caledonia, Fiji and Vanuatu triangle. Moreover, average  $N_2$  fixation rates are  $15 \text{ nmol N.l}^{-1}.\text{d}^{-1}$ , with  $26 \text{ nmol N.l}^{-1}.\text{d}^{-1}$  maxima. According to Garcia et al., (2007), Moutin et al (2008), fixation rates in the loyalty channel (between New Caledonia and Vanuatu) varies between 2 and  $50 \text{ nmol N.l}^{-1}.\text{d}^{-1}$ , which is in relative agreement. However, the chlorophyll values are still too high and the seasonality of the chlorophyll is still not correct (not shown).

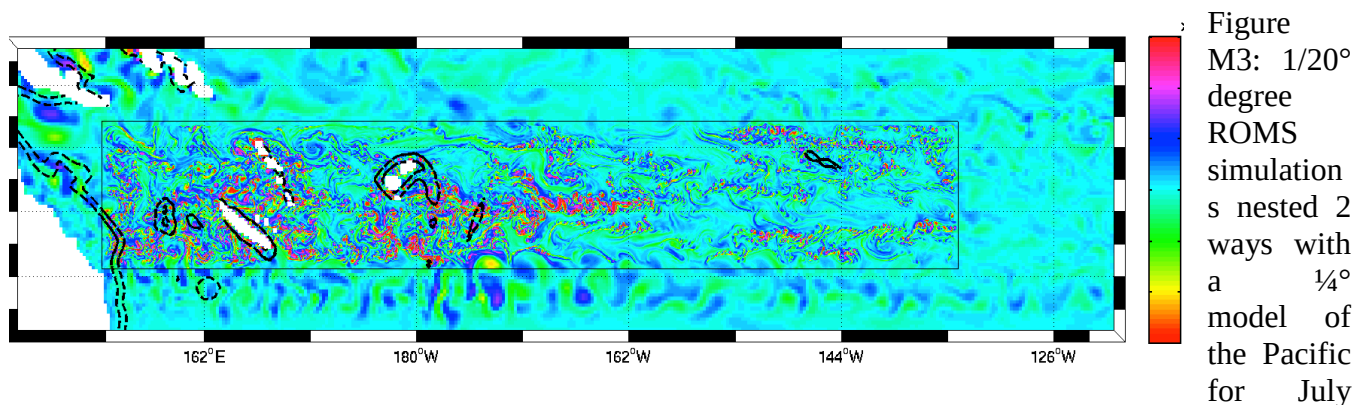


Of course, such crude validation calls for a more thorough evaluation of the model performances, which will be undertaken under the supervision of the data specialists of the project but the model already may provide a useful tool for process oriented studies such as OUTPACE.

### Specific tasks for OUTPACE

In the course of the project, we will attempt to include unicellular diazotrophs but also to improve our Trichodesmium parametrisation in concertation with a similar effort developed for the process studies modeling (Baklouti et al.). The OUTPACE cruise offers this unique opportunity provided that we can provide a framework comparable to the cruise. Toward that end, we will develop a 2-way nested configuration of ROMS-PISCES using the AGRIF tool that will also cover the period of the cruise at

high resolution. The chosen resolution is  $1/20^\circ$  degree, a compromise between sub-mesoscale resolving simulation and computer resources for the domain. The model in its Pacific configuration is currently used to understand the 2014 El Niño (Menkes et al., 2014) and is thus up-to-date. We will use that domain to embed the  $1/20^\circ$  degree grid. An example of the model outputs in the OUTPACE region is given on Figure M3. In that example, a simulation of the 31 July 2014 is shown with the large scale domain at  $1/4^\circ$  and a 2-way nesting over the OUTPACE domain at  $1/20^\circ$  degree. Vorticity stresses the fact that small scale structures and front are most prominent west of Fiji, as the SEC is diverted by islands. This also points out to the need of complementing the 1D studies by the type of 3D studies that we propose in this framework during OUTPACE.



### c) Marine $N_2$ fixation in global Models (A. Landolfi, A. Oschlies, et al.)

#### *Summary*

$N_2$  fixation is a fundamental process for the maintenance of the marine fixed nitrogen (N) inventory exerting a significant influence on the global carbon cycle by controlling marine productivity and the ocean's  $CO_2$  sequestration capacity. The environmental controls of this process are controversial and the pathways by which newly fixed N enters the marine food web have been poorly studied. This has hindered our ability to simulate realistic global patterns of marine  $N_2$  fixation and make predictions of N inventory changes in future climate. Here, we propose to incorporate the increased understanding of marine diazotrophy, obtained within the OUTPACE project, into a state-of-the-art global biogeochemical ocean circulation model (MOM4-NPZDDOM) to simulate realistic global spatial patterns of marine  $N_2$  fixation and assess its impact on marine biogeochemistry on large spatial and temporal scales.

#### *Motivation*

Fixed nitrogen is an essential nutrient for the maintenance of marine productivity. The variability of its inventory is thought to have driven past marine productivity changes (Falkowski 1997, Altabet et al. 2002) contributing to air-sea  $CO_2$  flux variability on long time-scales (Falkowski 1997). To date, large uncertainties remain on the balance of the marine N inventory (Codisopti et al. 2001, Gruber 2004). This is due, firstly to the still sparse (Luo et al. 2014), and often systematically underestimated (Grosskopf et al. 2012), observations of  $N_2$  fixation rates, and the not well known environmental controls of the growth of marine  $N_2$  fixers. While inter-basin differences of  $N_2$  fixation rates suggest a control of iron on  $N_2$  fixation both in the Atlantic and Pacific oceans (eg. Falcon et al. 2004, Moore et al. 2009), phosphate limitation of the important diazotroph *Trichodesmium* has been observed both in the North Atlantic (Sanudo-Wilhelmy et al. 2001) and in the South Pacific (Moutin et al. 2005, 2008). Phosphorus stress of *Trichodesmium* is well documented (Dyhrman et al. 2002) and can induce alternative P scavenging strategies such as phosphate luxury uptake and storage under  $PO_4$ -replete conditions (Krauk et al. 2006,

White et al. 2006), the hydrolysis of dissolved organic P (DOP) (Sohm and Capone 2006, Dyhrman et al. 2002, Mulholland et al. 2002) that can be regulated by cell-signalling (Van Mooy et al. 2012). Secondly, significant uncertainty exists also in the fate and pathways of the recently fixed N (eg. Mulholland 2007). A large part of recently fixed N can be released into the dissolved pool (Capone et al. 1994) which however, does not seem to accumulate in surface waters (Knapp et al. 2005) indicating it may fuel bacterial growth (Meador et al. 2007). On the other hand, isotopically light  $\delta^{15}\text{N}$  suspended particles and zooplankton indicate that fixed N is transferred to higher tropic levels and may be exported at depth (Montoya et al. 2002). These two pathways are not mutually exclusive but, their relative importance may lead to distinct diverging predictions on the N inventory dynamics (Landolfi et al. 2013) and on the long term effect on C export.

The limited, observationally-based, knowledge has hindered our ability to fully capture the distributional gradients of marine  $\text{N}_2$  fixation in global circulation-biogeochemical models making N inventory predictions hard to accomplish. The OUTPACE program offers a unique opportunity to fill out the gaps of the current knowledge on the environmental factors that control the source of N in the ocean and the pathway this new N enters the marine food-web on short temporal scales. The implementation of this novel understating within a global biogeochemical model will allow to improve the global representation of marine  $\text{N}_2$  fixation in global models and, assess the sensitivity of the N inventory and the cycling of C cycle to the input and fate of the newly fixed N, allowing to predict the unforeseen, far-reaching, feedbacks occurring at large temporal and spatial scales.

### ***Global models of marine $\text{N}_2$ fixation***

Coupled ecosystem-circulation models have a great potential for unraveling the biogeochemical interactions at basin and global scale.  $\text{N}_2$  fixation growth is modeled explicitly in global ecosystem-circulation models (eg. Moore et al. 2002, Moore and Doney, 2007) as a function of temperature constraints and phosphate and iron limitation. An important factor controlling  $\text{N}_2$  fixation in current models is the nitrate deficit relative to the Redfield-equivalent phosphate concentration, as highlighted already by earlier box models (Tyrrell 1999, Lenton and Watson 2000, Canfield 2006). The  $\text{NO}_3:\text{PO}_4$  control emerges from the low affinity for phosphate and low diazotrophic growth rates as compared to other phytoplankton. These factors make diazotrophs successful only in nitrate poor and phosphate replete environments. Decreased N availability occurs proximate to denitrifying areas where an excess of P ( $\text{P}^*$ ), relative to the Redfield ratio of 16:1 (Redfield 1963) occurs. In these regions, a tight spatial and temporal coupling between N sources and sinks is observed (Deutsch et al. 2007). Denitrification and anaerobic ammonium oxidation (Anammox) produce waters low in  $\text{NO}_3$ , which in turn are assumed to favor the growth of diazotrophs. The resulting nitrogen fixation would then enhance organic matter export and oxygen consumption at depth, further fueling denitrification. In such a tightly coupled system implicit in many current models, simulated  $\text{N}_2$  fixation can lead to a local run-away feedback with more N being lost than fixed (Landolfi et al. 2013). However, these potential N losses strongly depend on the fate of recently fixed organic N, for example direct or indirect N release into DON reduce N losses (Landolfi et al. 2013). The regional distribution of iron deposition is also important for simulating diazotrophs' growth (Moore and Doney 2007, Dutkiewicz et al 2012, Ward et al. 2014) and can decouple N sources from N sinks (Landolfi et al. 2013). To date the strength of the coupling between N source and sink is not known. This is due to both the sparse observational evidence of  $\text{N}_2$  fixation, particularly in the South Pacific and the lack of information on the fate of the recently fixed organic matter. Temperature constraints and phosphate and iron control, within state-of-the-art biogeochemical fully prognostic global 3D models, do not fully capture the high  $\text{N}_2$  fixation rates, inferred from geochemical evidence (Gruber 2004, Landolfi et al. 2008) and direct  $\text{N}_2$  fixation measurements, in the North Atlantic (eg. Luo et al. 2014). This can be related to the generally high  $\text{NO}_3:\text{PO}_4$  surface waters that suppress simulated diazotrophic activity. Regional models could reduce the  $\text{NO}_3:\text{PO}_4$  ratio of surface waters by increasing the availability of  $\text{PO}_4$  via preferential  $\text{PO}_4$  remineralization and by allowing for diazotrophic DOP uptake (Coles and Hood, 2006). However, sensitivity experiments (Landolfi et al. in prep.) with global models suggest that the same process

would also greatly (and unrealistically) enhance N<sub>2</sub>-fixation near denitrifying areas in the Eastern South Pacific Ocean, where the scarce observational data does not support such elevated N<sub>2</sub> fixation rates (Bonnet et al., 2013b; Luo et al. 2014). In consequence, the sensitivity of N<sub>2</sub> fixation to environmental factors is not currently well represented, making projections into the future role of marine N<sub>2</sub> fixation in a changing climate problematic (e.g., Boyd and Doney, 2002).

### **Objectives**

We here propose to incorporate the advances in our understanding of diazotrophic physiology and ecology from the OUTPACE project into a coupled biogeochemical ocean circulation MOM4-NPZDDDOM model. More specifically, we want to account for the insights, based on observations on role of iron, phosphate and DOP in controlling the distributional gradients of marine N<sub>2</sub> fixers, and assess the sensitivity of global N and C cycles to fate of N<sub>2</sub> fixation on decadal to centennial timescales. From their otherwise unpredictable combined effects, we expect to gain insight into the possible interactions between marine N<sub>2</sub> fixation, N inventory and biological production and C cycling and, how these interactions might affect the ocean's ability to mitigate climate change.

### **Methods**

The ocean-ice model corresponds to the one used by GFDL referred to as the CM2.1 setup of MOM4 by Griffies et al. (2005) and Gnanadesikan et al. (2006). The longitudinal resolution is 1° and the latitudinal resolution varies between 1° in the midlatitudes and 1/3° in the Tropics. The model has 50 vertical levels of which 22 are evenly spaced within the top 220 m. The model is driven by the Coordinated Ocean Reference Experiments (CORE) dataset which is based on the work of Large and Yeager (2004). The embedded ecosystem model, an NPZD-type model, is a modification of previous work (Schmittner et al. 2005). The main differences are the introduction of additional dissolved organic nitrogen, dissolved organic phosphorus and a detrital phosphorus compartment (NPZDD-DOM) which allow for differential cycling between phosphorus and nitrogen (Landolfi et al. 2013). The ten prognostic variables are: dissolved oxygen, nitrate, phosphate, (non-nitrogen-fixing) phytoplankton, nitrogen-fixing phytoplankton (Diazotrophs), zooplankton and particulate phosphorus detritus, particulate nitrogen detritus and semi-labile dissolved organic phosphorus and nitrogen. Diazotrophs are explicitly represented. Their maximum growth rate is temperature dependent and is lower than that of other phytoplankton. Their growth is light and phosphate limited, they can take up nitrate and are able to take up DOP as a source of phosphorus.

After 20 years of integration the circulation model will be coupled on-line with the ecosystem model with the default parameterizations of diazotroph's maximum growth-rate temperature dependence and phosphate uptake kinetics of Landolfi et al. (2013). This run will be taken as the reference experiment against which new (1) phosphorus (2) DOP and (3) iron parameterizations dependencies will be tested. Sensitivity of the global N and C cycle to the fate of recently fixed N will be carried out in a set of experiments with different zooplankton grazing preferences and DOM dynamics. Assessment of model output and observational N<sub>2</sub> fixation data comparison will be carried out. All global model experiments will be carried out over centennial timescales.

***Les References bibliographiques sont volontairement omises dans cette proposition à nombre de page limité***

## C) TABLEAU ET SYNTHÈSE DU BUDGET OUTPACE

Tableau représentant le budget par année de la campagne océanographique OUTPACE. La demande au programme LEFE est la suivante (2014 : 51 k€, 2015 : 51 k€, 2016 : 50 k€).

| FUNDINGS                               |            |            |            |           |            | Details (and corresponding budget lines in the LEFE document)                                  |
|----------------------------------------|------------|------------|------------|-----------|------------|------------------------------------------------------------------------------------------------|
| Fundings <b>acquired</b> or requested  | 2014       | 2015       | 2016       | 2017      | TOTAL      |                                                                                                |
| LEFE                                   | 51         | 51         | 50         | 0         | 152        |                                                                                                |
| Laboratory (Action for the South)      | 17         | 0          | 0          | 0         | 17         |                                                                                                |
| ACI IRD                                | 30         | 0          | 0          | 0         | 30         |                                                                                                |
| GOPS                                   | 18,5       | 0          | 0          | 0         | 18,5       |                                                                                                |
| CNES                                   | 5          | 20         | 5          | 0         | 30         |                                                                                                |
| GMMC (Argo)                            | 0          | 6          | 0          | 0         | 6          |                                                                                                |
| ANR                                    | 0          | 216        | 111        | 59        | 386        |                                                                                                |
| <b>TOTAL</b>                           | <b>122</b> | <b>293</b> | <b>166</b> | <b>59</b> | <b>639</b> |                                                                                                |
| EXPENSES                               |            |            |            |           |            |                                                                                                |
| Equipment                              | 6          | 0          | 0          | 0         | 6          | Specific irridium bouys equipment for the lagragian strategy (6)                               |
| Consummable and analyses               | 35,5       | 54         | 35         | 0         | 125        | Natural and unstable isotopes, laboratory consummable, budget for analyses (1,7)               |
| Trip expenses-Meetings                 | 42         | 8          | 20         | 0         | 70         | Pre et post cruise meetings, Airlines tickets, trip expenses (9,10,15,16,17,21)                |
| Material transport                     | 38         | 15         | 0          | 0         | 53         | Transport of scientific material, containers, dangerous goods, of frozen samples (11,12,13,19) |
| ANR Post-doctorate                     | 0          | 53,9       | 53,9       | 0         | 107,8      | 2 years of post-doctorate (32)                                                                 |
| Ph-D                                   | 0          | 29,0       | 29,0       | 29        | 87,1       | 3 years of doctorate (33)                                                                      |
| Master 2 student "gratifications"      | 0          | 4,4        | 0          | 0         | 4,4        | Gratification of 2 M2 internships (5)                                                          |
| Publications                           | 0          | 0          | 0          | 30        | 30         | Special issue in Biogeosciences and other publications (2,3) 15 papers from french partners    |
| Communications                         | 0          | 0          | 18         | 0         | 18         | Communications and special session dedicated at the Ocean Sciences Meeting (4,24)              |
| French ANR partners expenses (LOV anc  | 0          | 64         | 0          | 0         | 64         | Consummable, Equipment, travel of french ANR partners (HPLC Analyses and other)                |
| Trip expenses-Meetings for targeted wo | 0          | 0          | 10         | 0         | 10         | Meetings targeted workshops (22,23)                                                            |
| Additional analyses                    | 0          | 34,7       | 0          | 0         | 34,7       | Additional analyses (biological, biogeochemical, optical, physical)                            |
| Prélèvement laboratoire                | 0          | 30         | 0          | 0         | 30         | 15% prélèvement du laboratoire                                                                 |
| <b>TOTAL</b>                           | <b>122</b> | <b>293</b> | <b>166</b> | <b>59</b> | <b>639</b> | TOTAL (moins le prélèvement de 4% par l'ANR=12,9k€)                                            |

This table do not take into account fundings acquired by foreign participants (8,14,18,20,25,26,27,28,29,30,31)

Nous tenons à faire remarquer au CS LEFE que l'essentiel de la contribution de l'ANR va servir à financer un doctorant et un post-doctorant. Les contributions de LEFE en 2015 et 2016 sont nécessaires pour que toutes les analyses prévues dans le cadre de cette campagne puissent être réalisées. L'essentiel des analyses, dont le détail précis a été donné dans le document soumis à l'AO 2014 (annexe C du document complémentaire), est prévu d'être financé par le programme LEFE. Si on rapporte le budget global demandé à LEFE pour les analyses au nombre de participants français à terre et en mer, cela fait environ 3 k€ par participant pour 3 ans, ce qui paraît plus que raisonnable !

*Dossier scientifique*

**Nom du porteur du projet : Thierry Moutin & Sophie Bonnet**

**Titre du projet : OUTPACE (Oligotrophy to ULtra-oligotrophy PACific Experiment)**

Cette soumission constitue un **nouveau projet** visant à soutenir la réalisation d'une **campagne océanographique de 45 jours dans le Pacifique sud ouest** en 2015 pour profiter de la présence rare de l'Atalante dans le Pacifique SO le 1<sup>er</sup> semestre 2015 (Cf AO CNFH juin 2014).

Le porteur principal n'a pas obtenu de financement LEFE au cours des 3 dernières années. Le co-porteur a obtenu le financement du projet VAHINE pour une durée de 2 ans (**fin en 2014**). Le projet **OUTPACE** est un projet océanographique multi-disciplinaire d'envergure. Certains résultats obtenus lors de l'expérience en milieu contrôlé VAHINE (suivi de mésocosmes), en particulier de modélisation, seront utiles à la réalisation de OUTPACE.

**Intérêt scientifique et état de l'art**

The additional CO<sub>2</sub> in the atmosphere, mainly resulting from fossil fuel emissions linked with human activities (anthropogenic CO<sub>2</sub>), is the main cause of global warming. The ocean has acted as a major sink of anthropogenic CO<sub>2</sub> (Sabine et al., 2004) preventing a greater accumulation in the atmosphere and therefore a greater increase in the earth's temperature. The biological pump (Fig. 1) provides the main explanation for the vertical gradient of carbon (C) in the ocean. Its strength and efficiency depends upon the complex balance between organic matter production in the euphotic zone and its remineralisation in both the epipelagic and mesopelagic zones. The biological pump was thought to be in an equilibrium state with an associated near-zero net exchange of CO<sub>2</sub> with the atmosphere (Broecker 1991, Murnane et al., 1999). Climate alterations are beginning to disrupt this equilibrium and the expected modification of the biological pump will probably considerably influence oceanic C sequestration (and therefore global warming) over a decadal time scale (Sarmiento and Gruber, 2006). The long term decrease of phosphate availability and the shift from previously nitrogen (N) to phosphorus (P) limited production associated with increasing inputs of N by N<sub>2</sub> fixation was one of the first evidence of the biological pump alteration observed at the HOT time series station in the north Pacific gyre (Karl et al., 1997; 2008).

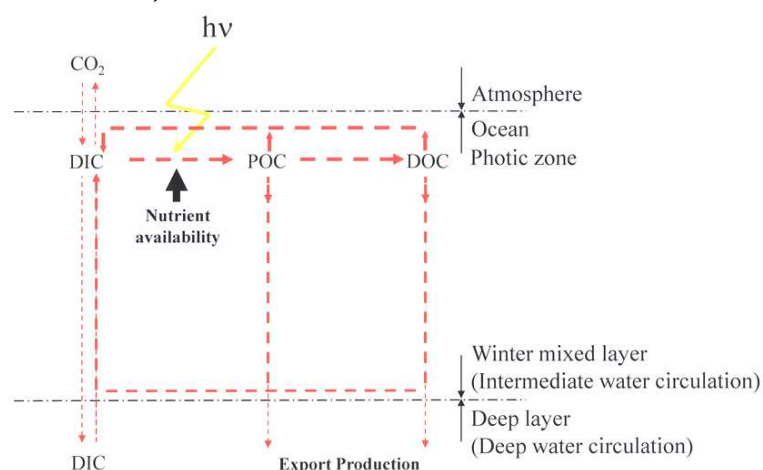


Fig. 1. Major C fluxes for a biological pump budget. Biological pump: C transfer by biological processes into the ocean interior. DIC: Dissolved Inorganic C, POC: Particulate Organic C, DOC: Dissolved Inorganic C. See Moutin *et al.*, (2012) for a detailed description.

The input of new N to the surface ocean through biological N<sub>2</sub> fixation represents a major link between the C and N biogeochemical cycles, i.e between the upper ocean nutrient availability and the biological pump, i.e between ocean and climate. This link was recently shown to play a central role in previous natural climate changes over long time scales (Galbraith et al., 2013, Dernières nouvelles de l'INSU

n°200: Le cycle océanique de l'azote face aux changements climatiques, Jeudi, 25 Juillet 2013). It is nevertheless clear that expected climate changes followed by anthropogenic atmospheric CO<sub>2</sub> changes may concern shorter time scales: the increase in atmospheric CO<sub>2</sub> over the past 200-y is equal to the increase in atmospheric CO<sub>2</sub> between glacial to interglacial conditions, which took place over several thousand y (Sarmiento and Gruber, 2006). It is therefore necessary to obtain a precise representation of the N<sub>2</sub> fixation process in global biogeochemical models. Even if considerable scientific progresses have been made over the last decades (see reviews from Sohm et al., 2011; Zehr, 2011), many questions remain regarding the impact of this process on biogeochemical cycles and C export.

### ***The role of N<sub>2</sub> fixation in the oligotrophic ocean and overview of previous cruises in the SW Pacific:***

The efficiency of oceanic C sequestration depends upon many factors, among which is the availability of nutrients to support phytoplankton growth in the photic zone of the surface ocean (Fig. 1). Large amounts of N are required for phytoplankton growth, as it is an essential component of proteins, nucleic acids and other cellular constituents. Fixed N in the form of nitrate (NO<sub>3</sub><sup>-</sup>) or ammonium (NH<sub>4</sub><sup>+</sup>) is directly usable for growth, but concentrations of fixed N are low (<0.1 μmol L<sup>-1</sup>) in the oligotrophic ocean and often growth-limiting in most of the open ocean euphotic zone (Falkowski et al., 1998). Dissolved dinitrogen (N<sub>2</sub>) gas in seawater, on the other hand, is very abundant in the euphotic zone (ca. 450 μmol L<sup>-1</sup>) and could constitute a nearly inexhaustible N source for the marine biota. Some prokaryotic organisms (Bacteria, Cyanobacteria, Archaea) called 'N<sub>2</sub>-fixers' (or diazotrophs) are able to use this gaseous N source since they can break the triple bond between the two N atoms of the N<sub>2</sub> molecule, and convert it into a usable form (i.e. NH<sub>3</sub>) for assimilation thanks to the nitrogenase enzyme system (Zehr et al., 1998; 2001). By doing this conversion, they release dissolved N in surface waters. At the global scale, they provide 100-200.10<sup>12</sup> g of N to the surface ocean every year (Mahaffey et al., 2005). It is the major external source of N for the ocean, significantly larger than atmospheric and riverine sources (Codispoti et al., 2001; Gruber and Sarmiento, 2002; Gruber, 2004). N<sub>2</sub>-fixing organisms act thus as 'natural fertilizers', and contribute to sustain life and potentially C export in coastal and oceanic environments.

Most of the ocean (60%, Longhurst et al., 1995) is comprised by low-nutrient, low-biomass oligotrophic ecosystems, which constitute the largest coherent biomes on our planet. They support a large part (40%) of the photosynthetic C fixation in the ocean (Antoine et al., 1996). This C fixation is mainly performed by pico-plankton (smaller than 2-3 μm in diameter) that are generally thought to represent a negligible fraction of the total particulate organic C (POC) export flux due to their small size, slow individual sinking rates, and tight grazer control that leads to high rates of recycling in the euphotic zone. Consequently, the efficiency of the biological C pump in these oligotrophic systems has long been considered to be low as the greatest proportion of fixed C is thought to be recycled in the surface layer and rapidly re-exchanged with the atmosphere.

Recent studies have challenged this view and indicate that all primary producers, including picoplanktonic cells, contribute to export from the surface layer of the ocean at rates proportional to their production rates (Richardson & Jackson, 2007). Export mechanisms differ compared to larger cells as picoplanktonic export is mainly due to packaging into larger particles and via grazing and/or aggregation processes (Jackson et al., 1990; 2001; Lomas et al., 2010). More recently, Close et al. (2013) pointed out that 40-70% of picoplanktonic cells are small enough to escape detection under the most common definition of suspended particulate organic matter (POM). Thanks to a coupling between lipid profiles, radiocarbon and stable isotopic signatures of lipids from the North Pacific Subtropical gyre (Hawaii Ocean Times-series station), they showed that the transfer of submicron POM from the surface to mesopelagic waters is an important contributor to the global biological pump. Besides submicron POM export, Karl et al. (2012) have reported an efficient summer export flux of C (3 times greater than the mean wintertime particle flux) at the same HOT station thanks to a 13-year sediment trap experiment. This summer export flux has been attributed to an increase in biomass and productivity of symbiotic N<sub>2</sub>-fixing cyanobacteria associated with diatoms, which have high sinking and low remineralization rates during downward transit. This efficient POM export was thus not related to submicron particles but rather to large-size phytoplankton that could grow in the oligotrophic waters of the NPG thanks to new N provided by N<sub>2</sub> fixation (Karl et al., 2012). **Consequently, the conventional**

**view of low POM export in oligotrophic areas should be revised to include alternative pathways for picoplankton export and the role of N<sub>2</sub> fixation in potentially promoting C export.**

The SW Pacific is an ideal location to study this question, as it is considered to be one of the highest areas of global N<sub>2</sub> fixation (Capone et al., 1997; Sohm et al., 2011). It exhibits among the highest abundances, diversity of N<sub>2</sub>-fixing organisms (Moisander et al., 2010) and activity (Montoya et al., 2004; Bonnet et al., 2009) in the world ocean. While average N<sub>2</sub> fixation rates range from 50-200  $\mu\text{mol N.m}^{-2}.\text{d}^{-1}$  in the tropical North Atlantic (Capone et al., 2005) and Pacific (Dore et al., 2002 ; Karl et al., 2011), they reach 126-4000  $\mu\text{mol N.m}^{-2}.\text{d}^{-1}$  (Montoya et al., 2004) and 50-360  $\mu\text{mol N.m}^{-2}.\text{d}^{-1}$  (Bonnet et al., 2009) in the SW Pacific, in the Arafura Sea (off Australia, TRICHONESIA cruise) and the western warm pool (EUC-Fe cruise), respectively. The seasonal DIAPALIS cruises (INSU-PROOF DIAPAZON programme) in the Loyalty channel off New Caledonia also reported high rates ranging from 151 to 703  $\mu\text{mol N.m}^{-2}.\text{d}^{-1}$  (Garcia et al., 2007), the highest being measured in austral summer which has been directly linked to phosphate availability (Van den Broeck et al., 2004; Moutin et al., 2005). The seasonal distribution of N<sub>2</sub> fixation is corroborated by *in situ* and satellite observations (TRICHOSAT algorithm, Dupouy et al., 2011) of recurrent large *Trichodesmium* blooms during the austral summer (October-March) during the 1998-2010 period in the Melanesian archipelagos around New Caledonia (Vanuatu, Fiji Islands). In addition to *Trichodesmium*, high abundances of unicellular diazotrophic cyanobacteria have been reported in the SW Pacific (Campbell et al., 2005; Bonnet et al., 2009; Hewson et al., 2009; Moisander et al., 2010). Campbell et al., (2005, TRICHONESIA cruise) reported  $1.6 \cdot 10^3$  cells.ml<sup>-1</sup> of Crocosphaera-like cells (UCYN-B) between New Caledonia and Fiji, which is on the same order of magnitude as *Synechococcus* abundance, the most widespread non-diazotrophic cyanobacterium in the ocean (Partensky et al., 1999). The newly discovered and uncultured UCYN-A (Zehr et al., 2001; 2008) also displays extremely high abundances ( $10^5$ - $10^6$  nifH copy.l<sup>-1</sup>, Moisander et al., 2010, KM0703 cruise; Bonnet et al., Unpublished, BIFURCATION cruise) in the SW Pacific (between New Caledonia and Australia), but they seem to have different ecological niches compared to *Trichodesmium* and UCYN-B (Moisander et al., 2010).

When going eastward towards the South Pacific gyre, Halm et al., (2011) have reported rates of 12–190  $\mu\text{mol N m}^{-2} \text{ d}^{-1}$  on the western border of the gyre and Raimbault and Garcia, (2008) and Moutin et al., (2008) reported rates of  $60 \pm 30 \mu\text{mol N m}^{-2} \text{ d}^{-1}$  in the central gyre during the BIOSOPE cruise (INSU-LEFE BIOSOPE programme), indicating a decreasing gradient of N<sub>2</sub> fixation from W to E and low N<sub>2</sub> fixation rates relative to other ocean gyre ecosystems. The organisms responsible for these fluxes are different from common autotrophic diazotrophs such as *Trichodesmium* or *Crocosphaera*, and are mainly affiliated with heterotrophic proteobacteria and low abundances of UCYN-A (Bonnet et al., 2008, BIOSOPE cruise; Halm et al., 2011).

**The W to E zonal gradient of N<sub>2</sub> fixation and the distinct diversity of N<sub>2</sub>-fixing organisms along this gradient provide a unique opportunity to study how production, mineralisation and export of organic matter depends upon N<sub>2</sub> fixation in contrasting nutrient conditions.** Comparisons between different systems along a zonal gradient of trophic status and N<sub>2</sub> fixation will provide new insights for identifying and understanding fundamental interactions between marine biogeochemical C, N, P, Si, Fe-cycles and oligotrophic ecosystems.

## **General objectives and associated strategy (detailed strategy in Annexe 1 – Document CNFH)**

### **OBJECTIVE 1: CHARACTERIZATION OF THE BIOGEOCHEMICAL AND BIOLOGICAL DIVERSITY IN THE SW PACIFIC DURING THE STRONGEST STRATIFIED PERIOD**

One of the main objectives of the **OUTPACE** (Oligotrophy to ULTra-oligotrophy **PAC**ific Experiment) cruise is to characterize the biogeochemical parameters and biological diversity/function along a W-E gradient from the North of New Caledonia, an area exhibiting high N<sub>2</sub> fixation rates, to the central South Pacific gyre, which has been characterized as the most oligotrophic oceanic area of the world ocean (Claustre et al., 2008), and exhibiting low N<sub>2</sub> fixation rates (e.g. Moutin et al., 2008) (Fig. 2).

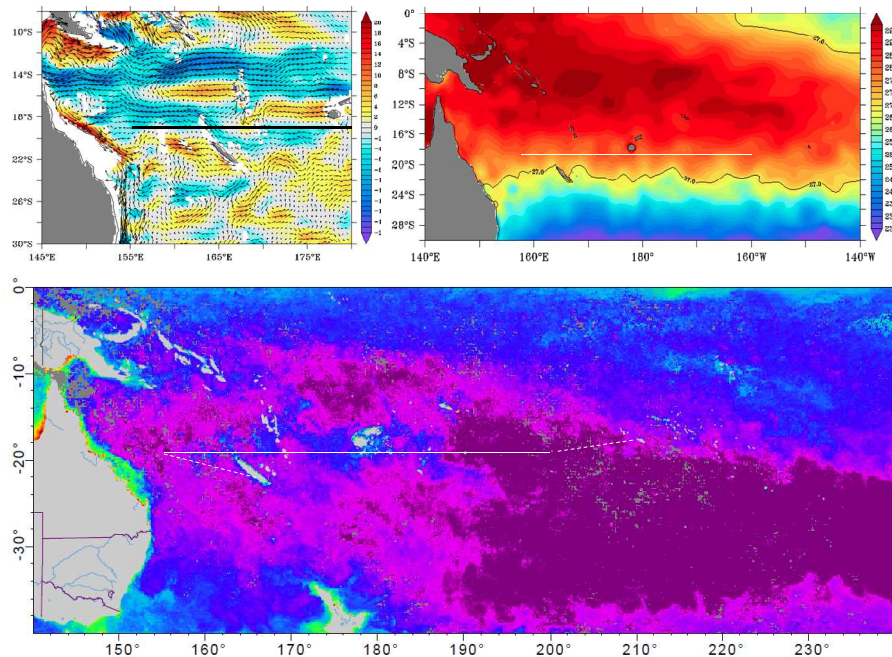


Fig. 2. Transect of the OUTPACE cruise superimposed on several maps (A) Absolute geostrophic currents (from CARS climatology ref to 1000 m, plus currents 0-200 m deduced from Argo floats drift, Kessler and Cravatte, 2013, JGR, accepted. Colorbar: zonal currents; Arrows: horizontal currents), (B) CARS2009 SST climatology (Ridgway and Dunn, 2003) (Dec-March), and (C) Surface chlorophyll concentrations (MODIS, composite Dec-March 2011-2012).

### Objective 1.1: Biogeochemical description

The classic variables temperature, salinity, dissolved oxygen concentration, along with alkalinity, pigments, organic and mineral C and other biogenic elements (N, P, Si) pools, and trace metals of biogeochemical importance (Fe, Zn, Cu, Co, Ni), will be measured, as well as other specific variables at selected depths ( $N_2$  fixation, diazotroph diversity, recycling times for dissolved phosphate...) in 18 short duration (SD, 8h) stations across the 3000 nm transect. The aim is to describe the variations in the biogeochemical characteristics (nutrient and trace metals availability in the photic layer, depth of the nutricline, ratios of dissolved/particulate and organic/mineral in the biogenic elements pools, drawdown of  $CO_2$  of anthropogenic origin, phytoplankton biomass and diversity,  $N_2$  fixation rates...). This biogeochemical description will extend to the gyre in the central South Pacific where oligotrophic conditions are the most extreme (Claustre and Maritorena, 2003). **Our recently acquired techniques for biogeochemical measurements in ultra-oligotrophic waters should enable us to significantly improve our understanding of the biogeochemistry of the SW Pacific.** We do not have a good insight into the spatial distribution of certain biogeochemical variables, such as  $N_2$  fixation, or absolute nutrients concentrations in surface waters, which have a predominant role in controlling planktonic production. Recent studies infer the importance of oligotrophic areas in the drawdown of anthropogenic C (e.g. Close et al., 2013). **It is necessary to quantify the C fluxes in the upper water of the SW Pacific** and their potential links with dinitrogen fixation.

**Specific question:** *What is the actual zonal distribution of main C, nutrients stocks/fluxes and trace metals in the SW Pacific?*

### Objective 1.2. Biological diversity

A major objective will be to produce an overall description of the planktonic community composition in the SW Pacific pelagic ecosystems, providing a “functional” structure for specific analysis. A detailed description of the biological diversity is essential as it is hypothesised that biodiversity increases the functional redundancy of marine ecosystems. Such redundancy may play an important role in an ecosystems ability to withstand natural and anthropogenic disturbances (Fonseca and Ganade, 2001). The need to describe a more “operational” functional structure should not replace having a “complete” description of the species composition. Variation in species composition probably remains the most effective tool in identifying natural and / or anthropogenic perturbations. A coupling of complementary techniques will be used along the transect, including surface continuous flow cytometry (CYTOSUB)

and discrete flow cytometry for bacteria, pico-, nano- and micro-phytoplankton (Marie et al., 1999), microscope determination of taxonomy for microphyto-, microzooplankton (Hasle et al., 1978) and heterotrophic nanoflagellates, and imagery (zooscan, flowcam) for metazooplankton taxonomy (Grosjean et al., 2006), associated with particle counters (UVP, LISST, LOPC) for spatial distribution and size-structure of particules and plankton.. The UVP will also assess the *Trichodesmium* spp. colony abundance in the water column (Guidi et al., 2012).

**A special attention will be given to studying the diversity of diazotrophs.** The scientific community made intense progress over the last decade thanks to the emergence of molecular methods, which allowed the discovery of an increasing diversity of N<sub>2</sub>-fixing organisms (Zehr et al., 2001; 2008), active in previously unsuspected ecological niches such as N-rich areas (e.g. Short and Zehr, 2007; Needoba et al., 2007; Moutin et al., 2008; Bonnet et al., 2011; Fernandez et al., 2011; Dekaezemacker et al., 2013). The filamentous photosynthetic cyanobacterium *Trichodesmium* spp. has long been considered the dominant marine diazotroph. Recent molecular studies have challenged this view as new evidence suggests that photosynthetic unicellular nano-planktonic diazotrophic cyanobacteria (UCYN; belonging to Group B or C, Zehr et al., 2001), photo-heterotrophic unicellular picoplanktonic cyanobacteria from Group A (Zehr et al., 2008) or heterotrophic bacteria are widespread in the oceans and capable of fixing N<sub>2</sub> (Halm et al., 2012; Riemann et al., 2010; Zehr et al., 2008; Zehr et al., 1998; Zehr et al., 2001). The discovery of heterotrophic and photo-heterotrophic (UCYN-A) diazotrophs implies that N<sub>2</sub> fixation is not necessarily linked with C fixation in the euphotic zone, and N<sub>2</sub> fixation has recently been reported in aphotic waters down to 800 m (Hamersley et al., 2011; Rahav et al., 2013) and 2000 m (Bonnet et al., Accepted). However, despite the increasing recognition of their abundance in the global ocean (Luo et al., 2012), very few studies have examined in details the precise role and regulation of N<sub>2</sub> fixation in photo-heterotrophic and heterotrophic (cyano)bacteria in marine waters. Their ubiquity has significantly widened the geographical limits where N<sub>2</sub> fixation was supposed to occur and, therefore, the potential amount of fixed N entering the oceans. The physiology of these (photo)-heterotrophic diazotrophs as well as the environmental factors controlling their distribution, their activity and their relation with other organisms in the ocean are still very poorly known. However, the little knowledge we have on these diazotrophs suggests that they are very different from *Trichodesmium* spp and are regulated in the SE Pacific by organic C and N availability (Bonnet et al., Accepted.). In the North Pacific Subtropical Gyre (NPSG) at station ALOHA, Dore et al. (2008) estimated that the highest integrated N<sub>2</sub> fixation rates occurred during summer while either *Trichodesmium* sp. or diatom blooms (mainly constituted by *Rhizosolenia* sp. or *Hemiaulus* sp. with *R. intracellularis*) were observed. However, the Southern Pacific exhibits a negative Si\* (SiOH<sub>4</sub>-NO<sub>3</sub>) whereas it is strongly positive in the Northern Pacific (Sarmiento and Gruber, 2006) and the relative importance of diatoms in N<sub>2</sub> fixation remains unknown in the proposed study area. We should not dismiss the role of diazotrophs living in association with diatoms as possible key players in N<sub>2</sub> fixation and C export (Karl et al., 2012), and offering a link between the N and silicon biogeochemical cycles. **There is a clear need to study all diazotrophs if we want to know how N<sub>2</sub> fixation and therefore the oceanic N budget will respond to global change in the ocean.**

The cruise transect will cross a gradient of phytoplanktonic biomass identified from satellite data, providing *in situ* observations to compare with such satellite-based analysis. It will be used to calibrate PHYSAT, which allows determining large organism's classes from space (Alvain et al. 2005; 2008), toward a better representation of N<sub>2</sub> fixing organisms. A comparison with vertical distribution of phytoplankton communities based on surface chlorophyll determination (Uitz et al., 2006) will also be undertaken. Ocean color remote sensing will also give a synoptic overview of the chlorophyll concentration in the South Pacific during the cruise. In parallel, synoptic observations of satellite SST and Sea Level anomaly will provide insights about the physical context of the upper water column at basin scale: i.e. variability of ocean stratification and thermocline depth (which can be used as a proxy of the nutricline depth) as well as horizontal and vertical advection from the surrounding waters of the transect (Wilson and Coles, 2005; Martinez et al., 2009). These physical observations will also be compared to *in situ* measurements to validate the use of this proxy.

**Specific questions:** What species currently characterize the SW Pacific's pelagic ecosystems during austral summer conditions? Which species or group is responsible for specific functions (N<sub>2</sub> fixation, dissolved organic phosphate utilization, bacterial production, silification rates)?

## **OBJECTIVE 2. PRODUCTION AND FATE OF ORGANIC MATTER IN CONTRASTING OLIGOTROPHIC ENVIRONMENTS WITH PARTICULAR EMPHASIS ON THE PRODUCTION SUSTAINED BY N<sub>2</sub> FIXATION**

**The major objective is to study how production, mineralisation and export of organic matter depends on the N<sub>2</sub> fixation process in contrasting oligotrophic areas: 3 long duration (LD: 7 days) stations.** We will quantify the flow of material (biogenic elements) through each ecosystem and particularly focus on the role of N<sub>2</sub> fixation in the production and fate of organic C. **The fate of newly fixed N by diazotrophs in the ocean has been poorly studied so far, despite the fact that N<sub>2</sub> fixation provides the major external source of N for the global ocean.** In most studies, the input of N brought to surface waters by N<sub>2</sub> fixation is converted using Redfield ratios in order to ‘theoretically’ evaluate the C production potentially sustained by this source of N. It is not clear whether this N fuels photoautotrophic phytoplankton and can be transferred along the trophic chain and rapidly exported out from the euphotic zone, and/or if it fuels heterotrophic growth and thereby contributes to remineralisation in surface waters. In addition, the plankton diversity and its evolution after the development of a diazotroph bloom (classical food web versus microbial loop) have been very poorly studied, mainly due to the difficulty of observing and monitoring a bloom in the natural environment.

### **Objective 2.1. Physical and biogeochemical characterization of contrasting oligotrophic environments**

A real added value for the project will be to sample 3 water masses along the trophic gradient characterized by different populations of N<sub>2</sub>-fixing organisms. We expect to locate the first LD station (A) in an area dominated by autotrophic diazotrophs *Trichodesmium* spp. and/or UCYN-B, which appear often spatially associated in the SW Pacific (Moisander et al., 2010). The previously developed algorithms **TRICHOSAT** (Dupouy et al., 2011) and **PHYSAT** (Alvain et al. 2005; 2008; the *Trichodesmium* algorithm is currently being developed in the framework of the SAVALEFER project, PI: C. Menkès) will help to locate a *Trichodesmium* spp. bloom from space. We expect to locate the second LD station (B) in an area associated with high abundances of UCYN-A, probably in the transition zone between the SW Pacific and the gyre (Halm et al. 2011). Because it will not be possible to identify it from space, we will use **diazotroph abundance, diversity and gene expression assessed onboard in near real time** using qPCR at each 18 SD stations to decide which one will be used for a process study (LD station). The Third LD station (C) will be located in the low N<sub>2</sub> fixation region (gyre), where heterotrophic diazotrophs dominate (Bonnet et al., 2008; Halm et al., 2011).

The physical dynamics of the regions around the 3 LD stations will be first characterized in terms of large scale general circulation through the analysis of satellite (SST, Ocean color, Altimetry) and operational model data. These will be automatically retrieved and processed (to derive Eulerian and Lagrangian diagnostics such as the Okubo-Weiss parameter and Lagrangian Coherent Structures), and then transmitted onboard by a dedicated server on land. These data will be analysed in near realtime both on board and on land in order to optimize the sampling strategy according to the local physical and ecological characteristics. This approach has already been successfully implemented by the proposed team and employed during several previous cruises: LATEX (Nencioli et al. 2011), KEOPS2 (d'Ovidio et al., 2012) and STRASSE. Local meso and submesoscale dynamics within each region (eddy, filaments and frontal processes and their impact on regulating vertical fluxes and structuring the ecological community) will be first characterized through multisensor MVP 200 mapping at the beginning and at the end of each survey. This will allow to accurately identify the homogeneous and coherent water masses which will be the focus of each LD station. For this purpose the MVP 200 will represent an extremely useful instrument to sample a large zone in a short time to preserve synopticity. If not available, a classical strategy with numerous CTD casts will be undertaken as during the BOUM cruise (Moutin et al., 2012). A glider mapping within a smaller region around the station will integrate the initial and final LD mappings. This will allow to investigate the impact of high-frequency small-scale physical phenomena on biomasses and biogeochemical fluxes. A recently improved version of the Lagrangian navigation software developed during the LATEX project (Doglioli et al., in press) will be used in order to take into account the translation, rotation and deformation of the studied water masses.

3 Bio-Argo floats, specifically equipped with low level phycoerythrin sensors will be deployed at each LD station. In addition to conventional sensors for monitoring physical and biogeochemical parameters,

they will allow to characterize *Trichodesmium* spp. blooms in the SW Pacific ocean during our cruise and on a longer time scale.

**Specific questions:** *How do physical processes structure the planktonic community? How accurately are phytoplanktonic communities, and specially N<sub>2</sub>-fixing organisms, represented by remote sensing data?*

## **Objective 2.2. Measurements of nutrient concentrations and/or availabilities in the photic zone, and identification and quantification of the major external fluxes.**

Nutrient availability (N, P, Si, Fe) in the photic zone controls primary production of organic matter, and thus exerts a strong influence on the species composition of the food web. It is dependent on several input, recycling and export fluxes. Nutrient concentrations in the upper layer represent the equilibrium state between all those fluxes and is the first indicator of nutrient availability. **Nevertheless, as nutrient concentrations are very low in marine oligotrophic habitats, these measurements represent a major observational challenge.** Nanomolar methods developed by the proposed team will be used for nutrient concentrations measurements when necessary (Rimmelin & Moutin, 2005, Rimmelin et al., 2007, Pascoa et al., 2012).

New nutrient inputs in the upper mixed layer include fluxes induced by ocean dynamics (meso- and submesoscale pumping; vertical turbulent mixing), interior sources (N<sub>2</sub> fixation) and surface fluxes (atmospheric deposition). The dynamics of meso- and submesoscale structures is often associated with strong vertical motion that can induce intense upwelling of deeper, nutrient-rich waters into the euphotic zone (Levy et al., 2012). The impact of those structures in regulating local nutrient availability and primary production will be investigated through the observations described in section 2.1.

Vertical turbulent mixing is a key process driving physical and biogeochemical fluxes between the mixed layer and the stratified ocean (Cuypers et al 2012) and can strongly impact primary production (Parad et al., in rev. 2013). While mixing in the mixed layer is mostly driven by direct atmospheric forcing, mixing in the stratified water column is mainly driven by internal wave breaking. Internal waves can be generated from inertial currents at the surface or tidal forcing at the bottom. The study area encompasses a large variety of environments regarding the internal waves (Internal tides notably vary from strong to negligible over the cruise area). As well we expect significant mixing resulting from instabilities of the strong currents of the western part. Therefore the measurements would provide a unique data set characterizing turbulent mixing in a poorly sampled area and under a variety of internal wave dynamical regimes and different current structures. Microstructure measurements that resolve the dissipative scales will be performed using a full ocean depth un-tethered profiling system, “VMP6000” (or a SCAMP that samples the first 100m). This profiler, that can profile down to 6000 m is equipped with microsensors for temperature and shear that enable two independent accurate estimates of the eddy diffusion coefficient K<sub>z</sub>. These two estimates will be used to test the relevance of the different indirect methods based on classical CTD and ADCP measurements. This validation will allow to estimate K<sub>z</sub> also when microstructure measurements are not performed. The same strategy has been used by the proposed team during the BOUM cruise (Cuypers et al., 2012).

**In addition to the input of nutrients by turbulent mixing, we will quantify the N input through biological N<sub>2</sub> fixation,** as it may be a major source in the SW Pacific. Gross and net N<sub>2</sub> fixation rates will be measured at every station (SD and LD) between 0 and 2000 m depth using both the acetylene reduction method (Capone, 1993) and the <sup>15</sup>N<sub>2</sub> labelling procedure, incubation (24 h) and mass spectrometry analyses (MIO Marseille) as described in Montoya et al., (1996) and Bonnet et al., (2011). The <sup>15</sup>N<sub>2</sub> enriched seawater method (Mohr et al., 2010; Wilson et al., 2012; Grosskopf et al., 2012) will be used during the cruise and several method inter-comparisons will be performed between the <sup>15</sup>N<sub>2</sub> bubble method and the <sup>15</sup>N<sub>2</sub> seawater enriched method, both performed under trace metal and DOM-clean (Bonnet et al., 2009) and non-trace metal clean and DOM-clean procedures.

In order to better understand the relationship between (photo)-heterotrophic diazotrophs and DOM, we will perform DOM characterization through FT-ICR MS (allows the detection of individual DOM compounds) simultaneously to N<sub>2</sub> fixation measurement and diazotrophs diversity.

Aeolian dust transport represents, on a global scale, the dominant source of iron, an essential micronutrient for phytoplankton growth, to the ocean (Jickells et al., 2005). Some of the melanesian archipelagos crossed during the transect (Vanuatu, Fiji) exhibit active volcanoes, which have been seen

(in the North Pacific) to provide sources of very soluble and bioavailable trace metals such as iron (Olgun et al., 2011) and potentially N and P. Consequently, dust inputs could stimulate *in situ* biological productivity in oligotrophic ecosystems and alter community composition, distribution of nutrients and finally the net sequestration of atmospheric CO<sub>2</sub>. **We will quantify the input of nutrients by the atmosphere and study the influence of dust deposits on the food web and their potential impact on N<sub>2</sub> fixation, using microcosm experiments.**

**Specific questions:** *Is N<sub>2</sub> fixation a major source of new N for primary production in the photic layer? Do direct measurements of eddy diffusion coefficients reasonably agree with previous estimations? What could be the role of dust?*

### **Objective 2.3. Organic matter production and food web structure**

Because of light/energy requirements, marine organic matter is almost entirely produced in the upper photic zone of the ocean. Variable primary production fuelled by physical processes acting over a wide range of scales interacts with predation to define the species composition of planktonic populations (Gargett & Marra, 2002). The basic principle of the conceptual food web structure, which has not been critically challenged since its original description (Johannes, 1965; Thingstad et al. 1999), is to consider two trophic strategies. Osmotrophy referring to organisms that feed by taking up dissolved nutrients and phagotrophy referring to organisms that feed by eating particulate matter. Osmotrophs include both heterotrophic bacteria and autotrophic phytoplankton, while the predatory food chain includes protozoa, mesozooplankton and higher predators (all heterotrophs).

#### Biogeochemical fluxes in relation to osmotrophic production

We will measure fluxes of biogenic elements (including C) and determine parameters that will help to represent these fluxes (P vs I parameters, K<sub>s</sub>, V<sub>max</sub>, affinity constants...). Classical approaches using stable (<sup>15</sup>N, <sup>13</sup>C) and unstable (<sup>14</sup>C, <sup>33</sup>P, <sup>32</sup>Si) isotopes for measuring biogeochemical fluxes will be used for bulk measurements, along with new techniques at the single-cell level, which address by which species (or group of species) a specific element has been taken up. **New methods concerning both dominant species separation as cell sorting by flow cytometry developed by the proposed team (Duhamel et al., 2009, Gregori et al., 2011; Talarmin et al., 2011) and low detection limits of chemical analyses will allow us to define species-specific uptake parameters.** The contribution of mixotrophy and photoheterotrophy to the biogeochemical fluxes of C and N will be studied together with the environmental factors that control mixotrophy and symbiosis by microbial eukaryotes. **The methods proposed will allow us to significantly improve our understanding of the factors controlling the distribution and activity of different groups of plankton functional types and to link their phylogenetic and functional diversity.**

Regarding N<sub>2</sub>-fixing organisms, recent major development in SIMS (Secondary Ion Mass Spectrometry) instrumentation (NanoSIMS 50TM) now allows for the measurement of isotopic ratios with a spatial resolution better than 100 nm (Guerquin-Kern et al., 2005; Slodzian et al., 1992). The capability of such instrument to measure isotopic composition makes it suitable to study environmental microbiology at the single-cell level (Lechene et al., 2006). Most of these recently discovered N<sub>2</sub>-fixing micro-organisms are uncultured and many unresolved questions remain about their physiology and their importance in term of input of new N in the ocean. The nanoSIMS technology associated with cell sorting (Bonnet et al., In Rev.) or FISH (HISM-SIMS, Thompson et al., 2012, Krupke et al., 2013) will be applied to assess the contribution of every diazotrophic group to overall fluxes, and determine organic/inorganic nutrients controlling their activity. We will focus on the recently described UCYN (Moisander et al. 2010, Thompson et al. 2012) group whose role in C and N cycling is not well understood thanks to recent methods developments realized by the proposed team (see references above).

The silicification process in marine diatoms will be investigated at the single-cell level using the PDMPO labelling technique (Leblanc & Hutchins, 2005).

#### Phagotroph production (secondary production)

The planktonic food web structure influences the fate of primary production in the ocean and has consequences for the CO<sub>2</sub> transfer process. It is generally understood that when small eukaryotes or prokaryotes dominate the microbial community, the grazers of picoplankton are small protozoa which do not produce rapidly sinking faecal pellets. **Despite their omnipresence and their pivotal role in the**

**energy flow in marine waters on a global scale, the physiological ecology of these organisms is poorly understood.** Several grazing steps are necessary to enable primary production to be incorporated into the upper trophic levels. Therefore, most of the C fixed by phytoplankton is respired and remineralised by the microbial community in the surface mixed layer and there is little or no net uptake of CO<sub>2</sub> from the atmosphere to the sea. In contrast, when large phytoplankton cells dominate, they can either sink to depth or be grazed by copepods and other mesozooplankton which produce rapidly sinking faecal pellets. However, Mesozooplankton, i.e. copepods, have recently been coupled to lower trophic levels in the eastern Mediterranean Sea (Thingstad et al. 2005) and their contribution to the C cycle of oligotrophic areas needs to be reconsidered. **The upper meso- and macrozooplankton, largely under-sampled by classical techniques, may also significantly contribute to the vertical transport of organic matter.**

An apparently high contribution of diazotroph N to zooplankton directly east of New Caledonia was recently evidenced (Hunt et al., in press), supporting similar findings in diazotroph rich areas of the Atlantic (Hauss et al. 2013). It is possible to trace the diazotroph signature through the macrozooplankton to the micronekton component of the food web. These are the first detailed stable isotope measurements of the pelagic community presented for the region. We will now increase the geographic coverage of zooplankton and stable isotope sampling across the SW Pacific diazotroph gradient, **specifically to investigate the relative contributions of diazotrophs to food web N and the their role in structuring community composition. We will apply bulk stable isotope analysis in combination with amino acid specific N isotope analysis (CSIA).** The latter method provides the added resolving power of direct estimate of source N isotope signature for any organism measured (McClelland et al. 2003). **A key question remains what are the pathways that fixed N takes to reach primary consumers (microzooplankton and zooplankton grazers) and upwards.** In part we can contribute to answering this question by detailed species level stable isotope analysis of all lower trophic level community components. Additional insights will be gained by experimental studies using labelled N tracers (Loick-Wilde et al. 2012).

#### Biogeochemical process from optical measurements

Integrated measurements of bio-optical properties and pigments will be made with instruments measuring hyperspectral radiometry in the UV-Visible domain, with UV-VIS Trios spectroradiometers (Murakami and Dupouy, 2013). Two consecutive profiles of downward irradiance [ $E_d(Z, \lambda)$ ] will be conducted at solar noon with a MicroPro free-fall profiler (Satlantic) that is equipped with pressure, temperature and tilt sensors and OCR-504 downward irradiance sensors for the UVR-B (305 nm), UVR-A (325, 340 and 380 nm) and PAR (412, 443, 490 and 565 nm) spectral domains, according to the protocol given in Tedetti et al. (2007) and Para et al. (2013). Surface (atmospheric) irradiance [ $E_s(\lambda)$ ], which is equivalent to the downward irradiance just above the sea surface [ $E_d(0^+, \lambda)$ ], will be continuously measured in the same channels from the ship deck with other OCR-504 downward irradiance sensors (surface reference). These measurements will be made to account for the variations in cloud conditions that occurred during measurement. Backscattering at the satellite channels will be obtained with the Hydroscat-6 (Dupouy et al., 2008; 2010; Loisel et al., 2011). Hyperspectral light absorption of particles and CDOM and FDOM characterization (with the identification of markers of bloom degradation in the surface, above and below the euphotic depth, etc...) in relation with pigment concentrations (Coble, 1996; Tedetti et al., 2011; 2012). Additionally, the characterization of optical properties of dominant species (Dupouy et al., 2008) encountered along the transect will be realized. In addition, inorganic aerosol quantification and atmospheric radiance measurements will be continuously done along the transect, especially around the Vanuatu archipelago. Simulations reveal that in Northern Atlantic atmosphere, dusts are able to induce a significant decrease of PP due to the attenuation of light by about 15-25% for dust optical depth (DOD) larger than 0.6-0.7 (at 550 nm).

**Specific questions:** *What are the characteristics of the dominant species concerning photosynthesis, N<sub>2</sub> fixation, nutrient uptake, osmotrophy and phagotrophy?*

#### **Objective 2.4. Organic matter mineralization and food web structure**

The study of organic matter degradation will focus on the relationship between mineralization and bacterial diversity. **Hence, examining the relationship between diversity and functionality within**

the bacterial community (ectoenzymatic activity, uptake of specific organic compounds representative of a chemical family) is a major challenge for understanding the impact of prokaryotic heterotrophic processes on mineralization of organic matter along the water column. We will also study the factors controlling bacterial production and the consequences of such controls on heterotrophic activity, and the hydrolysis rate of Dissolved Organic N and P.

The wide geographical and vertical (in the oceanic water column) distribution of heterotrophic diazotrophs suggests they are **bound to have a key role in global N<sub>2</sub> fixation**. However, failure to isolate and maintain these organisms in culture limits our knowledge on their activity and nutritional needs (Zehr, 2011). While the genetic information of these organisms reveals their dependence on organic matter (Riemann et al., 2010; Tripp et al., 2010), this relationship has not been studied so far. We will perform microcosm experiments at LD stations to test whether different sources of DOM (C-enriched, N-enriched or P-enriched commercially available sources, and natural *in situ* DOM) trigger N<sub>2</sub> fixation and/or the expression of *nifH* by different groups of heterotrophic diazotrophs. Additional nutrient amendment experiments will be performed using <sup>13</sup>C- and <sup>15</sup>N-labelled organic molecule and the **HISH-SIMS** (Thompson et al., 2012) method in order to identify which diazotrophs assimilate which organic molecule and in which quantity.

**Specific questions:** What are the changes in prokaryotic heterotrophic activity and community composition in relation with horizontal (west-east) and vertical (surface to depth) nutrient gradients and the composition/labability of the dissolved organic matter?

### **Objective 2.5. Organic matter export (particulate and dissolved matter)**

The structure of pelagic ecosystems influences the size and type of downward flux of biogenic C in the sea (Legendre and Rassoulzadegan, 1996). This vertical flux is composed mainly of large particles such as fecal pellets, hard parts of zooplankton, amorphous aggregates, marine snow (Fowler and Knauer, 1986; Silver and Gowing, 1991), and senescent diatoms, particularly in the aftermath of blooms (Billet et al., 1983). Fecal pellets can sink quickly: 20-900 m d<sup>-1</sup> for copepods (Lorenzen, 1983; Welschmeyer and Lorenzen, 1985), and up to 2700 m d<sup>-1</sup> for large gelatinous zooplankton (Bruland and Silver, 1981; Madin and Purcell, 1992). High sinking velocities can lead to the efficient export of biogenic C. The downward flux of biogenic C can therefore be largely dominated by C of zooplankton origin (Thibault et al., 1999). We will use the Underwater Vision Profiler (UVP; Picheral et al. 2010) to assess vertical POC flux from particle size distribution (PSD) (Guidi et al. 2008) and vertical distribution of zooplankton (Stemmann et al., 2008) that are directly linked to the vertical flux. We will also complement the zooplankton distribution by an indirect estimate of zooplankton vertical profiles through acoustics from the ship borne S-ADCP and Lowered ADCP on stations as well as from ER 60 (200kHz) echosounder. In addition, using images from the UVP, we will describe vertical, horizontal, and temporal evolution of the *Trichodesmium* spp. colonies similar to what has been done recently in the North Pacific (Guidi et al. 2012). ***Trichodesmium* spp. colonies will be spatially and temporally correlated to vertical particle flux and their potential contribution will be estimated.**

**We will study the C/N/P/Si stoichiometry of settling particulate matter because despite its biogeochemical relevance, there are few direct measurements available** (Geider & La Roche, 2002). We will also examine the role of larger particulate matter responsible for the marine snow rarely observed in oligotrophic areas and quantify instantaneous DOC export.

It has been recently reported that bacteria living within colonies of the N<sub>2</sub>-fixing cyanobacterium *Trichodesmium* spp. use cell-cell signaling to regulate the degradation of organic phosphorus compounds (Van Mooy et al., 2012). In a separate study, it has been found that bacteria living on sinking particles may also use cell-cell signaling to regulate organic matter degradation (Hmelo et al., 2011). Finally, rates of N<sub>2</sub>-fixation were also found to be affected by cell-cell signaling (Van Mooy and Dyhrman, unpublished). We will combine these three findings and ask the next question: **How does cell-cell signaling** via acylated homoserine lactone (AHL) and autoinducer-2 (AI-2) molecules in senescent *Trichodesmium* spp. colonies **affect the export of new organic N in sinking particles?**

**Specific questions:** What is the ratio of particulate vs dissolved organic matter export? What is the role of *Trichodesmium* spp., and of cell-cell signalling in the export?

## Objective 2.6. Fate of N<sub>2</sub> fixation

Although direct evidence of trophic transfer from diazotrophs through planktonic food webs is lacking, studies based on natural abundance of <sup>15</sup>N in suspended particles and zooplankton suggest that N from diazotrophy would support a part of primary and secondary production in the subtropical Atlantic Ocean (Montoya et al., 2002; Landrum et al., 2011). In addition, a low delta <sup>15</sup>N signature of exported material at the time series station HOT (Karl et al., 1997; 2002; Casciotti et al., 2008), and of nitrate accumulating in the upper thermocline of the Sargasso Sea (Knapp et al., 2008) would indicate that recently fixed N is transferred out of the euphotic zone. As fragments of *Trichodesmium* spp. are rarely recovered in sediment traps (Walsby, 1992) and are mostly ungrazed (O'Neil, 1999), transfer of fixed N is probably done through the dissolved pool (Mulholland, 2007). Indeed, *Trichodesmium* spp. has been seen in the natural environment and in cultures to release up to 50-80% of recently fixed N<sub>2</sub> as dissolved organic N (DON) and ammonium (NH<sub>4</sub><sup>+</sup>) (Capone et al., 1994; Mulholland & Bernhardt, 2005) potentially available for surrounding planktonic communities. Alternatively, Brandes et al., (1998) suggested that some material derived from N<sub>2</sub> fixation can be remineralized in surface waters, lightening the isotopic nitrate signal, which may be propagated along the trophic chain.

This lack of knowledge is essentially due to the lack of techniques which allow us to trace the N transfer through the different compartments of the food web. We propose here to overcome these difficulties by using a combination of powerful techniques previously used by the proposed team in the framework of the VAHINE project (INSU-LEFE – ANR JCJC PI: S. Bonnet), including: 1/ high-resolution nanometer scale secondary ion mass spectrometry (NanoSIMS) coupled to 2/ flow cytometry cell sorting and 3/ <sup>15</sup>N<sub>2</sub> isotopic labelling (Bonnet et al., In Rev.).

Previous studies have shown that increased TEP production by *Trichodesmium* was coupled with caspase activation and programmed Cell Death (PCD, Berman-Frank et al., 2004, 2007). The concentration of TEP will be correlated to PCD of diazotrophs using diagnostic genetic markers currently developed by I. Berman-Frank (Univ. Bar Ilan, Israel).

In order to quantify the part of export production that has been sustained by N<sub>2</sub> fixation, a δ<sup>15</sup>N budget will be performed at LD stations, based on <sup>15</sup>N natural abundance (collaboration with A. Knapp, Florida State University). δ<sup>15</sup>NO<sub>3</sub>, δ<sup>15</sup>DON will be measured along the vertical at LD stations. Using the PN sinking flux and PN sinking δ<sup>15</sup>N, we will construct a 1-D (vertical) isotope budget, where the water mass at LD stations is treated as a single box. We will assume that there are two sources of new N to the surface waters, the first of which being total dissolved N (TDN) (TDN = NO<sub>3</sub><sup>-</sup>+NO<sub>2</sub><sup>-</sup>+DON) mixed up from subsurface waters, using the δ<sup>15</sup>N of TDN from the base of the seasonal mixed layer as the δ<sup>15</sup>N for this source term. The other source of new N will be assumed to be N from N<sub>2</sub> fixation, with a δ<sup>15</sup>N of -1‰ (Hoering and Ford, 1960; Minagawa and Wada, 1986; Carpenter et al., 1997). First we will construct a δ<sup>15</sup>N budget assuming that the TDN supplied from the subsurface is the only important source of new N, and assume that the two dominant export terms are represented by 1) the gradient in [TDN] and TDN δ<sup>15</sup>N from the surface to subsurface and 2) the PN sinking flux and its δ<sup>15</sup>N. We will then ask whether the δ<sup>15</sup>N budget can be balanced without N<sub>2</sub> fixation, or whether the δ<sup>15</sup>N of sediment trap material is too low to be explained by the TDN source alone, and the δ<sup>15</sup>N budget thus requires an additional, low δ<sup>15</sup>N source of new N, such as from N<sub>2</sub> fixation (see similar calculations in Altabet, 1988; Karl et al., 1997; Knapp et al., 2005). Using this δ<sup>15</sup>N budget, we will determine what fraction of export production is supported by N<sub>2</sub> fixation. By multiplying this fraction of export production supported by N<sub>2</sub> fixation by the PN sinking flux, we can calculate an absolute N<sub>2</sub> fixation rate for the duration of the sediment trap deployment and compare them with direct measurements.

**Specific questions:** What is the fate of N<sub>2</sub> fixation? Does programmed cell death ((PCD), induced in some of the dominant diazotrophs – *Trichodesmium*) induce enhanced vertical export as we see from lab experiments? Can we identify changes in physiological status of the populations using PCD markers (Bar-Zeev et al. 2013)?

## OBJECTIVE 3. REPRESENTATION OF MAJOR BIOGEOCHEMICAL FLUXES AND OF THE DYNAMICS OF THE PLANKTONIC TROPHIC NETWORK

The multidisciplinary field data gathered during the OUTPACE cruise will be available to the international community as soon as possible (CYBER data base) in order to help represent the interactions between climate, biogeochemical cycles and marine ecosystems. **A multi-scale modelling**

**approach is proposed from the beginning of the OUTPACE project.** Its objective is to complement the observations with the analyzed data in order to understand the interactions between the biogeochemical cycles of the biogenic elements and the dynamics of the planktonic trophic network. Modelling will also be used in interaction with observation and measurements at several scales and at several moments of the project in order to optimize the potential of each of these tools.

### **Objective 3.1. Process scale studies**

We first plan to focus on some physical and biogeochemical processes through (i) the zero-dimensional (0D) modelling of microcosm and through (ii) the simplified 1D vertical modelling of the physical and biogeochemical processes at the long duration stations. These stations will be positioned in regions where the horizontal variability of the hydrological and biogeochemical properties is supposed to be low over the duration of sampling making it possible to consider a 1DV modelling approach. Moreover, the majority of the model's needs, in terms of state variables and parameters, will be acquired through in situ measurements or by controlled activities i.e. sampling and microcosms during the cruises.

Microcosm experiments and modelling (0D) are important for several reasons, the main one being that they provide a vision of the biogeochemical and trophic web functioning deconvoluted from hydrodynamic aspects. This is a crucial step towards the understanding of the ecosystem. Microcosms are also useful to acquire certain model parameters (characteristics of nutrient assimilation, photosynthetic parameters, respiration rates, mineralisation...) and to test or develop new mathematical formulations for processes where current formula are not validated or represented. It is also important that these microcosm experiments are undertaken during the cruises (or field studies) with in situ water samples in order to obtain parameters which are representative of the studied ecosystem.

In practice, a biogeochemical model relevant for SW Pacific waters and developed during the VAHINE project will be used to model the 0D experiments. It is based on a previous version of a biogeochemical model developed for the Mediterranean sea which included 6 functional types, namely bacteria, two phytoplankton size-classes, HNF, ciliates and mesozooplankton and which was already implemented in the Eco3M modelling tool. In this model, organisms have flexible stoichiometry and are represented in terms of C, N, P biomasses as well as in abundances. During the VAHINE project, the diazotroph *Trichodesmium* spp. has been added and the introduction of other unicellular diazotrophs will be done in near future (Post Doc ANR JCJC VAHINE). This model will be validated in the frame of the VAHINE project but it can already be used as an a priori model to help in designing the microcosm experiments that will be held on board. The measurements will in turn be useful to refine the a priori model.

In terms of processes, this model gives a mechanistic or semi-mechanistic representation of the key processes of plankton dynamics (photosynthesis, photoacclimation, nutrient absorption, DOM release, respiration, grazing, mineralization, excretion,...). The majority of these processes have been validated using microcosm and mesocosm experiments (Baklouti et al., 2006b, Baklouti et al., 2011, Alekseenko et al., subm.). Among the processes that specifically need to be worked on, the diazotrophy process is certainly the main one. For *Trichodesmium* spp., the mechanistic model of Stephens et al. (2003) adapted to *Trichodesmium* spp. by Rabouille et al. (2006) has been used and adapted to our model. However, for unicellular diazotrophs, existing models for diazotrophy are scarce and rely on empirical laws deduced from macroscopic observations. In order to improve such models, the underlying mechanisms of diazotrophy need to be understood, especially the interaction between N<sub>2</sub> fixation and nutrient uptake of other N sources, phosphate, and iron.

### **Objective 3.2. In situ experiments at long duration station (1DV)**

**The major objective of 1DV modelling is to represent fluxes of biogenic elements in the surface layer and provide an accurate budget on the fate of the organic C produced (Re-mineralisation, export).** The use of a flexible stoichiometry model (non Redfieldian) will potentially make it possible to illustrate the decoupling of the biogenic element cycles in the oligotrophic ocean. The objective is to understand the production and fate of organic matter in the oligotrophic food web, and their role in the global C cycle. To achieve this, we will proceed to the extraction of those criteria which appear essential in providing a satisfactory representation of the marine pelagic ecosystem and biogenic element fluxes. The complexity of the trophic relationships will be simplified by focusing on the groups of species

having similar biogeochemical functions (i.e. functional groups). The relevance of the 1DV modelling will depend on in situ measurements carried out at the long duration stations. Indeed, a certain number of parameters which will be measured are essential in achieving a realistic representation of the dynamics of the pelagic ecosystem and thus a precise estimate of the C budget. These parameters include all the parameters related to phytoplankton photosynthesis and to nutrient uptake by the plankton. In coupled physical-biogeochemical models, the parameterization of turbulence is frequently a source of error, because it is based on empirical laws, and because these laws are not then re-calibrated for each studied site (typical values found in the literature are used to parameterize these laws). Here, it will be possible to use realistic vertical profiles in the hydrodynamic 1DV model to accurately estimate the coefficients of turbulent vertical diffusion -  $K_z$ - (cf obj 2.1). The use of such realistic profiles is an essential step in representing the mixing processes in the surface layer and the nutrient fluxes at the base of the euphotic layer. However, a simplified 1D version could be achieved instead if the 1DV model is too difficult to parametrize. This simplified model would consider that the water column is divided in a number of boxes that would receive different light intensities according to the vertical profile of PAR, and which would exchange matter only through the sinking of detrital matter. The biogeochemical model that will be used for the 1DV configuration is the same as the 0D one except that it will benefit from the refining that will have been done thanks to the microcosm experiments. This type of model enables us to obtain a representation of the biogeochemical cycles of the principal biogenic elements (C, N, P) as well as representing organisms ("functional" groups) that play a key role in the dynamics of organic C production and transfer. The whole data set collected from the long duration stations, and in particular the data obtained in a quasi continuous manner such as nitrate fluxes and stocks, will offer the possibility of validating the coupled model which up to this point has never been carried out due to the strong mismatch between experiments and modelling.

**Specific questions:** *Is it possible to reproduce, with only one uniquely structured model, the principal physical and biogeochemical characteristics of the different oligotrophic areas studied?*

**Liens avec les programmes nationaux et internationaux :** [Annexe A](#) (dossier CNFH, p. 4).

**Résultats attendus plus détaillés :** [Annexe A](#) (dossier CNFH, p. 5).

#### **Calendrier :**

Février 2014: Réunion pré-campagne avec tous les participants (Marseille, France)

Septembre 2014: Réunion pré-campagne du comité organisateur (Marseille, France)

Octobre 2014-Février 2015: Préparation de la campagne, envoi du matériel sur l'Atalante à Nouméa

Février-Mars 2015: Mission dans le Pacifique SO (description détaillée dans l'annexe 1)

Décembre 2015: Réunion post-campagne avec tous les participants (Marseille, France)

Février-Mars 2016: Atelier de travail focalisé sur la physique (~10 personnes)

Mai-Juin 2016: Atelier de travail focalisé sur la biogéochimie (~10 personnes)

2016: Demande pour une session spéciale à l'OSM 2016, Nouvelle Orléans, Louisiane (USA)

2016-2017: Publication des résultats scientifiques (Issue spéciale, Journal à déterminer)

**Valorisation des travaux antérieurs :** [Annexe A](#) (dossier CNFH, p. 30-31).

**Références :** [Annexe A](#) (CNFH) p. 25-30 pour la communauté OUTPACE et p. 51-53 pour les autres

**Lettres de soutien :** IMBER ([Annexe A](#), dossier CNFH, p. 54) et B. Van Mooy (dossier CNFH, p. 55).

[Annexe A](#) (p. 1-55): dossier CNFH simplifié, [Annexe B](#) (p. 56): Tableau budget (postes de dépenses),

[Annexe C](#) (p. 57-58): Coûts analytiques, [Annexe D](#) (p. 59): devis transport personnel, [Annexe E](#) (p. 60-

61): devis transport matériel, [Annexe F](#) (p. 62): demande cellule piège.

**Remarque : une contribution aux réseaux ARGO et Bio-ARGO** est souhaité dans cette campagne. La demande sera réalisée en temps voulu. Le largage de 3 flotteurs Bio-ARGO à chaque station de longue durée a déjà été discuté avec Hervé Claustre du LOV. Il est envisagé notamment si ces flotteurs peuvent être équipés de capteurs de fluorescence capables de détecter certains diazotrophes : l'étude est en cours.

**Ressources nécessaires à la réalisation du projet** (Cf Annexe 4 pour Tableau correspondances N° de postes). NB : ce tableau inclue une 4<sup>ème</sup> année (2017) qui ne figure pas sur le site INSU et explique le différentiel des postes ‘fonctionnement’ et ‘salaires’ par rapport au tableau INSU.

| N° POSTE DE DEPENSE (Cf Tab. Annexe 2)               | 2014          | 2015          | 2016          | 2017         | TOTAUX        | TOTAL ACQUIS (Or salaires perm.) | TOTAL DEMANDE LEFE | TOTAL DEMANDE ANR |
|------------------------------------------------------|---------------|---------------|---------------|--------------|---------------|----------------------------------|--------------------|-------------------|
| <b>FONCTIONNEMENT Total --&gt;</b>                   | <b>16000</b>  | <b>10240</b>  | <b>22740</b>  | <b>12500</b> | <b>61480</b>  | <b>FONCTIONNEMENT</b>            |                    |                   |
| 1                                                    | 16000         | 0             | 0             | 0            |               |                                  |                    |                   |
| 2                                                    | 0             | 0             | 7500          | 7500         |               |                                  |                    |                   |
| 3                                                    | 0             | 0             | 5000          | 5000         |               |                                  |                    |                   |
| 4                                                    | 0             | 2500          | 2500          | 0            |               |                                  |                    |                   |
| 5                                                    | 0             | 7740          | 7740          | 0            |               | 0                                | 16000              | 45480             |
| <b>PETIT EQUIPEMENT (&lt;15K€) Total --&gt;</b>      | <b>6000</b>   | <b>0</b>      | <b>0</b>      | <b>0</b>     | <b>6000</b>   | <b>PETIT EQUIPEMENT</b>          |                    |                   |
| 6                                                    | 6000          | 0             | 0             | 0            |               | non évalué ici mais > 1M€        | 6000               | 0                 |
| <b>ANALYSES (incluant consommables) Total --&gt;</b> | <b>113760</b> | <b>113760</b> | <b>45880</b>  | <b>0</b>     | <b>273400</b> | <b>ANALYSES</b>                  |                    |                   |
| 7                                                    | 43760         | 43760         | 21880         | 0            |               |                                  |                    |                   |
| 8                                                    | 70000         | 70000         | 24000         | 0            |               | 164000                           | 43760              | 43760             |
| <b>MISSIONS + TRANSPORT DE MATERIEL Total --&gt;</b> | <b>121760</b> | <b>53840</b>  | <b>35200</b>  | <b>0</b>     | <b>210800</b> | <b>MISSIONS</b>                  |                    |                   |
| 9                                                    | 8800          | 0             | 0             | 0            |               |                                  |                    |                   |
| 10                                                   | 2780          | 0             | 0             | 0            |               |                                  |                    |                   |
| 11                                                   | 12660         | 14320         | 0             | 0            |               |                                  |                    |                   |
| 12                                                   | 5520          | 8320          | 0             | 0            |               |                                  |                    |                   |
| 13                                                   | 8000          | 0             | 0             | 0            |               |                                  |                    |                   |
| 14                                                   | 25000         | 0             | 0             | 0            |               |                                  |                    |                   |
| 15                                                   | 42000         | 0             | 0             | 0            |               |                                  |                    |                   |
| 16                                                   | 0             | 1800          | 0             | 0            |               |                                  |                    |                   |
| 17                                                   | 0             | 8000          | 0             | 0            |               |                                  |                    |                   |
| 18                                                   | 17000         | 0             | 0             | 0            |               |                                  |                    |                   |
| 19                                                   | 0             | 4000          | 0             | 0            |               |                                  |                    |                   |
| 20                                                   | 0             | 6000          | 0             | 0            |               |                                  |                    |                   |
| 21                                                   | 0             | 11400         | 0             | 0            |               |                                  |                    |                   |
| 22                                                   | 0             | 0             | 4100          | 0            |               |                                  |                    |                   |
| 23                                                   | 0             | 0             | 4100          | 0            |               |                                  |                    |                   |
| 24                                                   | 0             | 0             | 27000         | 0            |               | 72000                            | 85600              | 35200             |
| <b>SALAIRES NON PERMANENTS Total --&gt;</b>          | <b>49949</b>  | <b>152497</b> | <b>88598</b>  | <b>32568</b> | <b>323612</b> | <b>SALAIRES</b>                  |                    |                   |
| 25                                                   | 18677         | 0             | 0             | 0            |               |                                  |                    |                   |
| 26                                                   | 7004          | 7004          | 4669          | 0            |               |                                  |                    |                   |
| 27                                                   | 4669          | 4669          | 0             | 0            |               |                                  |                    |                   |
| 28                                                   | 9338          | 28015         | 0             | 0            |               |                                  |                    |                   |
| 29                                                   | 0             | 9770          | 0             | 0            |               |                                  |                    |                   |
| 30                                                   | 3257          | 9770          | 0             | 0            |               |                                  |                    |                   |
| 31                                                   | 7004          | 14009         | 4669          | 0            |               |                                  |                    |                   |
| 32                                                   | 0             | 46692         | 46692         | 0            |               |                                  |                    |                   |
| 33                                                   | 0             | 32568         | 32568         | 32568        |               | 132524                           | 0                  | 191088            |
| <b>TOTAUX</b>                                        | <b>598938</b> | <b>650434</b> | <b>362096</b> | <b>77636</b> | <b>TOTAUX</b> | <b>368524</b>                    | <b>151360</b>      | <b>315528</b>     |
| <b>TOTAL ACQUIS</b>                                  | <b>185949</b> | <b>149237</b> | <b>33338</b>  | <b>0</b>     |               |                                  |                    |                   |
| <b>TOTAL DEMANDE A LEFE</b>                          | <b>81640</b>  | <b>69720</b>  | <b>0</b>      | <b>0</b>     | <b>151360</b> |                                  |                    |                   |
| <b>TOTAL DEMANDE A L'ANR</b>                         | <b>0</b>      | <b>111380</b> | <b>159080</b> | <b>45068</b> | <b>315528</b> |                                  |                    |                   |
| <b>TOTAL DEMANDE ACI IRD</b>                         | <b>18000</b>  | <b>0</b>      | <b>0</b>      | <b>0</b>     | <b>18000</b>  |                                  |                    |                   |
| <b>COUT GLOBAL (4 ans)</b>                           | <b>285589</b> | <b>330337</b> | <b>192418</b> | <b>45068</b> | <b>853412</b> |                                  |                    |                   |
| <b>COUT GLOBAL France (4 ans)</b>                    | <b>135581</b> | <b>228655</b> | <b>159080</b> | <b>90136</b> | <b>613452</b> |                                  |                    |                   |

**Co-financements acquis ou soumis (hors INSU).** Le tableau ci-dessus dresse la liste des besoins financiers pour le projet OUPACE. En vert figurent les co-financements acquis, en bleu, le co-financement demandé à LEFE. **Afin de réduire au minimum le budget demandé à LEFE**, des co-financements (en rouge) ont été acquis ou vont être demandés par ailleurs (cf section 4. Liens avec les programmes nationaux et internationaux doc CNFH en Annexe). **Co-financements acquis.** L'ensemble des coûts humains/missions/équipements/analyses afférents à la participation des 9 universités étrangères est pris en charge par nos partenaires étrangers. Pour la partie française, 2 doctorants et 6 postdoctorants impliqués dans ce projet sont déjà financés (bourses MRT, Postdoc Marie Curie, LABEX CORAIL). Une demande 'Banque Sud' MIO à hauteur de 24 K€ est acquise sur le principe (co-financement missions). **Co-financements demandés.** Une ACI IRD est demandée en septembre à hauteur de 18 K€ dans la mesure où ce projet implique plus de 10 scientifiques de l'IRD de 5 unités IRD (MIO, LEGOS, LOCEAN, EIO). Une demande ANR sera déposée le 23/10/2013 (Cf Annexe CNFE).

|                                                                                                                         | coût total            | Cofinancements (préciser la source pour chaque case concernée) |                      |                   |                       |                              |                     | Total demande INSU (préciser l'action concernée en cas de projet multi-actions) |                         |                         |
|-------------------------------------------------------------------------------------------------------------------------|-----------------------|----------------------------------------------------------------|----------------------|-------------------|-----------------------|------------------------------|---------------------|---------------------------------------------------------------------------------|-------------------------|-------------------------|
|                                                                                                                         |                       | Acquis<br>année 1                                              | Acquis<br>année 2    | Acquis<br>année 3 | Demandés<br>Année 1   | Demandés<br>année 2          | Demandés<br>année 3 | demande INSU<br>année 1                                                         | demande INSU<br>année 2 | demande INSU<br>année 3 |
| Moyens nationaux ( <i>rempli automatiquement sur le formulaire informatisé</i> )                                        | 1170000<br>(Atalante) | 0                                                              | 0                    | 0                 | 0                     | 1170000<br>(Cf dossier CNFH) | 0                   | 0                                                                               | 0                       | 0                       |
| Personnel permanent ( <i>rempli automatiquement sur le formulaire informatisé</i> )                                     | 1107743               | 205466                                                         | 507358               | 394919            | 0                     | 0                            | 0                   | 0                                                                               | 0                       | 0                       |
| Personnel temporaire ( <i>rempli automatiquement sur le formulaire informatisé</i> )                                    | 291044                | 49949                                                          | 73237                | 9338              | 0                     | 79260                        | 79260               | 0                                                                               | 0                       | 0                       |
| Fonctionnement<br>- Consommables<br>- Petit équipement<br>- Missions Analyses Publications<br>- Gratifications de stage | 517300                | 136000<br>(MIO+étrangers)                                      | 76000<br>(étrangers) | 24000             | 99640<br>(LEFE + IRD) | 101840<br>(LEFE + ANR)       | 79820<br>(ANR)      | <b>81640</b>                                                                    | <b>69720</b>            | 0                       |
| Equipement > 15 k€ ( <i>joindre devis</i> )                                                                             | 0                     | 0                                                              | 0                    | 0                 | 0                     | 0                            | 0                   | 0                                                                               | 0                       | 0                       |

**Récapitulatif (doivent correspondre aux cases du tableau du formulaire informatisé)**

Total cofinancements acquis : 368 524 euro (hors salaires permanents)

Total cofinancements demandés : 310 340 euro (1 480 340 euro avec demande Atalante)

**Total demande INSU : 151 360 euro**

